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Fibroblast Growth Factor23 as a Novel Marker of Renal Impairment in Multiple Myeloma

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ABSTRACT

Background: Osteoblasts, the cells that make up bone, produce and secrete fibroblast growth factor 23 (FGF23). People with chronic renal disease had a higher-than-normal levels of FGF23 in their blood.

Objective: The goal of this study was to examine FGF23's possible predictive function for renal impairment (RI) in patients with multiple myeloma (MM).

Patients and Methods: Intact FGF23 serum levels were measured in four groups of patients: 1st Group: 20 MM patients with RI either on dialysis or not; 2nd Group: 20 MM patients without RI; 3rd Group: 30 healthy individuals; 4th Group: 10 RI patients without MM.

Results In this study, we found that both MM patients with and without RI had elevated levels of FGF23 (mean=158.50 and 94.75, respectively) than in healthy individuals (mean=25.17). The difference in results between the MM and healthy individual groups was highly significant ($p<0.01$). Patients with MM and RI have greater serum iFGF23 levels compared to those with MM and no RI ($p<0.05$). Significant direct link exists between iFGF23 and creatinine ($p<0.01$), ($r=0.689$). There is a strong inverse correlation between eGFR and iFGF23 in the serum ($p<0.01$).

Conclusion: FGF23 levels increase significantly in MM with RI patients and are higher in MM patients with RI than those without RI. Renal impairment in MM may be diagnosed and predicted with the help of FGF23.

Keywords: Fibroblast Growth Factor 23, glomerular filtration rate, biomarker, Multiple Myeloma, Renal Impairment.

Footnote: This abstract was presented at the 2021 annual meeting of the Society of Hematologic Oncology (SOHO)..

INTRODUCTION

According to the World Health Organization (WHO), multiple myeloma (MM) is a type of plasma cell neoplasm that develops in the bone marrow and causes haematological blood cancer. Multiple myeloma is characterised by the transformation of normal plasma cells into malignant myeloma cells and the subsequent overproduction of an aberrant immunoglobulin known as monoclonal protein or M protein[(1)]. As part of the spectrum of plasma cell dyscrasias that includes monoclonal gammopathy of undetermined significance (MGUS) and progresses to overt plasma cell leukaemia and extramedullary myeloma, MM is the second most frequent hematologic malignancy. Morbidity is high in MM patients since the disease destroys vital organs. The elderly are disproportionately affected by this illness[(2)].

Bone cells, especially osteoblasts, produce and secrete a protein called fibroblast growth factor 23 (FGF-23), which is composed of 251 amino acids and has a molecular weight of 26 kDa[(3)]. The phosphate metabolism-regulating FGF23 is well-known. Elevated blood FGF23 concentrations have been reported for a variety of mesenchymal malignancies, including those that cause oncogenic osteomalacia[(4)].

Non-mesenchymal tumours are less common causes of hypophosphatemic osteomalacia. Serum paraprotein and beta-2 microglobulin concentrations were substantially correlated with FGF23 levels, which were shown to be increased in some patients with myeloma and MGUS[(5)]. Similar to the cytoplasmic localization of FGF23 observed in mesenchymal tumours associated with oncogenic osteomalacia, malignant plasma cells in bone marrow trephines from individuals with myeloma demonstrated cytoplasmic expression of FGF23. Myeloma is characterised by aberrant signalling involving lFGF and FGF receptors[(5)]. Serum FGF23 rises with declining renal function, according to the findings of multiple studies on the involvement of FGF23 in FGF and secondary hyperparathyroidism[(6)].

Elevated FGF23 levels in renal failure presumably result from a compensatory mechanism for hyperphosphataemia, while retention of FGF23 in the circulation and calcitriol medication are also possible causes [(6)]. In conclusion, elevated blood FGF-23 concentrations were associated with higher mortality in hemodialysis maintenance patients and accelerated disease progression in chronic kidney disease (CKD) patients not on dialysis. Therefore, FGF-23 may become an important therapeutic target for the treatment of CKD[(3)]. In multiple myeloma, however, details on FGF23's function are scarce. Therefore, this study reports the level of serum iFGF23 and investigates predictability in renal impaired individuals with multiple myeloma.

Objective:

The goal of this study is to examine FGF23's possible predictive function for renal impairment (RI) in patients with multiple myeloma (MM).

