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Research Article

Cardioprotective role of Fetuin-A in patients with end-stage renal disease on regular hemodialysis

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Abstract

Background: Cardiovascular pathology remains the leading cause of morbidity and mortality in end stage renal disease patients on regular hemodialysis. We aimed to evaluate the cardioprotective role of Fetuin-A in patients with ESRD on regular hemodialysis**.**Patients and Methods: This cross sectional study included eighty patients fromNational Institute of Nephrology and Urology, Cairo, Egypt. Fetuin-A was measured by ELISA .Mitral and Aortic valves calcificationswere asessd by a standard 2-dimensional transthoracic echocardiogram.Routine laboratory ,hsCRP and PTH were done.Framingham risk score (FRS)was measured.Patients were stratified into 3 tertiles according to serum fetuin-A concentration [those with serum fetuin-A= 100 - < 350 µg/ml (26 patients), 350 - <430 µg/ml (27 patients), ≥ 430 µg/ml (27 patients)] respectively. Results: Aortic valve calcification was seen in 23 (88.5%) vs. 16 (59.3%) vs. 6 (22.2%),(*P*<0.01) while mitral valve calcification was seen in 9 (34.6%) vs. 5 (18.5%) vs. 2 (7.4%), (P<0.045) in the 3 tertiles respectively. Statistically significant values of hsCRP (16.85 ± 14.80 vs. 14.22 ± 16.65 vs. 5.78 ± 11.22) (*P* value = 0.008) and serum albumin (*P* value < 0.001) were observed across the three tertiles of increasing serum fetuin-A. No significant differences between low risk (42) and moderate risk(30) high risk (8) patients according FRS regarding Fetuin levels. Conclusion :Fetuin-A levels is inversily correlated to valvular calcification and hs-CRP levels and hence may be cardioprotective in in hemodialysis patients.

Keywords: Fetuin-A, Valve calcification, Framingham score, Hemodialysis.

Introduction

Calcification of cardiac valves in hemodialysis patients is associated strongly with incident all-cause and Cardiovascular mortality (Panuccio *et al.*,2004).Valvular calcification is closely associated with increased carotid intimal arterial thickness (Leskinen et al.,2009) and it is a good marker of underlying atherosclerotic burden especially in hemodialysis patientsother than reflecting an excess calcium × phosphorus load*(Wang et al., 2005)*. Human Fetuin-A (alpha2-Heremans Schmid glycoprotein), a 59 kDa glycoprotein synthesized in the liver, has been identified as a potent systemic inhibitor of vascular and soft-tissue calcification (Schafer *etal.,* 2003).Fetuin-A is highly effective in the formation and stabilization of protein-mineral colloids, referred to as calciprotein particles (CPPs) (Heiss *et al.,* 2008). Fetuin-A may have a regulatory role in atherogenesis via its anti-inflammatory property and inhibition of calcification, it has been shown to antagonize transforming growth factor and bone morphogenetic proteins that are potent osteogenic growth and differentiation factors possibly involved in atherosclerotic calcification (Binkert *et al.,* 1999).Serum Fetuin-A is decreased in patients with

moderate to severe chronic kidney disease, especially dialysis patients(Ix*et al.,* 2006).Hemodialysis patients with calciphylaxis had depressed Fetuin –A serum levels accompanied by a reduced capacity of their serum to inhibit calcium phosphate precipitation (kettele*retal.,* 2003).This study aims at studying the cardioprotective role of Fetuin-A in patients with ESRD on regular hemodialysis**.**

Patients and methods

This is a single center cross sectional study comprised Eighty ESRD patients ,age 18-60 years old, undergoing conventional regular hemodialysis for at least 6 months, thrice weekly 4 hours per session , using bicarbonate dialysis and low reflux dialyzers. Patients were recruited from dialysis center of National Institute of Nephrology and Urology, Cairo, Egypt with strict adherence to its ethical committee.
All the studied patients subjected to full history and clinical examination including age, sex,BMI,and duration of dialysis, cause of renal failure, other comorbid conditions and medications. Patients with evident history of ischemic, cerebrovascular or peripheral disease,malignant hypertension, and clinical signs of overt infection, decompensated liver disease, parathyroidectomy or previous renal transplantation were excluded. Blood pressure was calculated as the average value of all recordings (12 measurements, ie, 3 measurements/wk) performed predialysis during the month preceding the study.

