Research Article

The relation between fibroblast growth factor 23 level and anemia in chronic kidney disease patients.

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Abstract
Background: Anemia is commonly observed in the course of chronic kidney disease (CKD) and it is associated with diminishing the quality of a patient's life. It also increases morbidity and mortality and CKD progression rate, so it is critical to continue investigations that help explain risk factors involved in the development of anemia in CKD. FGF23 is a promising biomarker of adverse outcomes in patients with CKD. Several studies have suggested a possible association between FGF23 and anemia in these patients. The aim of the current study was to detect level of FGF23 in CKD patients and clarify the relation between of FGF23 level and anemia in CKD patients. **Subjects and Methods: Subjects**: This case - control study was conducted on 90 subjects at the nephrology department of Minia University Hospital through the period from March 2019 to November 2019 selected and divided into three groups containing both control and patients groups. Group I included 30 patients with chronic kidney disease, not on hemodialysis, **Group II** included 30 patients with chronic kidney disease on Hemodialysis and both are with anemia. **Group III** included 30 individuals who are the healthy group. **Exclusion criteria:** Pregnancy, liver cirrhosis, Polycystic kidney disease, Renal cancer, Recent chemotherapy or immunosuppressive therapy, New York Heart Association class 3 or 4 heart failure, Multiple myeloma, Overt gastrointestinal diseases such as untreated gastric cancer and ulcers, Abnormalities of the white blood cell count and differential or platelet count. **Laboratory methods: A)Blood sampling protocol:** 6 ml of blood was withdrawn by sterile venipuncture and before dialysis session in the second group, left to be clotted then centrifuged and the separated serum was divided into liquates. One was designated for the immediate assessment of routine chemistry, The rest of serum was stored at -5 c for subsequent assay of specific labs. **B) Routine laboratory Investigations:** Using the commercially available kits, all patients underwent full laboratory investigation including Complete blood count (CBC) and Renal function tests (serum urea and creatinine). **C) Specific investigation:** eGFR, HS-CRP, Iron profile (serum Iron, Ferritin, TIBC), calcium (Ca), phosphate (P), intact (i)-PTH level and i-FGF23 level. **D) Imaging studies:** Abdominal ultrasound was performed by General Electric ultrasound and transducer with a frequency of 3.5 megahertz (MHz), USA. **Statistical analysis:** Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 25). **Results:** Elevated FGF23 inversely correlated with iron deficiency anemia in patients with CKD dialysis patients of statistical significance (P=0.012). **Conclusion**: FGF23 levels in CKD are higher than levels observed in healthy patients, elevated FGF23 inversely correlated with iron deficiency anemia in patients with CKD patients, and with high sensitivity and specificity of FGF23 as a promising marker for prediction of disease progression.
Key Words: CKD, Anemia, FGF23, Hemodialysis. All authors have no conflict of interest.

Introduction
The prevalence of chronic kidney disease (CKD) is growing in most countries (Glassock et al., 2017).

Anemia is a frequent complication of kidney disease. When it became severe, it causes symptoms that can be debilitating. The course of anemia tends to track the decline in kidney function, with prevalence increasing in more advanced disease (Fishbane & Coyne, 2020).

It is associated with poor quality of life, increased risk of cardiovascular disease, and mortality in patients with CKD (Eriksson et al., 2016).

There are various causes of renal anemia such as decreased production of erythropoietin (EPO), resistance to EPO, shortened survival of red blood cells (RBCs), and bone marrow fibrosis (Tanaka et al., 2018), other potential factors such as chronic inflammation, iron deficiency, malnutrition, increased destruction of red blood cells, and vitamin D deficiency also contribute to the pathogenesis of renal anemia (Patel et al., 2010), so studies suggest that there may be a link between markers of mineral metabolism and hemoglobin parameters (Tsai et al., 2016).

These factors released by osteocytes may mediate changes in the bone marrow microenvironment and therapy, influence hematopoietic cell outcomes (Cain et al., 2012). One of these factors is Fibroblast growth factor-23 (FGF23).

FGF23 is an established biomarker of adverse outcomes in patients with CKD. Several studies have suggested a possible association between FGF23 and anemia in these patients (Nam et al., 2018).
It is a bone-derived hormone regulating phosphate and vitamin D metabolism (Fukumoto, 2020).

FGF23 levels increase gradually with diminishing renal function, and it seems to be an adaptive mechanism to prevent hyperphosphatemia (Kovesdy and Quarles, 2013).