PATIENTS AND METHODS

Eighty participants, 40 males and 40 females, were analysed in this cross-sectional study (Fifty

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patients and thirty controls). The work location was the Internal Medicine Department's Clinical Haematology and Oncology Unit at Ain Shams University Hospital. Patients with a recent multiple myeloma diagnosis who meet diagnostic criteria established by the International Myeloma Working Group (IMWG) [7]. Patients with a newly diagnosis of multiple myeloma who have renal impairment as measured by their growth factor receptors (GFR)[8] and patients with a recent diagnosis of multiple myeloma who had no evidence of renal impairment were eligible for inclusion. Patients receiving chemotherapy for their multiple myeloma and those whose renal function was compromised for any reason were not included in this analysis.

Ethical approval: An approval of the study was obtained from Ain Shams University Faculty of Medicine's Ethical Committee with number and date MS 444/2019. All procedures were carried out in our study involving human subjects according to the code of Ethics of the Word Medical Association[Ref] (1964 Helsinki Declaration). Every patient signed an informed written consent for participation in the study.

Measurement of FGF23:

The concentration of iFGF23 was evaluated using an enzyme-linked immunosorbent test (ELISA) on blood samples. Moreover, we employed (iFGF23) assays from Kainos and Immutopics, which recognise only the full-length form of FGF23 (32kDa). The obtained blood samples were centrifuged at 2000-3000 RPM for 20 minutes after being allowed to clot at room temperature for 10-20 minutes. After that, serum samples were kept in the freezer. **Breda *et al*.**[9] estimated that the unit of measuring cFGF23 is Ru/ml, and the unit of measuring iFGF23 is pg/ml. We used the iFGF23 assay, which measures only full active intact (~32KDa).

Statistical methodology:

Data analysis was done by IBM computer using SPSS (USA, IBM 2009) (the Statistical Program for Social Sciences) version 20.0, using a p value of 0.05 as the threshold for statistical significance. There was no one-tailed p-values. Information was displayed and properly analysed based on the nature of the information collected for each metric. For parametric numerical data, the mean and standard deviation were used for descriptive statistics; for nonparametric data, the median and interquartile range (IQR) were used instead. Methods such as the t-test, Chi-squared test, one-way analysis of variance (ANOVA), the Pearson correlation coefficient (r), and the receiver operating characteristic (ROC) curve were utilised in the statistical analysis.

RESULTS

Eighty adult subjects were categorized into four groups of patients: 1st Group: 20 MM patients with RI either on dialysis or not; 2nd Group: 20 MM patients without RI; 3rd Group: 30 healthy individuals; 4th Group: 10 RI patients without MM. The mean of FGF23 in groups (1, 2, 3, and 4) is (158.50 pgl/ml SD ± 62.64, 94.75 pgl/ml SD ± 56.97, 25.17 pgl/ml SD ± 19.50 and 461 pgl/ml SD ± 190.70, respectively) *(P=0.000)*. The difference in FGF23 levels between each of the patient groups (1, 2, 4) and the control group (3) is statistically significant *(p<0.01)* as shown in Fig (1). Additionally, the difference in FGF23 level between each MM group (1, 2) and CKD group (4) is highly significant with *(p<0.01)*, and the difference in FGF23 level between the two MM groups (1, 2) is significant with *(p<0.05)*, as depicted in Table (**1**). Correlation between FGF23 and other parameters in each patient groups (Group 1, 2, 4) are shown in Table (**2**, **3**, **4**).

Table (1): Comparison between the studied groups regarding FGF23 level.

Variables	Groups								ANOVA	P-value	Post-Hoc test
	Group 3		Group 4		Group 2		Group 1				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
FGF23 (pg/ml)	25.17	19.50	461.00	190.70	94.75	56.97	158.50	62.64	78.283	0.000**	(3,4)**, (3,2)**, (3,1)**, (4,1)**, (2,4)**, (2,1)*

FGF23: fibroblast growth factor 23; Group 1=New diagnosed MM with RI, Group 2=New diagnosed MM without RI, Group 3=Healthy control, Group 4=Renal impairment; *Statistically significant at P<0.05; **Highly statistically significant at P<0.01