Fasting blood samples in midweek non dialysis day were collected after an overnight fast of at least 12 hours.The serum to be tested were stored at −80°C until analysis, and supernatant sera were diluted to a ratio of 1: 10 000. Fetuin-A was measured in diluted serum solutions by means of a human enzyme-linked immunosorbent assay (ELISA) kit *(Biovendor Laboratory Medicine Inc, Brno, Czech Republic)*with a minimal detectable concentration of approximately 0.035 g/L and ninety-five percentile normal range was found to be 0.5 to 1.0 g/L. The assay utilizes a two-site sandwich technique with two selected polyclonal antibodies that bind to different epitopes of human Fetuin-A.Patients were stratified into 3 tertiles according to serum fetuin-A concentration [those with lower tertile of serum Fetuin-A= 100 - < 350 µg/ml, middle tertile 350 - <430 µg/ml and upper tertile ≥ 430 µg/ml].Routine laboratories were done included creatinine , urea , CBCserum levels of albumin, Calcium, phosphorus, triglyceride (TG), total cholesterol, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c),were estimated using standard laboratory techniques.Corrected serum calcium was calculated (corrected calcium [mg/dl] =serum calcium [mg/dl] +0.8 [4- serum albumin [g/dl]).

A standard 2-dimensional transthoracic echocardiogram was performed and aortic and mitral valves calcifications (VC) were assessed. Cardiac valve calcification was considered present when bright echoes of more than 1-mm thickness where seen on 1 or more cusps of the aortic valve, mitral valve or mitral valve annulus *(*Wong *et al.,*1983*).*The Framingham risk score (FRS) formula was calculated based on a model comprising diabetes , age, sex, total cholesterol, HDL-cholesterol, systolic blood pressure, treatment of hypertension and cigarette smoking to derive an estimated risk of developing Coronary heart disease within 10 years (D'Agostino *et al.,* 2008).The FRS is used to identify individuals categorically as 'low' (<6% 10-year risk), 'intermediate' (6–20% risk) or 'high' risk (>20% risk).

Data are entered into an excel sheet and transferred to SPSS (Statistical Package for Social Sciences) ver. 20. Quantitative data are presented as mean and standard deviation. Comparison of means are done using independent t-test between two groups or ANOVA test between more than two groups. Qualitative data are presented as count and appropriate percentage. Comparison between different groups is done using chi-square test or Fisher exact test if required. Association between quantitative variables is done using Pearson "r" and tested by appropriate t-test. In all hypotheses testing a value of $P \leq 0.05$ is considered statistically significant.

Results

The enrolled patients were 39 males and 41 females, with mean age 44.51 ± 10.41 and mean hemodialysis duration was 5.55 ± 4.30308 years. Causes of renal failure were hypertension in 35(43.75%) of patients , 7 (8.75%) diabetic kidney disease ,6 (7.5%) APKD , 3 (3.75%) analgesic nephropathy , 4 (5 %) chronic glomerulonephritis , 2(2.5%) lupus nephritis, 3(3.75%)chronic pyelonephritis and vesicoureteric reflux , 3 (3.75%) obstructive uropathy,2 (2.5%) amyloidosis and unknown etiology in 15 (18.75%) of studied patients. 61.25% of all patients were

hypertensive and were treated with one of calcium channel blockers, angiotensin converting enzyme inhibitors and /or α - and β -blockers, achieving a satisfactory blood pressure control.The demographic, anthropometric characteristics and laboratory results of all patients shown in **(Table 1).**Patients were stratified into 3 tertiles according to serum Fetuin-A concentration [those with serum fetuin-A= 100 - < 350 µg/ml (26 patients), 350 - <430 µg/ml (27 patients), ≥ 430 µg/ml (27 patients)] respectively,comparison between the 3 tertiles groups regarding studied parameters by ANOVA test shown in (Table 2).The group of patients with the lower tertile of serum Fetuin levels had significantly low albumin and high hsCRP levels than other groups ,also there was low Fetuin A levels with increase age of patients significantly.

