The Effect of High Flux versus Low Flux Dialyzers on Serum Fibroblast Growth Factor-23 (FGF-23) and Its Cardiovascular Implications in Prevalent Hemodialysis Patients

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ABSTRACT

Background: Cardiovascular disease is the main cause of death in patients with end stage renal disease. Fibroblastic growth factor- 23 (FGF-23) is a novel phosphaturic hormone produced in the osteocyte. Patients with kidney disease complain of elevated levels of FGF-23 in response to hyperphosphatemia.

Patients and Methods: This was a cross-over prospective study conducted on 30 patients on regular hemodialysis. The study period was 6 months, laboratory investigations for FGF-23, complete blood picture, s. urea (pre-dialysis and post-dialysis), serum albumin, serum electrolytes (Ca^{++} & PO_4), alkaline phosphatase, PTH, serum ferritin, and lipid profile as well as echocardiography were obtained in the start of the study, after 3 months on maintained low-flux hemodialysis and 3 months after switching the patients to high-flux hemodialysis.

Results: There was a highly significant reduction of FGF-23 level with high-flux dialyzers than with low-flux dialyzers. The adjusted difference in the absolute change in FGF-23 levels between the three months period of low-flux dialysis and the three months period of high-flux dialysis showed a highly significant reduction with high-flux than with low-flux (p= 0.000). There was a significant reduction of serum levels of PTH, calcium, phosphorus levels with high-flux dialyzers than with low-flux dialyzers, the adjusted difference was (p= 0.022), (p= 0.000), and (p= 0.006) respectively.

Conclusion: FGF-23 levels were significantly higher in hemodialysis patients than in normal population. FGF-23 was better eliminated by high-flux dialyzers than low-flux dialyzers and high FGF-23 levels were associated with increased incidence of cardiac valve calcification. FGF-23 positively correlated with right ventricular systolic pressure.

Keywords: Cardiovascular, Low-flux, High-flux, FGF-23, Hemodialysis patients

INTRODUCTION

Patients with end stage renal disease, treated with hemodialysis, have an annual mortality rate of approximately 20%. Cardiovascular disease is the main cause of death, with the annual death rate from CVD at 9%, which is approximately 10–20 higher than in the general population [1]. Vascular calcification is also common in patients with end-stage renal disease (ESRD) who are treated with regular dialysis and it may contribute to the very high mortality rate from cardiovascular causes in such patients. Arterial calcification can result from the deposition of mineral along the intimal layer of the arteries in conjunction with atherosclerotic plaques or from calcium deposition in the medial layer of the arteries that is due, at least in part, to disturbances in mineral metabolism [2].

fibroblastic growth factor 23 (FGF23) is a novel phosphaturic hormone produced in the osteocyte [3]. In patients with kidney disease, normal serum phosphate levels are maintained despite a declining nephron mass, in part by progressive "secondary" increases in FGF-23 levels [4]. Increases in FGF23 levels help maintain serum phosphate in the normal range in CKD. Prospective studies in populations of pre-dialysis CKD, incident and prevalent ESRD and kidney transplant recipients demonstrate that elevated FGF23 levels are independently associated with progression of CKD and development of cardiovascular events and mortality [5].

Aim of study to compare between the effect of low-flux versus high-flux dialyzers on serum level of FGF-23, and to study the association between FGF-23 and echocardiographic parameters in hemodialysis patients.

PATIENTS AND METHODS

This was a cross-over prospective study conducted on 30 patients on regular hemodialysis maintained on thrice weekly sessions in Dar Elshefaa hemodialysis unit, 4 hours each. The study Period was 6 months (from March to August 2016). Patients were on low flux polysulfone filters (Fresinius F6), then switched to high-flux polysulfone filters (F6). Laboratory investigations included FGF-23, complete blood picture, s. urea (pre-dialysis and post-dialysis), serum albumin, serum electrolytes (Ca^{++} & PO_4), alkaline phosphatase, PTH, serum ferritin, and lipid

profile as well as echocardiography were obtained in the start of the study, after 3 months on maintained low-flux hemodialysis and 3 months after switching the patients to high-flux hemodialysis.

Inclusion criteria: The patients should be ≥ 18 years old and maintained on regular HD for more than 6 months.