Elevated FGF23 levels have been shown to be associated with adverse outcomes, such as kidney disease progression (Isakova et al., 2011), vascular calcification (Khan et al., 2012), left ventricular hypertrophy, immunesuppression, inflammation, (Kondo et al., 2020) cardiovascular events, (Scialla, et al., 2014), and increased mortality (Kendrick et al., 2011).

It is well known that iron deficiency is an important factor that can promote anemia in CKD, In clinical studies, iron deficiency results in increased intact FGF23 concentrations (Imel et al., 2020).

Results

Table (1): Demographic data in studied groups:

		Group I (CKD)	Group II (ESRD)	Group III (Control)	P value		
		N=30	N=30	N=30			
Age	Median IQR	47 (33.8-60.5)	42 (33.8-57)	27 (24-32.3)	<0.001*		
					I vs II	I vs III	II vs III
					0.717	0.001*	<0.001*
Sex	Male Female	5(16.7%) 25(83.3%)	12(40%) 18(60%)	14(46.7%) 16(53.3%)	0.037*		
					I vs II	I vs III	II vs III
					0.045*	0.012*	0.602
Residence	Rural Urban	13(43.3%) 17(56.7%)	0(0%) 30(100%)	19(63.3%) 11(36.7%)	<0.001*		
					I vs II	I vs III	II vs III
					<0.001*	0.121	<0.001*
Marital status	Single Married	3(10%) 27(90%)	7(23.3%) 23(76.7%)	18(60%) 12(40%)	<0.001*		
					I vs II	I vs III	II vs III
					0.166	<0.001*	0.004*
Weight	Range Mean± SD	(50-100) 73.4±13.5	(49-126) 79.5±21.5	(48-93) 68.6±12.8	0.042*		
					I vs II	I vs III	II vs III
					0.334	0.488	0.032*
Height	Range Mean± SD	(150-180) 166.4±7.6	(155-185) 166.4±7.5	(154-180) 166.9±7.9	0.954		
					I vs II	I vs III	II vs III
					1	0.966	0.959
BMI	Range Mean± SD	(20.9-35.2) 26.4±4.1	(20.3-44.9) 28.6±7.2	(19.7-29.4) 24.4±3.1	0.010*		
					I vs II	I vs III	II vs III
					0.240	0.287	0.007*
Smoking	No Yes	29(96.7%) 1(3.3%)	21(70%) 9(30%)	27(90%) 3(10%)	0.017*		
					I vs II	I vs III	II vs III
					0.006*	0.612	0.053

Table (1): shows the results of demographic data of the studied groups.

There was female predominance in diseased groups than control one of statistical significance. (p= 0.037), significant increase in age in diseased goups than control (p<0.001), Also in married than single of diseade one. (p<0.001).

There was increase in urban than rural of statistical significance among 1 vs 2 and 2 vs 3, (p<0.001) and group 2 is the highest. (p<0.001), significant increase in BMI in 2 vs 3. (p<0.010), significant increase in smoking in 2 vs 1. (P< 0.017*)*.

Table (2): Correlations between FGF23 and iron prpfile:

ESRD group	IRON		TIBC		TSAT		Ferritin	
	R	P value	R	P value	R	P value	R	P value
FGF23	-0.454	0.012*	0.081	0.677	-0.461	0.012*	0.352	0.056

- Pearson's correlation
- *: Significant level at P value < 0.05.

Table (2) shows Correlations between FGF23 and iron prpfileIn ESRD dialysis patients
There was inverse correlation between FGF23 and TSAT of statistical significance (P=0.012) and positively with ferritin.

CKD group	IRON		TIBC		TSAT		Ferritin	
	R	P value	R	P value	R	P value	R	P value
FGF23	-0.072	0.705	-0.108	0.569	-0.026	0.891	0.077	0.686

Table (3) In CKD non dialysis patients
There was inverse correlation between FGF23 and TSAT and positively with ferritin but of no statistical significance.

Control group	IRON		TIBC		TSAT		Ferritin	
	R	P value	R	P value	R	P value	R	P value
FGF23	0.018	0.926	0.116	0.541	-0.036	0.851	-0.292	0.117

Table (4) In control group
There was inverse correlation between FGF23 and TSAT and ferritin of no statistical significance.