Table 1: Characteristics of studied patients (N=80).

Studied parameters	**(Mean ± SD)**
Age (Year)	44.51 ± 10.41
Sex : MaleN (%) **Female** N (%)	39 (48.8%) 41 (51.3%)
BMI (kg / m ²)	26.54 ± 5.98
Mean SBP(mmHg)	138.4 ± 29.8
Mean DBP(mmHg)	82.9 ± 15.8
Hemoglobin(g / dl)	9.96 ± 2.04
Albumin(g / dl)	3.56 ± 0.54
Calcium (mg / dl)	8.95 ± 1.14
PO4(mg / dl)	5.81 ± 1.84
Ca × PO4 (mg² / dl²)	51.95 ± 17.90
PTH(pg / dL)	511.12 ± 443.27
Cholesterol (mg / dl)	165.60 ± 48.40
TG(mg / dl)	83.79 ± 18.53
LDL*(mg / dl)*	104.31 ± 40.94
HDL(mg / dl)	41.90 ± 12.64
hsCRP (mg/L)	19.83±11.59
Fetuin A(µg / ml)	421.13 ± 168.55

Table 2:Characteristics of patients according to different levels of Fetuin-A.

Variables	**Fetuin A in Tertile**			**P value**
	lower tertile (N=26) 100 - < 350	**middle tertile (N=27) 350 - < 430**	**upper tertile (N=27) >430**	
Age (Year)	47.65 ± 8.20	45.67 ± 10.52	40.33 ± 11.16	0.031*
Dialysis Duration (Year)	6.56 ± 5.07	5.52 ± 4.36	4.61 ± 3.26	0.253
SBP(mmHg)	141.9 ± 30.2	134.8 ± 29.4	138.5 ± 30.6	0.691
DBP(mmHg)	83.8 ± 16	80.7 ± 14.7	84.1 ± 17.2	0.695
BMI (kg/m²)	26.12 ± 5.03	27.58 ± 7.86	25.89 ± 4.54	0.628
Hemoglobin (g / dl)	9.71 ± 2.65	10.23 ± 1.73	9.93 ± 1.66	0.666
Albumin (g/dl)	3.17 ± 0.62	3.64 ± 0.44	3.86 ± 0.28	<0.001*
Calcium(mg/dl)	9.14 ± 1.32	8.72 ± 0.74	9.00 ± 1.27	0.312
PO4(mg / dl)	5.95 ± 2.02	6.03 ± 1.64	5.45 ± 1.86	0.457
Ca × PO4 Product (mg² / dl²)	53.80 ± 19.10	52.85 ± 16.32	49.27 ± 18.58	0.648
PTH (pg / dL)	506.27 ± 521.18	559.44 ± 376.59	467.47 ± 435.42	0.708
hsCRP(mg/dl)	16.85 ± 14.80	14.22 ± 16.65	5.78 ± 11.22	0.008*
Cholesterol(mg/dl)	170.00 ± 52.68	161.89 ± 43.68	165.07 ± 50.08	0.833
TG (mg / dl)	81.54 ± 18.19	90.37 ± 16.89	79.37 ± 19.24	0.065
LDL (mg / dl)	103.88 ± 40.80	102.15 ± 38.05	106.89 ± 45.07	0.917
HDL (mg / dl)	41.65 ± 12.72	41.74 ± 11.05	42.30 ± 14.40	0.983

**Statistically significant P<0.05*

The mean Fetuin-A level showed significant negative correlations with age *(r=-0.303,p < 0.006)* and hs CRP *(r=-0.303,p < 0.006)* (Figure 3) but showed a significant positive correlation with serum albumin (*r=0.479,p < 0.001).*However, no significant correlation was observed between serum Fetuin- A levels and the biochemical parameters of hemoglobin, Ca, phosphorus , Ca X phosphorus product , lipid profile for other parameters such as, sex , BMI,dialysis duration or mean systolic and diastolic blood pressure *(p>0.05).*No statistically significant difference between mean of Fetuin levels in hypertensive patients 412.04±170.86 vs 435.48±166.59 in normotensive patients (P=0.548).