Exclusion criteria: Active infection, active autoimmune disease, malignancy, decompensated liver disease, and heart failure class III and IV.

All patients were subjected to full history taking including medical co-morbidities, etiology of renal failure, regimen of hemodialysis, duration on dialysis and complications related to hemodialysis.

Ethical consideration and written informed consent:
An approval of the study was obtained from Ain Shams University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis
Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:
▪ Independent-samples t-test of significance was used when comparing between two means.
▪ Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.
▪ The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:
▪ Probability (P-value)
- P-value <0.05 was considered significant.
- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS
Among the included 30 patients, there was 16 male patients (53.3%) and 14 female patients (46.7%) and the mean hemodialysis duration was 5.22 ± 3.39 years with hemodialysis duration ranging from 1 year to 13 years. Etiology of ESRD was hypertension (HTN) 18 patients (60.0%), diabetes mellitus (DM) 4 patients (13.3%), polycystic kidney disease (PKD) 3 patients (10.0%), glomerulonephritis (GN) 2 patients (6.7%), obstructive uropathy 1 patient (3.3%) and

unknown cause was in 2 patients (6.7%). The baseline FGF-23 levels were higher than the normal range (Median 400 & IQR: 266.5 – 531)

There was a highly significant reduction of FGF-23 level with high-flux dialyzers than with low-flux dialyzers. The difference in the absolute change in FGF-23 levels between the three months period of low-flux dialysis and the three months period of high-flux dialysis showed a highly significant reduction with high-flux than low-flux (p= 0.000) as shown in table (2) and figure (6).

There was a significant reduction of serum levels of PTH, calcium and phosphorus levels with high-flux dialyzers than with low-flux dialyzers. The adjusted difference was (p= 0.022), (p= 0.000), and (p= 0.006) respectively (Table 2 & figures 3, 4 & 5).

Also there was significant increase in serum albumin, and significant reduction in total cholesterol level with high flux dialyzers than with low flux dialyzers. When we compared the adjusted difference of serum albumin level during the low-flux period and the high-flux period, (median -0.1, IQR (-0.2 – 0.1)) and (median 0.05, IQR (-0.1 – 0.3)) respectively, it showed a significant increase in serum albumin when using high-flux compared to low-flux dialyzer, (p= 0.049) (Table 1 & figure 1).

As regards lipid profile, when using the adjusted difference between the low-flux and high-flux period there was a significant reduction of the total cholesterol during the high-flux period (p = 0.038), [median 8 (IQR -5 – 31)] and [median 4 (IQR -10 – 13)] respectively (Table 1 & figures 2).

As regards echocardiography findings, with the use of high-flux dialyzer, there was a decrease in the number of patient with diastolic dysfunction although it did not reach a significant value. The adjusted difference between low-flux and high-flux periods showed a significance improvement in ejection fraction value (p= 0.031) (Table 3).

FGF-23 showed a positive correlation with RVSP at baseline, after 3 months on low-flux dialysis, and after 3 months on high-flux dialysis with P-value (0.000), (0.001), and (0.000) respectively (Figure 8).

In our study, we found a highly significant correlation between FGF23 level and valvular calcification with low-flux dialyzer. Also, there was a significant correlation between FGF23 level and valvular calcification with the high-flux dialyzer (Table 3 and figures 7 & 8).

In our study. FGF-23 showed a negative correlation with baseline total cholesterol and LDL levels (p= 0.048) and (p= 0.036) respectively (Table 5 and figures 10 and 11).

Table (1): Comparison between the difference in laboratory results of the patients during the period of low-flux dialysis and the difference during the high-flux dialysis.

