Int. J. Adv. Res. Biol. Sci. 2(10): (2015): 124–129

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

Fetuin A, Bone Alkaline Phosphatase and Aortic Calcification in Prevalent Hemodialysis Patients

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Abstract

Vascular calcification is frequent in prevalent hemodialysis (HD) patients. Both fetuin A (a natural calcification inhibitor) and bone alkaline phosphatase (a natural calcification inducer) are frequently reported to be altered in HD patients. The aim of this study is to assess the possible association of aortic calcification and both fetuin A and bone alkaline phosphatase in prevalent HD patients. The study involved forty stable prevalent HD patients who were divided into two groups according to aortic calcification index (ACI) using non contrast spiral CT scan, group A with no or minimal calcification (ACI < 2) (22 patients) and group B with more advanced calcification (18 patients). All cases were studied by CBC, routine chemistry in addition to serum fetuin A, bone alkaline phosphatase. PTH (Intact), and ultrasensitive CRP. We did not detect significant differences between the 2 groups in all studied parameters (including fetuin A, bone alkaline phosphatase, intact PTH, and ultrasensitive CRP) except statistically significant older age and higher total corrected calcium level in patients with more advanced calcification (group B). On the other hand, we detected statistically significant negative correlation between ACI and fetuin A level and statistically significant positive correlation between ACI and duration of HD in group B. Multivariate analysis showed only positive correlation between ACI and age. It may be concluded that aortic calcification in prevalent HD patients is multifactorial and that aging, longer duration of HD, possibly changes in fetuin A level may be major contributing factors.

Keywords: hemodialysis, fetuin A, bone alkaline phosphatase, CRP, PTH, HD.

Introduction

The mortality rate among patients with ESRD who are undergoing dialysis is approximately seven times greater than for similar individuals in the general population and is largely attributed to cardiovascular causes (1).Decades of clinical observations have noted deposition of extra osseous calcium in patients with ESRD (2). Medial calcification of large vessels, intimal calcification of coronary arteries, and calcification of the cardiac valves are each associated with higher mortality in patients with ESRD (4). They may be initiated by the uremic milieu, as well as comorbid factors, such as diabetes, inflammation, and hyperphosphatemia (3). Animal models have identified osteoprotegerin (OPG), osteopontin (OPN), bone morphogenic protein-7 (BMP-7), and fetuin-A as factors that may regulate calcification in the vasculature. Fetuin-A is secreted by hepatocytes into the circulation, where it forms soluble complexes with calcium and phosphate (4). Fetuin- A accounts for approximately 50% of the capacity of serum to inhibit the spontaneous apatite formation from solutions supersaturated in calcium and phosphate. The inhibition is achieved by rapid formation of soluble colloidal Fetuin-A calcium phosphate complexes, termed calciprotein particles (CPPs) (5). In this study we evaluated the the possible association of aortic calcification and both fetuin A and bone alkaline phosphatase in prevalent HD patients.

Materials and Methods

40 ESRD patients on regular hemodialysis were enrolled in our study. They were divided into two groups according to aortic calcification index (ACI)

using non contrast spiral CT scan, group A with no or minimal calcification (ACI <2) (22 patients) and group B with more advanced calcification (18 patients). Diabetics, chronic smokers, those known to have collagen diseases, and patients with active inflammatory or infectious illness or malignancy were excluded from the study. We obtained history, clinical examination and laboratory investigations from all patients which included Serum fetuin-A, high-sensitivity C reactive protein (hs-CRP) assay, serum calcium and corrected calcium, phosphorus, total protein, albumin, ALT, AST and total bilirubin, PT, PTT, INR, intact parathormone, Bone alkaline phosphatase and total alkaline phosphatase. Vascular calcification was assessed by non contrast CT scan to detect the aortic calcification index. Calcification of the abdominal aorta above its bifurcation was evaluated semi-quantitatively in 10 CT slices at 1 cm intervals, with a slight modification of a technique previously reported by others. In brief, the cross-section of the abdominal aorta on each slice was divided into 12 sectors, and the number of sectors with calcification was counted in each slice. The number of sectors with calcification was divided by 12. The values thus obtained for the 10 slices were added together. The totals were then divided by 10 (the number of slices inspected) and multiplied by 100 to express the result as a percentage (6).