Echocardiography revealed an evidence of valvular calcification (VC) in 47 patients (58.75%), among which 33 (41.25%) had isolated one calcified valve [31with aortic valve calcification and two patients with mitral valve calcification] and 14 (17.5%) showed calcification of both valves. Compared with the non-calcification group,there was significant difference between both groups regarding age, duration of dialysis, hsCRP and albumin *(P<0.05).*The mean of Fetuin in group with valvular calcification was significantly less than group with no valvular calcification(*P< 0.001*) .No significant relation between VC and the other parameters as shown in (Table 3).

Table 3: Comparison between patients with valve calcification and patients without

Variables	No valvular calcification (n = 33)	Valvular calcification (n = 47)	P value
Age in years (mean ± SD)	41.061 ± 10.365	46.936 ± 9.841	0.012*
Sex (m/f) N (%)	14(42.4)/19(57.6)	25(53.2)/22(46.8)	0..343
Dialysis duration in years(mean ±SD)	3.424 ± 2.372	7.043 ± 4.729	<0.001*
Systolic BP (mm/Hg)	136 ± 26.9	140 ± 31.9	0.561
Diastolic BP (mm/Hg)	83 ± 16.3	82.8 ± 15.7	0.942
BMI (kg/m²)	26.847 ± 5.337	26.318 ± 6.434	0.699
hemoglobin (g / dl)	10.006 ± 1.789	9.921 ± 2.215	0.856
Albumin (g / dl)	3.882 ± 0.310	3.334 ± 0.557	< 0.001*
Calcium (mg / dl)	8.755 ± 1.314	9.088 ± 0.993	0.690
PO4(mg / dl)	5.567 ± 1.825	5.974 ± 1.850	0.333
Ca × PO4 Product (mg² / dl²)	48.788 ± 17.638	54.173 ± 17.940	0.187
PTH (pg / dL)	492.242 ± 402.07	524.374 ± 473.86	0.752
hsCRP (mg/dl)	3.455 ± 7.198	18.383 ± 15.993	<0.001*
Cholesterol (mg / dl)	170.333 ± 48.223	162.277 ±48.763	0.467
TG (mg / dl)	84.182 ± 20.525	83.511 ±17.226	0.875
LDL (mg / dl)	113.182 ± 42.139	98.085 ±39.327	0.105
HDL (mg / dl)	40.939 ± 12.983	42.574 ±12.490	0.572
Fetuin A (µg / ml)	541.21 ± 179.83	336.80 ±93.16	<0.001*

**Statistically significant P<0.05.*

Low tertile of Fetuin A levels not only significantly correlated to valve calcification but also to the numberof calcified valves and relation of status of calcification cross tertiles of Fetuin-A levels was shown in (Table 4) and figures (1, 2).Bymultiple logistic analysis with calcification of valves as a dependent variable and selected independent variables. There were independent effect of duration of dialysis, hsCRP, LDL and tertiles of Fetuin-Awas shown in (Table 4).

Table4:Relation of status of calcification cross tertiles of Fetuin -A levels.