|		Diff. between 0-3 (low-flux)	Diff. between 3-6 (high-flux)	Test value ‡	P-value	Sig.
HGB (gm/dl)	Median (IQR)	-0.2 (-1 – 0.7)	0.05 (-1.1 – 0.6)	-0.141	0.888	NS
Albumin (mg/dl)	Median (IQR)	-0.1 (-0.2 – 0.1)	0.05 (-0.1 – 0.3)	-1.972	0.049	S
Ferittin (ng/ml)	Median (IQR)	-15 (-176 – 40)	-10 (-120 – 100)	-0.991	0.322	NS
Total Cholesterol (mg/dl)	Median (IQR)	8 (-5 – 31)	4 (-10 – 13)	-2.07	0.038	S
Triglyceride (mg/dl)	Median (IQR)	-2 (-27 – 16)	-19.5 (-37 – 12)	-1.198	0.231	NS
LDL (mg/dl)	Median (IQR)	1.8 (-4.8 – 22.6)	6.4 (-6 – 14.4)	-0.488	0.626	NS
HDL (mg/dl)	Median (IQR)	-0.5 (-3 – 4)	3.5 (0 – 8)	-1.912	0.056	NS
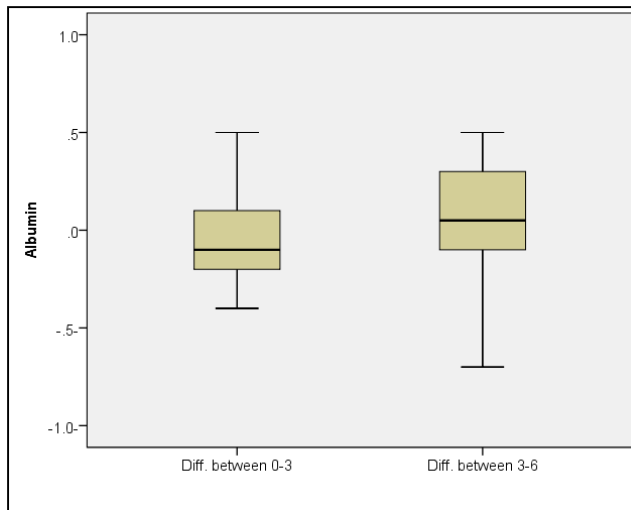
Figure (1): Comparison between the difference in serum albumin level during the period of low-flux dialysis and the difference during the high-flux dialysis.
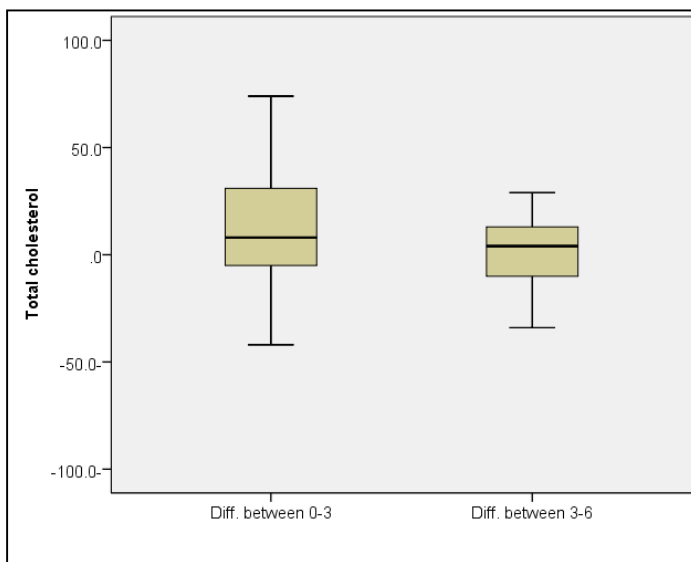

Figure (2): Comparison between the difference in total cholesterol level during the period of low-flux dialysis and the difference during the high-flux dialysis.

Table (2): Comparison between the differences in laboratory results of the patients during the period of low-flux dialysis and the difference during the high-flux dialysis as regard the specific biomarkers of mineral and bone metabolism.