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences, Version 17.0 (SPSS, Inc., Chicago, III, USA) for Windows. Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Rates and proportions were calculated for categorical data. For categorical variables, differences were analyzed with χ^2 (chi square) test and Fisher's exact test when appropriate. Differences among continuous variables with normal distribution were analyzed by Student's T-test; for continuous variables without normal distribution, we used non-parametric tests and differences were analyzed by the Mann–Whitney U-test. Differences among the three groups (control subjects, group A and group B) were analyzed with Univariate ANOVA and Bonferroni post hoc test. Correlations were determined by using spearman rho. P value of ≤0.05 was considered statistically significant.

Results

Table 1 showed the demographic and laboratory parameters for both groups. We can notice that there was significant increase in age and corrected Ca in group B more than in group A suggesting that these two factors are important determinant of vascular calcification in HD patients.

Table (1): Demographic and laboratory characteristics of group A and group B

	Group A (n=22)	Group B (n=18)	P value
	Mean ±SD	Mean ±SD	
Age (years)	38.2±14.3	49.5±14.3	0.018
Cr. (mg/dl)	7.5±1.7	8.0±3.1	0.496
BUN (mg/dl)	85.9±22.5	78.2±14.6	0.221
High scrp(ug/ml)	7.02±3.014	6.8±2.8	0.978
Albumin(g/dl)	3.7±0 .4	3.7±0.3	0.982
Corr. ca (mg/dl)	8.0±0.8	8.6±0.78	0.035
PO4 (mg/dl)	5.4±1.3302	5.±1.3	0.687
Ca x PO4	42.9±9.3	47.8±11.9	0.158
T. protein(g/dl)	7.1±0.8	7.3±0.8	0.965
INR	1.1±0 .2	1.1±0.	0.878
ALT(IU/L)	24.9±8.4	22.2±10.7	0.379
AST(IU/L)	29.2±8.3	26.1±9.9	0.282
Total Bilirubin (mg/dl)	0.9±0.2	0.9±0.2	0.715
Fetuin A(ng/ml)	105.0±33.2	110.0±25.4	0.784
T. alkaline phosphatase (U/L)	281.0±157.3	334.5±624.7	0.673
Bone alkaline phosphatase (U/L)	90.0±29.5	87.5±21.8	0.661
Intact Parathormone (pg/ml)	438.8±459.5	635.4±631.3	0.314

As shown in table 2, multivariate analysis was done to measure the independent effect of all factors that affect ACI status, and it showed that age remain in the model and founded to be positively associated with higher ACI. The higher the age, the higher the ACI or you can say with every year increase in age there were an increased risk by 1.1 times than its previous year. Correlative analysis of Fetuin A and other variables in both groups are shown in tables 3,4.

Table (2): Age was founded to be positively associated with higher ACI.

	B	SE	P value	OR	95.0% CI OR	
					Lower	Upper
Age	0.055	0.024	0.025	1.056	1.007	1.108
Constant	-2.591	1.124	0.021	0.075		

B=Regression coefficients, SE=Standard error of the coefficient, OR=Odds Ratio,
95% CI for OR = 95% confidence interval for the =Odds Ratio. P-value≤0.05 is considered significant.

Table (3): Correlations between Fetuin-A and other variables in group A

Fetuin-A	Rho	P value
Age (years)	0.052	0.819
hscrp (ug/ml)	0.353	0.107
Corrected Ca(mg/dl)	-0.148	0.510
PO4 (mg/dl)	0.150	0.506
Ca x PO4	0.024	0.917
Bone alkaline phosphatase(U/L)	-0.128	0.569
Total alkaline phosphatase(U/L)	-0.232	0.299
Intact parathormone (pg/ml)	0.054	0.812
ALT(IU/L)	-0.268	0.228
AST(IU/L)	-0.503	0.017
Total bilirubin(mg/dl)	-0.426	0.048
INR	-0.381	0.080
Total protein (g/dl)	-0.154	0.493
Serum albumin(g/dl)	-0.312	0.157
Creat(mg/dl)	0.365	0.094
BUN(mg/dl)	-0.149	0.509
Dialysis duration	-0.294	0.184

Table (4): Correlations between Fetuin-A and other variables in group B.