		Tertiles						P value
		lower tertile (N=26) 100 - <350		Middle tertile (N=27) 350- <430		upper tertile (N=27) 430 ->1000		
		No	%	No	%	No	%	
Aortic	Calcified	23	88.5%	16	59.3%	6	22.2%	<0.001
Mitral	Calcified	9	34.6%	5	18.5%	2	7.4%	0.045
Calcification of Either Valves	Calcified	24	92.3%	17	63.0%	6	22.2%	<0.001
Status of Calcification of Valves	Both Free	2	7.7%	10	37.0%	21	77.8%	<0.001
	One calcified	16	61.5%	13	48.1%	4	14.8%	
	Both calcified	8	30.8%	4	14.8%	2	7.4%	

**Statistically significant P<0.05*

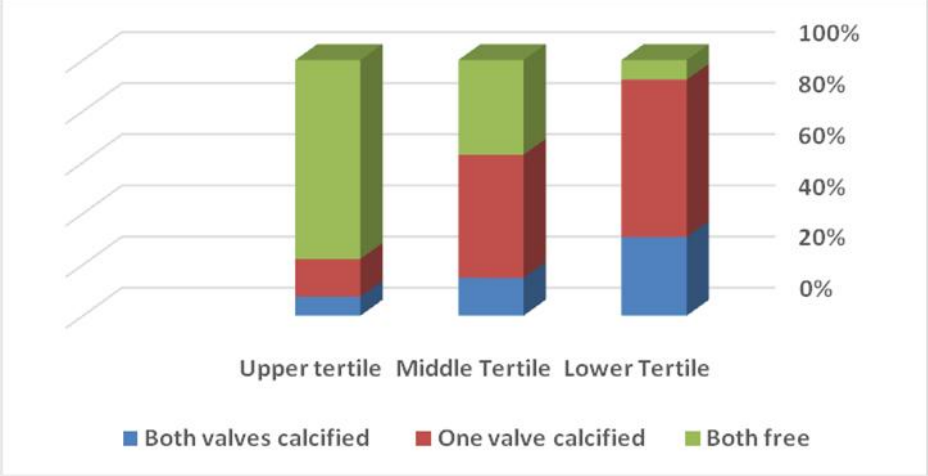

Figure 1: Status of calcified valve samong hemodialysis patients with different tertiles of Fetuin -A levels.

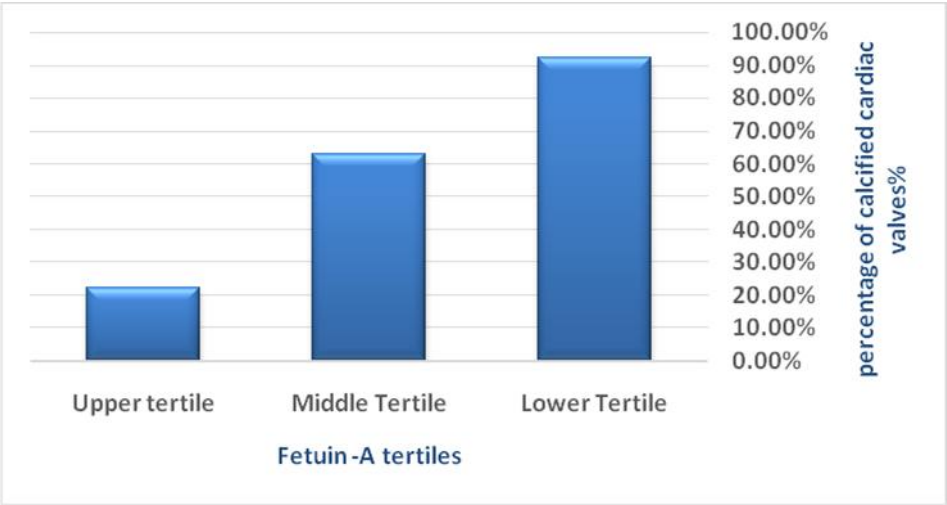

Figure 2: Percentage of calcified valves among hemodialysis patients with different tertiles of Fetuin-A levels.

Table 5: Multiple logistic regression analysis with calcification of valves as a dependent variable.

Variables in the equation	B	Sig.	Odds ratio	95% C.I. for Odds ratio	
				Lower	Upper
Age (Year)	-0.046	0.365	0.955	0.865	1.055
Dialysis Duration (year)	0.493	0.005	1.638	1.156	2.319
Albumin (g/dl)	-2.359	0.115	0.095	0.005	1.771
hsCRP(mg / dl)	0.222	0.008	1.248	1.060	1.470
LDL (mg / dl)	-3.716	0.033	0.024	0.001	0.747
Tertiles of Feutin-A(µg / ml)	3.784*	0.020	44.001	1.811	1068.938
Hypertension	1.203	0.266	3.330	0.400	27.750

* *statistically significantP <0.05*.

multiple logistic analysis was performed with calcification of valves as a dependent variable and selected independent variables. There were independent effect of duration of dialysis, CRP, LDL and tertiles of fetin-A.