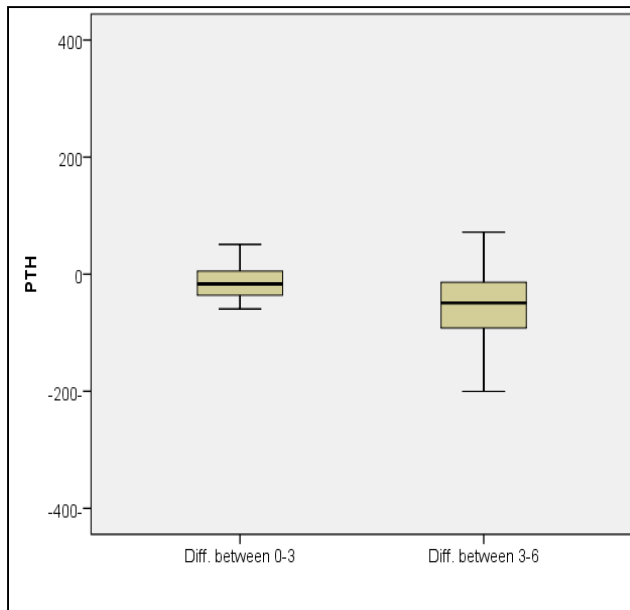|		Diff. between 0-3 (low-flux)	Diff. between 3-6 (high-flux)	Test value ǂ	P-value	Sig.
Intact PTH (pg/ml)	Median (IQR)	-16.65 (-36 – 5.3)	-49 (-92 – -13.70)	-2.284	0.022	S
Corrected Ca (mg/dl)	Median (IQR)	0.5 (0.3 – 0.8)	-0.1 (-0.3 – 0.4)	-4.135	0	HS
PO₄ (mg/dl)	Median (IQR)	0.45 (-0.6 – 1.2)	-0.5 (-1.6 – 0.2)	-2.745	0.006	HS
ALK.Ph (U/L)	Median (IQR)	-4 (-37 – 17)	-28.5 (-50 – 10)	-1.871	0.061	NS
FGF-23 (pg/ml)	Median (IQR)	-14 (-45 – 0)	-105.3 (-205.1 – -59)	-3.692	0.001	HS

Figure (3): Comparison between the difference in PTH level during the period of low-flux dialysis and the difference during the high-flux dialysis
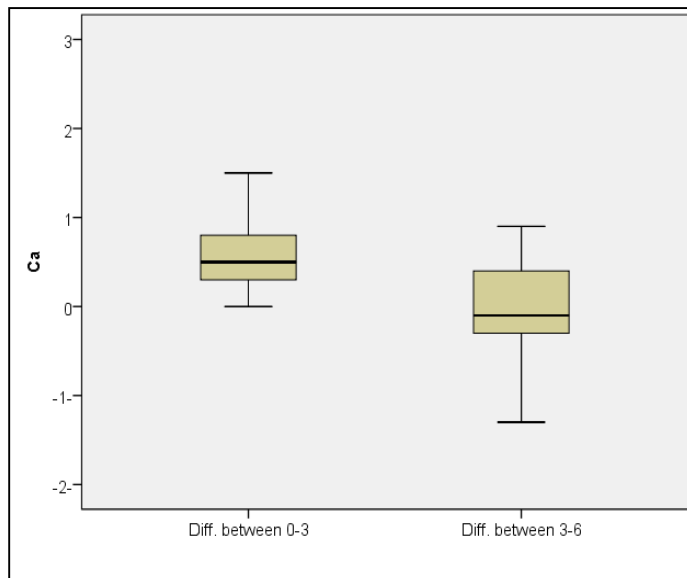