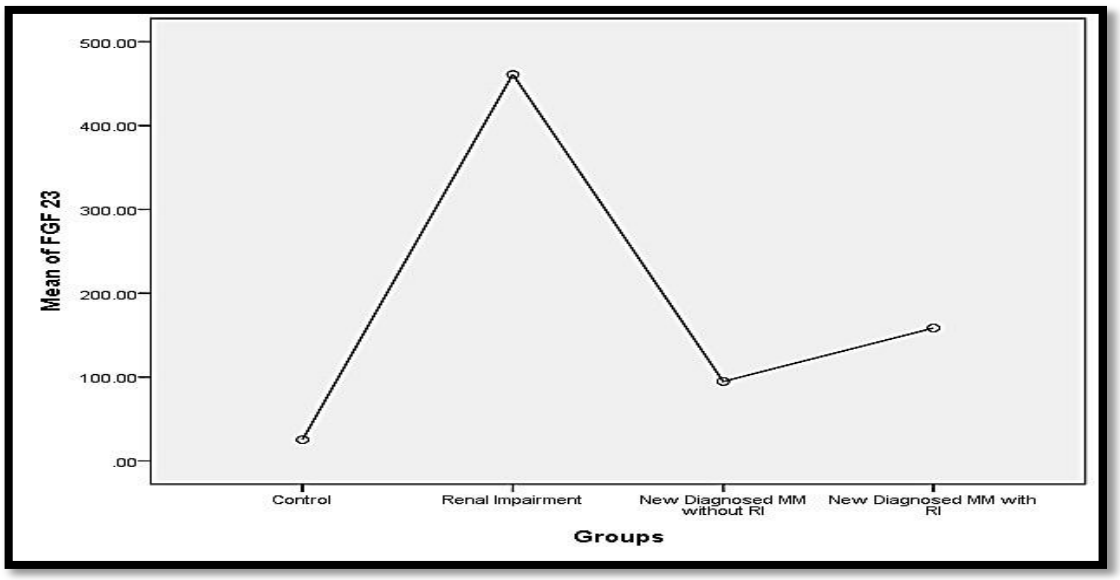

Figure (1): The mean of iFGF23 in each Group

Table (2): Correlation between FGF23 and all other parameters in patients with renal failure group (group 4)

Variables	FGF 23 (pg/ml)	
	R	P-value
Age (year)	.216	0.549
Hemoglobin (g/dL)	.184	0.611
Creatine (mg/dL)	-.171	0.638
Sodium (mEq/L)	-.633	0.049*
Potassium (mEq/L)	-.539	0.108
Alanine aminotransferase (ALT) (U/L)	-.201	0.577
Aspartate aminotransferase (AST) (U/L)	.319	0.370
Direct Bilirubin (µmol/L)	.713	0.021*
Phosphorous (mg/dL)	.087	0.810

*Statistically significant at P<0.05

Table (3): Correlation between FGF23 and other parameters in multiple myeloma patients without renal impairment (Group 2).

Variables	FGF 23 (pg/ml)	
	R	P-value
Age (year)	.107	0.653
Hemoglobin (g/dL)	-.525*	0.017*
Creatine (mg/dL)	.189	0.425
Sodium (mEq/L)	.055	0.817
Potassium (mEq/L)	.313	0.179
ALT (U/L)	.221	0.350
AST (U/L)	-.140	0.555
Direct Bilirubin (µmol/L)	.276	0.239
Phosphorous (mg/dL)	-.074	0.757
Bone marrow plasma cells	.337	0.146
B2 Microglobulin	.021	0.931
Lactate dehydrogenase (LDH) (U/L)	.135	0.571
Calcium (mg/dL)	-.258	0.272
eGFR (mL/min/1/.73m^2)	-.357	0.122

*Statistically significant at P<0.05

Table (4): Correlation between FGF23 and other parameters in multiple myeloma patients with renal impairment (Group 1).

Variables	FGF 23	
	R	p-value
Age (year)	.029	0.902
Hemoglobin (g/dL)	.127	0.593
Creatine (mg/dL)	.688	0.001**
Sodium (mEq/L)	-.014	0.952
Potassium (mEq/L)	.468	0.037*
ALT (U/L)	.029	0.905
AST (U/L)	-.070	0.769
Direct Bilirubin (µmol/L)	-.268	0.253
Phosphorous (mg/dL)	-.035	0.882
BM Plasma cells	.168	0.479
B2 Microglobulin	.036	0.879
LDH (U/L)	.143	0.547
Calcium (mg/dL)	-.095	0.690
Egfr (mL/min/1/.73m^2)	-.224	0.342

*Statistically significant at P<0.05; **Statistically highly significant at P<0.01