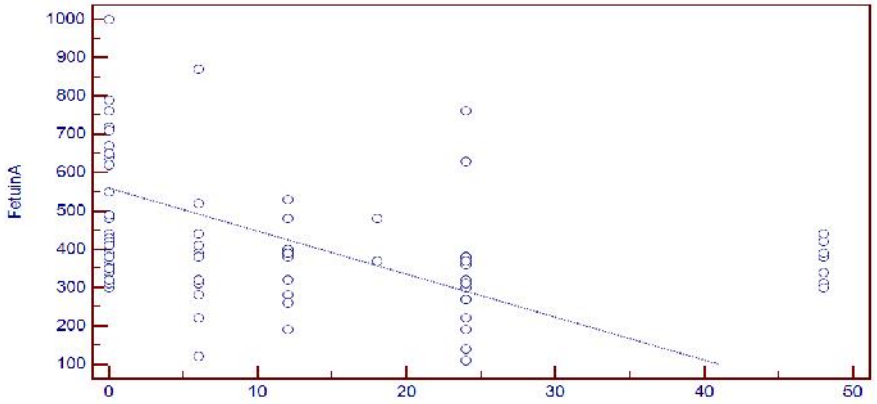

Figure 3: Relationship of serum Fetuin-A levels with serum hsCRP.

By calculating FRS in all the patients , we divided the patients into 3 groups ; low (< 6 %) , moderate (6 – 20 %) and high (20 %) risk groups , according to calculated points of risk .In FRS 2008 model , 42 patients were with low risk score and 30 patients with moderate risk and 8 patientwith high risk. There there wasn't any significant relation detected with the risk score Fetuin -A levels or status of calcification (p>0.05) (Table 6).

Table 6: Correlation of Framingham risk score and evaluated parameters:

	FRS 2008						P value
	Low risk (N=42)		Intermediate risk (N=30)		High risk (N=8)		
	Mean	SD	Mean	SD	Mean	SD	
Dialysis Duration (years)	5.46	4.21	5.53	3.79	6.06	6.74	0.938
HB (g/dl)	10.11	1.86	9.72	2.37	10.01	1.73	0.723
Albumin (g/dl)	3.64	0.51	3.49	0.57	3.40	0.59	0.367
Calcium (mg/dl)	8.71	1.09	9.20	1.22	9.27	0.96	0.140
PO4 (mg/dl)	5.75	1.77	6.01	2.0	5.36	1.70	0.655
Ca×PO4 (mg^2/dl^2)	50.01	16.50	55.26	19.92	49.74	17.39	0.446
PTH (pg/dl)	371.88	291.16	740.85	563.01	380.63	213.94	0.001*
hsCRP(mg/dl)	11.43	16.80	13.40	13.34	12.00	11.56	0.862
Fetuin-A (µg/ml)	426.67	183.05	427.00	160	370.00	122.36	0.670
Cardiac valve calcification N (%)	24(57.1%)		18 (60.0%)		5 (62.5%)		0.946