Figure (4): Comparison between the difference in serum calcium level during the period of low-flux dialysis and the difference during the high-flux dialysis.
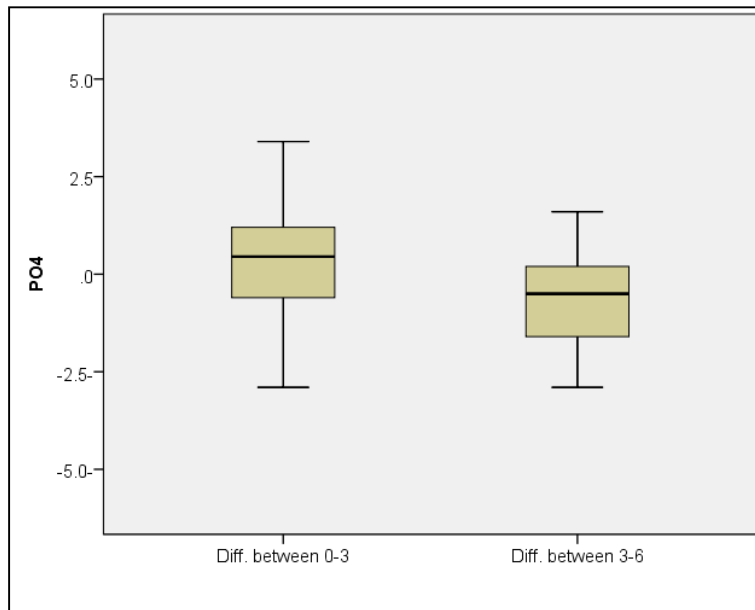
Figure (5): Comparison between the difference in serum phosphorus level during the period of low-flux dialysis and the difference during the high-flux dialysis
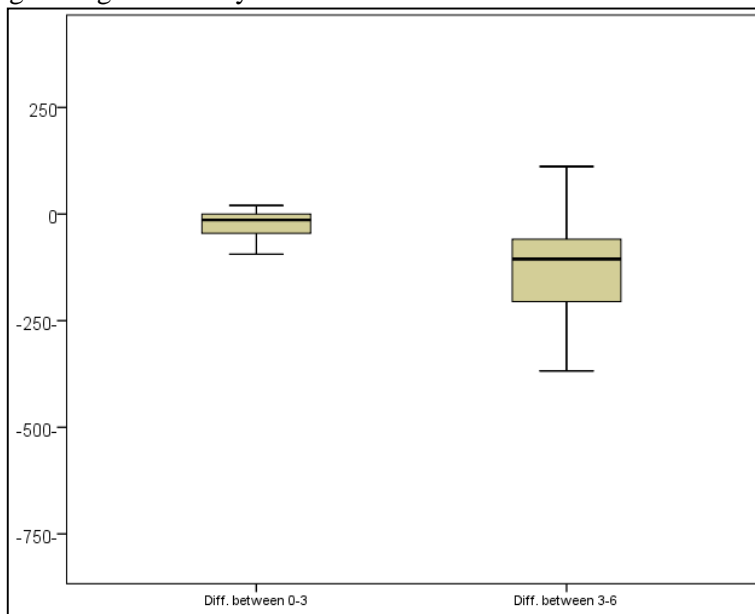

Figure (6): Comparison between the difference in serum FGF-23 level during the period of low-flux dialysis and the difference during the high-flux dialysis

Table (3): Comparison between the difference in echocardiography findings during the period of low-flux dialysis and the difference during the high-flux dialysis as regard the ejection fraction and right ventricular systolic pressure.

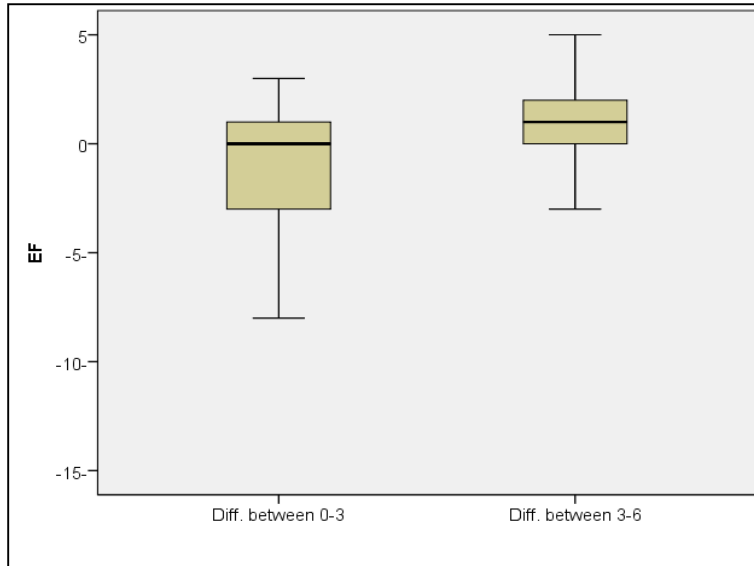|		Diff. between 0-3	Diff. between 3-6	Test value ‡	P-value	Sig.
EF	Median (IQR)	0 (-3 – 1)	1 (0 – 2)	-2.158	0.031	S
	Range	-8 – 3	-12 – 5			
RVSP	Median (IQR)	-1 (-3 – 2)	1 (-3 – 3)	-0.840	0.401	NS
	Range	-8 – 27	-9 – 15			