Determination of the optimum cut-off value for serum FGF 23 levels

The optimal cut-off value for dichotomizing serum FGF 23 level in both MM patients' groups and the control group was determined using the receiver operating characteristic (ROC) curve and it was > 62.5 pgl/ml using the ROC curve (AUC= 0.948, 80% sensitivity, and 93% specificity) (Fig 2). According to this cut-off point, in the control group, (90%) were negative and (10%) were positive, while in both MM cases groups (80%) of the cases were positive and (20%) were negative, with a highly statistically significant difference in FGF 23 level between two groups (both MM cases and control groups) *(p<0.000)* as shown in Fig (2). Utilizing the ROC curve, the optimal cut-off value for dividing MM patients into those with renal impairment and those with renal failure according to serum FGF 23 level was > 215 pgl/ml

(AUC= 0.980, 100% sensitivity and 90% specificity). This cutoff point resulted in a highly statistically significant difference in FGF 23 levels between the two groups, with 90% of MM patients with renal impairment testing negative and 10% testing positive. *(p <0.000)*. Using the ROC curve, we found that a blood FGF 23 level of > 135 pg/ml best separated patients with and without renal impairment in those with multiple myeloma (AUC= 0.780, 70% sensitivity and 75% specificity). Using this threshold, there was a highly statistically significant difference in FGF 23 level between the MM patients with renal impairment and the MM patients without renal failure groups: 25% were negative and 75% were positive (Groups of people with MM who have renal impairment and those who have renal failure) *(p < 0.002)*.

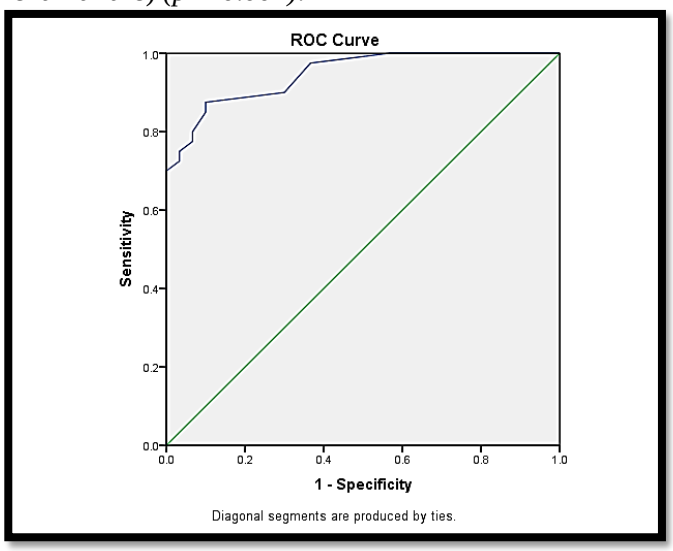

Figure (2): ROC Curve displaying diagnostic accuracy of iFGF23 to differentiate between Myeloma and control groups.

DISCUSSION

With a total of 251 amino acids and a molecular weight of 32 kilodaltons, FGF23 is a member of the fibroblast growth factor (FGF) family. It functions as a hormone because of its poor affinity for heparin sulphate. Increased expression of FGF23 is a novel factor presented to medicine because of its association with renal impairment and multiple myeloma[10]. Bone osteocytes, the most common bone cells, produce the hormone FGF23[11]. Intact fibroblast growth factor-23 (iFGF 23) and C-terminal (cFGF 23) versions of FGF 23 were found to be included in the assessment of FGF 23 concentration by Chudek et al. Full-length iFGF 23 is the most prevalent form in the bloodstream, and it also happens to be the most physiologically active form. In contrast, cFGF23 are ineffective fragments[12]. **Smith *et al.*[13]** reported that FGF23 has a cleavage pathway and estimated that there is an enzyme (type I precursor convertase) called furin responsible for cleaving FGF23 at R179, yielding N and C-terminal fragments. They also reported that currently, intact FGF23 (iFGF23) assays from Kainos and Immutopics are the only commercial ELISA options for measuring FGF23 in human plasma., which only pick up full-length FGF 23 (32kDa), and the C-terminal FGF23 (14kDa) test, also by Immutopics, which picks up both iFGF23 and cFGF23.