Discussion

Valvular calcification represents a marker of atherosclerosis in dialysis patients other than reflecting an excess calcium phosphorus load (Wang *et al.,* 2005).Our study included Forty seven hemodialysis patients (58.75%) with valvular calcification with significant low Fetuin level than patients without valvular calcification. By dividing the patients into tertiles of serum Fetuin- A 92.3% of the patients with lower tertile had valvular calcification, 63% of patients with medium tertile and 22.2% of the patients with upper tertile with VC. These results are in agreement with (Coen *et al.,* 2006) study who also found an association between lowlevels of Fetuin-A,and severely increased calcium score in hemodialysis patients. Also the same results were observed in patients on peritoneal dialysis (Wang *et al.,* 2005).In our study no significant difference between the 3 tertile group regarding calcium, phosphorous or Ca×PO4product. Low levels of Fetuin-A may enhance cardiovascular calcium deposition (Coen *et al.,* 2006).
In our study we observed that the lower Fetuin tertile significantly related to increase number of calcified valves, about 30.8% of patients with lower tertile had both mitral and Aortic valves vs 14.8% of middle tertile group and 7.4% of upper tertile group. Serum Fetuin-A had independent protective effect *(odds ratio=44.00,P=0.020)* on valvular calcification by multiple logistic analysis.

We reported a significant decrease of albumin and increase hsCRP titre with the lower Fetuin tertile .This raises an interesting hypothesis of whether Fetuin-A have a regulatory role in atherogenesis via its anti-inflammatory property and inhibition of calcification **(***Wang et al., 2005*).In agreement with (kettele*r et al.,* 2003) who performed a study on 312 HD reported a reverse association between serum fetuin-A and CRP in these patients, and (Oikawa *et al.,* 2007) study included 40 HD patients and 20 controls, it has been shown that the mean of serum Fetuin-A in the HDpatients had a negative association with hs-CRP.

In the present study was performed with calcification of valves as a dependent variable and selected independent variables,there was independent effect of duration of dialysis, hsCRP titre and LDL as risk factors for valvular calcification by multiple logistic analysis.

In **(Koos *et al.*,2009**)a longitudinal, prospective study investigating an association of serum fetuin-A levels with the progression of aortic valve calcification (AVC) revealed that low levels of the systemic calcification inhibitor serum fetuin-A are associated with an accelerated progression of AVC in patients with established and the impact upon AVC progressionwas independent of the renal function, inflammation, baseline calcification level, as well as 'traditional' risk factors such as hypercholesterolaemia in non-dialyzed patients.

No statistically significant correlation was found between Fetuin level and duration of hemodialysis in our study, this in agreement with (Wang *et al.*,2005) study results and against (Oikawa et al., 2007) study, it has been shown that the mean of serum Fetuin-A in the HD patients group has a negative association with dialysisduration.

In the present study there was no statisticaly significant correlation between fetuin level and calcium,phosphorous,Ca X phosphrous product or iPTH ,the similar as Oikawa et al., 2007 study results among hemodialysis patients.

Atherosclerosis is highly prevalent in HD patients and may be significantly associated with malnutrition, aggravated inflammation, vascular calcification, coagulation pathways alterations, oxidative stress and infection, which may worsen outcomes(**Chen *et al.*, 2010).*(*Huang *et al.*,2013**)evaluated the association between FRS category and overall and cardiovascular mortality.

The 10-year coronary heart disease risk was estimated by FRS 2008 and we reported 52.5% were with low FRS vs 37.5% with intermediate FRS and 10% with high FRS. We didn't observe statistically significant difference between low, intermediate or high FRS patients group regarding mean of Fetuin levels or valvular calcification,maybe due to limited number of patients in our study. (**Mehrotra *et al.*, 2005**) study observed a direct relationship between serum Fetuin-A levels and Coronary Artery Calcium Score (CACS) as a marker for atherosclerotic plaque among patients with pre-dialysis stage of diabetic nephropathy (r = 0.22, P = 0.038).

In Pateinakis et al., 2013 study on81 chronic hemodialysis patients ,Fetuin-A was independently

inversely associated with arterial stiffness measured carotid-to-femoral pulse wave velocity (cfPWV) and common carotid intima-media thickness (ccIMT), a surrogate of early atherosclerosis**.**

We didn't found in the present study any significant difference between hypertensive and non-hypertensive patients regarding Fetuin level or significant correlation between mean of Fetuin level and systolic or diastolic BP.

In conclusion, Fetuin-A levels is inversily correlated to cardiac valve calcification, number of calcified valves and hs-CRP levels and hence may be cardioprotective in chronic hemodialysis patients.

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