Figure (7): The difference in ejection fraction during the period of low-flux dialysis and the difference during the high-flux dialysis
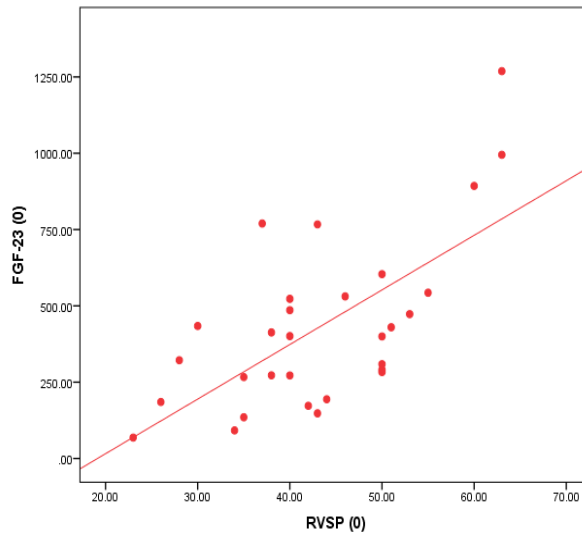

Figure (8): Correlation between FGF-23 and RVSP.

Table (4): Incidence of cardiac valve calcification in patients with higher FGF-23 levels:

Baseline		Negative Valvular Calcification	Valvular Calcification	Test value	P-value	Sig.
		No. = 19	No. = 11			
FGF-23 (pg/ml)	Median (IQR)	283 (185 – 413)	543 (429.9 – 893)	-2.862‡	0.004	HS

Table (4) showed that the group of patients with cardiac valve calcification showed higher levels of serum FGF-23 (Figure 9).
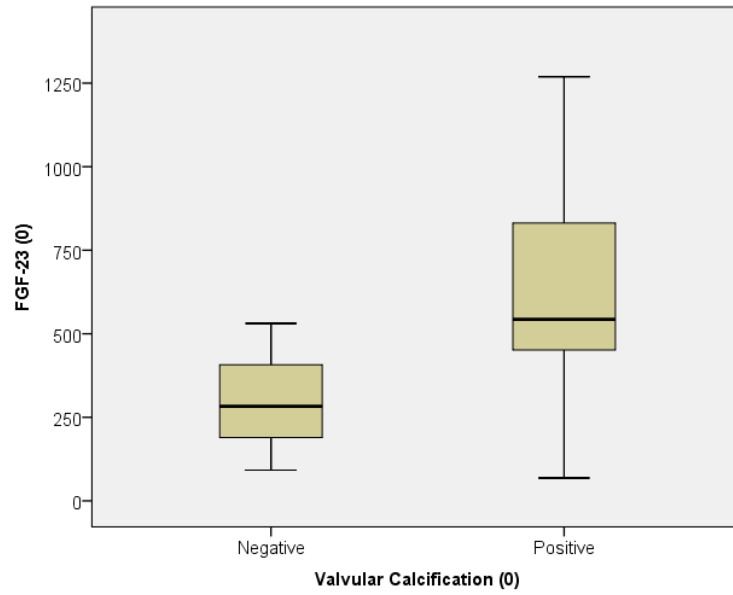
Figure (9): a Box plot showing a higher FGF-23 levels in patients with cardiac valve calcification.

Table (5): Correlation coefficient (r) between FGF-23 and Lipid profile:

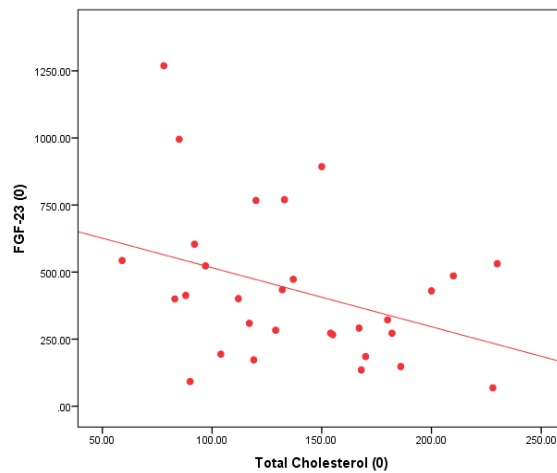| Correlation		
Baseline	**FGF-23**	
	R	**P-value**
Total Cholesterol (mg/dl) (up to 200)	-0.365*	0.048
Triglyceride (mg/dl) (< 150)	-0.146	0.441
LDL (mg/dl) (< 100)	-0.384*	0.036
HDL (mg/dl) (40-60)	-0.091	0.633