Fetuin-A	Rho	P value
Age (years)	-0.030	0.907
hscrp (ug/ml)	0.027	0.915
Corrected Ca(mg/dl)	0.114	0.654
PO4 (mg/dl)	-0.262	0.293
Ca x PO4	-0.228	0.364
Bone alkaline phosphatase (U/L)	-0.232	0.354
Total alkaline phosphatase (U/L)	0.120	0.637
Intact parathormone (pg/ml)	0.031	0.902
ALT (IU/L)	-0.099	0.695
AST (IU/L)	0.007	0.977
Total bilirubin (mg/dl)	-0.331	0.180
INR	-0.023	0.929
Total protein (g/dl)	0.297	0.231
Serum albumin (g/dl)	0.527	0.025
Creat (mg/dl)	0.405	0.095
BUN (mg/dl)	-0.146	0.563
Dialysis duration	-0.242	0.334

Significant inverse correlation between Fetuin A and AST (figure 1), total bilirubin and INR was noticed in group A. On the other hand, positive correlation between Fetuin A and creatinine was found and it was significant. In group B, significant positive correlation between Fetuin A and albumin was found. Also positive correlation was noticed between Fetuin A and creatinine, and it was significant (p=0.025). In group B, with ACI more than 2, we found significant inverse correlation between ACI and Fetuin A (Table 5). Significant direct correlation between ACI and dialysis duration was also noticed.

Figure (1): correlation between Fetuin-A and AST in group A

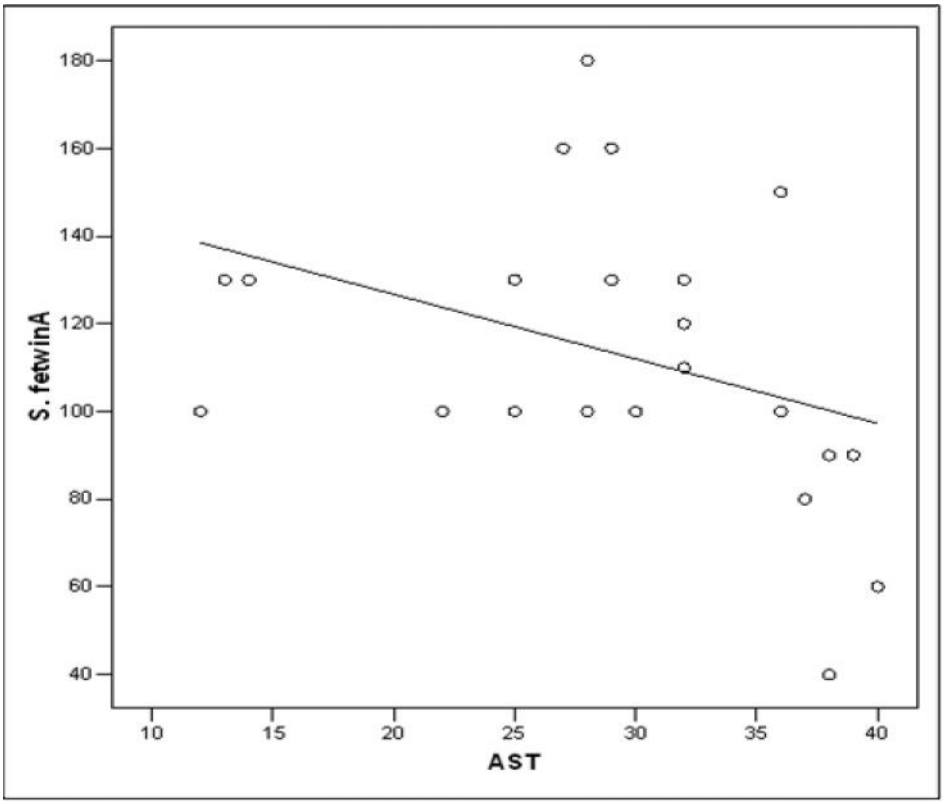

Table (5): Correlations between ACI and other variables in group B.