Choosing the best assay approach for patient research is contentious. Compared to healthy individuals (mean=25.17), patients with multiple myeloma (mean=158.50) and those without the disease (mean=94.75) had significantly greater levels of FGF23, as seen in table (1). The results were highly significant between MM groups and the healthy individual group with (p<0.01) (however, we used iFGF23 assay, which measures only full active intact (~32KDa). This result agrees with several studies, such as **Stewart and colleagues[14]** found that serum FGF23 levels were significantly higher in myeloma patients compared to healthy controls**.** Nevertheless, the authors used ELISA with polyclonal A bag against active intact FGF23 and inactive c-terminal fragment FGF23·

Suvannasankha *et al.* [15] increases in both total and active FGF23 were detected in MM serum, although the existence of inactive FGF23 fragments could not be ruled out. Furthermore, our investigation indicated that FGF23 levels were initially elevated in MM patients with renal impairment. (mean=158.50) than in those without renal impairment (mean=94.75), and these results were significant (p<0.05). Moreover, we found that the FGF23 level is higher in renal impairment (mean=416) than in healthy individuals (mean=25.17), with a highly significant difference (p<0.01). To correlate serum FGF23 with other laboratory parameters, we found that FGF23 was correlated directly to phosphate (p=0.058), creatinine (p<0.01), and inversely correlated to eGFR (p<0.01). These results agree with **Liu Z *et al.*[16]** in 2019, they found that CKD patients had higher levels of creatinine and iFGF23 than healthy controls (*P*<0.01), Phosphate levels are also elevated in CKD patients compared to healthy controls. (*P*<0.05). **Noonan *et al.*[17]** showed that an increase in bioactive, intact FGF23 generated by bone is a systemic response to a decrease in serum phosphate in CKD (iFGF23). Renal phosphate reabsorption is decreased by FGF 23 via its co-receptor Klotho (KL) and fibroblast growth factor receptors (FGFRs).

Quarles and colleagues[10] demonstrated that FGF23 levels in CKD increase as a compensatory mechanism as it increases phosphorous excretion, but the decline in vitamin D levels in CKD is thought to be independent of high FGF23 levels. This finding aligns with our results as iFGF23 was elevated in renal impairment, but we did not measure c-terminal FGF 23 because we used only iFGF 23 assays. However, we found that FGF 23 increases with renal impairment and is directly proportional to creatinine, phosphate, and degree of renal impairment and inversely proportional to eGFR. **Shimad *et al.*[18]** showed that only full-length FGF23 was found in plasma of ESRD patients receiving peritoneal dialysis, suggesting that the elevated concentration of FGF23 seen in RI patients is

attributable to accumulation of iFGF23.

Additionally, we discovered that iFGF 23 is elevated in renally impaired patients compared to healthy individuals, and that iFGF 23 is up in renally impaired patients with multiple myeloma compared to patients without renal impairment. **Wolf *et al.*[(19)]** found that increased levels of FGF 23 are linked to increased serum phosphate and decreased glomerular filtration rate, and these associations are strengthened in CKD compared to healthy person**s.** According to this study, iFGF23 serum levels are inversely correlated to ca serum levels (p=0.507) and (r=-0.108), which contradicts the findings of **Haad *et al.*[(20)]** as they estimated that iFGF 23 serum levels are directly correlated to ca serum levels, which might be due to a difference in sample size.

In our study, the cut-off point for iFGF23 was greater than or equal to 135 pg/ml. This threshold has an excellent sensitivity of 70% and a good specificity of 75% for distinguishing between multiple myeloma patients with and without renal impairment. Moreover, we noticed that FGF23 has a cut-off point greater than or equal to 62.5 pg/ml at this cut-off point; With an impressive sensitivity of 80% and specificity of 93%, it can reliably distinguish between patients with multiple myeloma and healthy controls., which is consistent with **Suvannasankha *et al*.[(15)]**. There are a few limitations to this study, including the limited sample size and the fact that only one form of FGF23 was measured.

CONCLUSION

We concluded that FGF23 levels increase significantly in MM and RI patients and are higher in MM patients with RI than those without RI. FGF23 levels are directly proportional to creatinine. FGF23 can be used as a diagnostic and predictive marker for renal impairment. Because of this, we propose that larger-scale research is required to confirm the importance of serum FGF23 levels in patients with MM. Assays for measuring cFGF23 and iFGF23 in serum from MM patients are currently lacking, hence more study is required.

RECOMMENDATION

We recommend that larger-scale investigations are needed to confirm the importance of blood FGF23 levels in individuals with MM. To better understand the relationship between cFGF23 and iFGF23 in MM patients, serum FGF23 levels should be measured using an iFGF23 assay in future research with using western blotting to measure cFGF23 only to compare and determine which one is better used as a diagnostic marker and which one is more related to MM morbidity. In addition, more studies to determine if FGF23 and its axis (FGF23, klotho, heparanase) can be targeted in the treatment of MM.

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