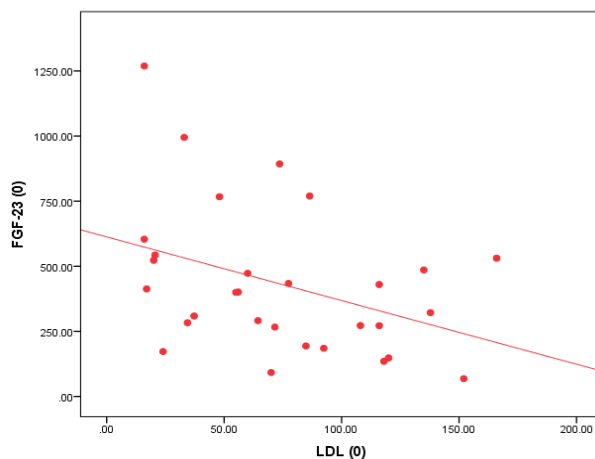**Figure (10):** Correlation between FGF-23 and total cholesterol.

Figure (11): Correlation between FGF-23 and LDL.

DISCUSSION

Up to 40–50% of patients with end-stage renal disease (ESRD) die because of cardiovascular disease (CVD), reflecting their rapidly progressing atherosclerotic burden and heavy arterial calcification [6]. Altered calcium, phosphorus, parathyroid hormone (PTH), and vitamin D levels in ESRD induce mediators of bone-like metabolism and mineralization in the vascular tunica media [7].

Fibroblast growth factor-23 (FGF-23) levels also start rising early in patients with chronic kidney disease and is implicated in cardiovascular and overall mortality of hemodialysis patients [8]. Elevated phosphate, calcium, PTH, and FGF23 levels are all associated with vascular calcification in patients with CKD and may directly promote calcification [9].

Our study was conducted on 30 randomly selected prevalent hemodialysis patients aged 25 – 69 years old with mean age of 50.10 ± 11.54 years. Male patients were 16 (53.3%) and mean hemodialysis duration was 5.22 ± 3.39 years with hemodialysis duration ranging from 1 year to 13 years.

In our study, the baseline FGF-23 levels were higher than the normal range and this observation is in line with other reports of **Gutierrez et al.** [10] and **Jean et al.** [11].

We found that there was a highly significant reduction of FGF-23 level with high-flux dialyzers than with low-flux dialyzers. The difference in the absolute change in FGF-23 levels between the three months period of low-flux dialysis and the three months period of high-flux dialysis showed a highly significant reduction with high-flux than with low-flux (p= 0.000). This is in agreement with previous studies by **Fu et al.** [12] and **Zhang et al.** [13].

There was no significant change in PTH during the three months of the low-flux period, the baseline and after three months PTH median was 330.85 with (IQR) (163 – 559.5) and 369.5 with (IQR) (230 – 500) respectively (p= 0.072). When using high-flux dialyzer, PTH levels showed a significant reduction from the start and after three months of the high-flux dialysis, from median (IQR) 369.5 (230 – 500) to 274.9 (183.8 – 482) (p= 0.021). The difference in the absolute change in PTH levels between the three months period of low-flux dialysis and the three months period of high-flux dialysis showed a significant reduction with high-flux than with low-flux (p= 0.022). This is in agreement with **Makar et al.** [14]. This result was contradicting with the results of **Fu et al.** [12] where their study showed no significant difference in calcium, phosphorus, PTH, and alkaline phosphatase between low and high flux groups.

The difference in the absolute change in serum calcium level between the low-flux and the high-flux period showed significant reduction, (p= 0.000). Also, the difference in the absolute change in serum phosphorus level between the low-flux and the high-flux period showed significant reduction, (p= 0.006).