Discussion

Our studied subjects were 5 males (16.7%) and 25 females (83.3%) in group I, 12 males (40%) and 18 females (60%) in group II, 14 males (46.7%) and 16 females (53.3%) in group III, There was a significant increase in age in diseased groups than control ($p<0.001$) agreeing with the suggestion that amending the CKD definition to include age-specific thresholds for GFR (Delanaye et al., 2019).

In contrast to the incidence of the end-stage kidney disease (ESRD) 50% higher in adult men than in women, even though there is a higher prevalence of chronic kidney disease (CKD) in women (Harris & Zhang, 2020), our study show a significant female predominance with a significant change among groups ($p=0.037$).

As regard residence, there was an increase in urban than rural of statistical significance among 1 vs 2 and 2 vs 3 ($p<0.001$) and group 2 is the highest ($p<0.001$), which agree with the association between metabolic syndrome and chronic kidney disease in a Chinese urban population (Juan Chen et al.,2017).

As regard the body mass index (BMI), its mean was 26.4±4.1 kg /m2 in group I, 28.6±7.2 kg /m2 in group II, 24.4±3.1 kg /m2 in group III. There was significant increase in BMI in 2 vs 3.($p<0.010$), Patients with (ESRD) on dialysis have protein-energy imbalances (Fouque et al., 2008), which can lead to muscle wasting (Kim et al., 2014).

Given the increasing prevalence of obesity among ESRD patients over the last decade, recent studies have examined the presence of low muscle mass concurrent with a high-fat mass in the dialysis population (sarcopenic obesity) (Bučar Pajek & Pajek, 2018), and this is consistent with our study result of the significant increase in BMI in ESRD.

There was a significant increase in smoking in 2 vs. 1.($P< 0.017$), which agreed with some but not all reporting cigarette smoking as an independent risk factor for loss of kidney function over time (Ricardo et al., 2015).

Although FGF23 regulates phosphate and vitamin D levels, its actions extend beyond maintaining mineral metabolism homeostasis (Grabner et al., 2015).

In our study we demonstrate an inverse relationship between FGF23 and TSAT in both ckd dialysis and non-dialysis patients but not hemoglobin level.

Our findings are of great clinical importance, as anemia is a common complication in CKD patients and is associated with adverse outcomes.
Preclinical data suggest a novel role of elevated FGF23 in hematopoiesis through impairment of erythropoiesis in bone marrow cells, deletion of FGF23 in wild-type mice resulted in increased erythropoiesis in bone marrow independent of vitamin D, and exogenous FGF23 adminis-

tration resulted in a rapid decrease in erythropoiesis (Coe et al., 2014). FGF23 levels in the latter studies were parallel to levels detected in murine models with early CKD, supporting the hypothesis that moderate elevations of FGF23 observed in early CKD may contribute to the development of anemia.

Human studies of FGF23 and red cell parameters are confirmatory (Breda et al., 2015), Studies that have shown the relationship between FGF23 and anemia in CKD of conflicting results.

It is well known that iron deficiency is an important factor that can promote anemia in CKD. Interestingly, animal and human studies demonstrated that absolute and functional iron deficiency stimulates FGF23 production (David et al., 2016), In line with these findings, our data showed that FGF23 was inversely correlated with iron profiles, including iron and TSAT which thus assures power to detect statistical significance in dialysis patients so it can be presumed that iron deficiency induces anemia either directly or indirectly through a negative impact of FGF23 on erythropoiesis, and positively correlated with ferritin, ferritin is an acute-phase reactant and can be elevated in response to uremic inflammatory condition despite the presence of iron deficiency.

Inflammation leads to increase FGF23 levels via suppression of FGF23 degradation, FGF23 could increase inflammatory cytokine production (S. Singh et al., 2016).

Thus, high FGF23 levels may be accompanied by inflammation, resulting in the development of functional iron deficiency.

The emerging link between iron deficiency and FGF23 regulation offers yet another possible explanation pathway for the relationship between elevated FGF23 and anemia that observed and further clinical studies are required to confirm the association between iron metabolism and FGF23.

Conclusions

Elevated FGF23 inversely correlated iron deficiency anemia in patients with CKD dialysis patients.

Recommendations

Further studies are needed on other populations with a different background and geographical origin and more clarification needed about the relation of FGF23 and anemia and more explanation for the mechanisms.

Limitations of the study

There are potential limitations of our study which include a small sample size and a single-point measurement of FGF23 and hemoglobin, it would be interesting to see whether a change of FGF23 level is concordant to that of hemoglobin level.

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