Hypoalbuminemia is common in patients with ESRD. It is caused by a combination of a reduced synthesis and an increased degradation of albumin. The altered albumin homeostasis in ESRD patients is caused by a systemic inflammatory state which correlates closely with mortality [15]. We found no significant change of serum albumin after the use of high-flux filters, as serum albumin levels changed from 3.85 ± 0.55 at start of high-flux period to 3.90 ± 0.46 at the end of the 3-months high-flux period (p= 0.351). When we compared the difference in the absolute change of serum albumin level during the

low-flux period, which showed no significant change and the high-flux period (median -0.1, IQR (-0.2 – 0.1)) and (median 0.05, IQR (-0.1 – 0.3)) respectively , it showed a significant increase in serum albumin when using high-flux compared to low-flux dialyzer (p= 0.049). The improvement in serum albumin with the use of high flux dialyzer could be due to a degree of enhanced serum albumin and nutritional state with high-flux dialyzers. This could be due to improved dietary intake as a result of potential removal of plasma substances that inhibit appetite. Leptin, which is one of middle-sized uremic toxins that weights 16000 D could be removed with high flux dialyzer [16]. This is not in agreement with **Makar et al.** [14] and **El Arbagy et al.** [17].

As regards lipid profile, our patients had almost normal or high normal values of total cholesterol (138.50 ± 46.39 mg/dl), normal LDL (median 70.8 (IQR 34.4 – 116 mg/dl)), elevated triglyceride (164.70 ± 62.65 mg/dl) and decreased HDL (36.40 ± 7.37 mg/dl). When using the difference in the absolute change between the low-flux and high-flux period there was a significant (p= 0.038) reduction of the total cholesterol during the high-flux period [median 8 (IQR -5 – 31)] and [median 4 (IQR -10 – 13)] respectively, but there was no significant difference in the absolute change of LDL, triglyceride, or HDL between low-flux and high-flux periods. This non-significant change of lipid profile in our study is contradicting with the findings observed by **Wanner et al.** [18] who found that with high-flux polysulphone membranes, there was a higher reduction of triglyceride and total cholesterol than with high-flux modified cellulose membrane, while oxidized LDL decreased with high-flux polysulphone but not with high-flux modified cellulose. This could be explained by the fact that polysulphones are more biocompatible than cellulose membranes causing less oxidative stress and not due to the flux permeability. Also, **Schiffl & Lang** [19] found that the improvement of uremic dyslipidemia is due to use of ultra-pure dialysate and not due to the flux permeability.

In our study, FGF-23 showed a negative correlation with baseline total cholesterol and LDL levels (p= 0.048) and (p= 0.036) respectively. This is in agreement with **Yan et al.** [20]. This correlation is thought to be due to the association of FGF23 with factors other than mineral metabolism as it belongs to the FGF19 subfamily and shares structural and biological similarities with the other family members (FGF19 and FGF21) that are involved in lipid and glucose metabolic homeostasis [21].

In our study, there was no significant change in serum hemoglobin level or serum ferritin between the low-flux and the high-flux period (p= 0.888) and (p= 0.322) respectively.

As regard, echocardiography findings, with the use of high-flux dialyzer there was a decrease in the number of patient with diastolic dysfunction even it did not reach a significant value. The adjusted difference between low-flux and high-flux periods showed a significance improvement (p= 0.031) in ejection fraction value. This is in agreement with **Francisco et al.** [22]**.** FGF-23 showed a positive correlation with RVSP at baseline and after 3 months on high-flux dialysis with the same P-value (0.000). There was a highly significant correlation (p= 0.004) between FGF23 level and valvular calcification with low-flux dialyzer, and a significant correlation (p= 0.023) with the high-flux dialyzer. This is in agreement with **Fu et al.** [12]**.**

CONCLUSION

FGF-23 levels were significantly higher in hemodialysis patients than in normal population. FGF-23 is better eliminated by high-flux dialyzers than low-flux dialyzers. FGF-23 is associated with increased incidence of cardiac valve calcification. FGF-23 positively correlates with right ventricular systolic pressure. There was a higher reduction of PTH, calcium, and phosphorus levels with high-flux dialyzers. There was a significant reduction of the total cholesterol with the high-flux dialyzer, but no significant change with LDL, HDL, or triglyceride was noticed.

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