ACI (%)	Rho	P value
Age (years)	0.130	0.607
Fetuin-A (ng/ml)	-0.509	0.031
hscrp (ug/ml)	-0.204	0.417
Corrected Ca (mg/dl)	0.022	0.931
PO4 (mg/dl)	-0.045	0.858
Ca x PO4	-0.031	0.902
Bone alkaline phosphatase (U/L)	0.086	0.733
Total alkaline phosphatase (U/L)	0.039	0.879
Intact parathormone (pg/ml)	0.197	0.433
ALT (IU/L)	0.246	0.326
AST (IU/L)	0.162	0.521
Total bilirubin (mg/dl)	-0.033	0.897
INR	-0.142	0.573
Total protein (g/dl)	-0.214	0.393
Serum albumin (g/dl)	-0.322	0.192
Creat(mg/dl)	0.038	0.882
BUN(mg/dl)	-0.124	0.625
Dialysis duration	0.571	0.013

Discussion

The principal determinant of ACI in HD patients as shown in our study by a multivariate analysis was the age because we had a significant difference in age between group A and B. It was noticed that with every year increase in age there was an increased risk by 1.1 times in ACI, also a positive correlation was observed as regards ACI and dialysis duration in group B. Both findings are similar to those observed by Lee et al. (7) who stated that HD patients with arterial intimal and medial calcification were older than HD patients without calcification. Another crucial factor affecting ACI was Fetuin A, as a significant inverse relationship has been noticed in our study between serum Fetuin A and HD patients with ACI> 2 (group B). This inverse relationship was also observed by other investigators (8, 9, and 10) who reported that lowest levels of Fetuin A were associated with highest prevalence of valvular calcification and atherosclerotic vascular disease (AVD) in dialysis patients. Clinical observational study by London et al. (11) has demonstrated that valvular calcification (VC) represents a marker of atherosclerosis and arterial calcification in dialysis patients. Additionally, hemodialysis patients with arterial intimal calcification (AIC) were reported to have higher mortality than those with arterial medial calcification (AMC). Fetuin-A seems to protect from precipitation of calcium phosphate under extra-osseous calcification stress by organizing a fetuin–mineral complex (FMC).Vitamin D has been demonstrated to promote ectopic calcifications by different mechanisms that also include fetuin-A exhaustion as a result of FMC formation (12). Parameters such as fetuin-A and CRP levels were only assessed at a single point in time instead of having time-averaged values and related to markers of arterial stiffness which develop over many years. Furthermore, arterial calcification is a complex phenomenon and just represents a surrogate parameter of medial calcification. Nevertheless, since fetuin-A is strongly deposited at sites of vascular calcifications, fetuin-A may be a functional defense system against overt unwanted calcifications in populations without or with early stages of CKD (10).An assumption that a protective mechanism allows an upregulation of fetuin-A in the early stages of CKD and dialysis, and only severe or prolonged exposure to a pro-inflammatory and/or pro-calcific environment eventually leads to low levels due to reduced production and/or increased consumption (13).Another finding of a prospective study conducted by Brandenburg et al., (14) was that the administration of the non-calcium-containing phosphate binder sevelamer over 8 weeks was associated with a significant increase of serum fetuin-A levels in haemodialysis patients. More interestingly, fetuin-A levels remained relatively high for an additional 8 weeks despite cessation of sevelamer. It can only be speculated that chronic modification of the systemic (micro-) inflammatory state in dialysis patients by sevelamer contributes to serum fetuin-A changes. We were unable to find a significant correlation between fetuin-A and features of bone turnover including total alkaline phosphatase, Ca, P, Ca × P, or iPTH levels, this could be due to the small number of subjects available. On the contrary, other studies (15, 16) documented an inverse correlations between serum fetuin-A and serum iPTH, total alkaline phosphatase in HD patients, also they found that serum Fetuin-A level inversely correlated with basal calcium-phosphorus product in HD, It is conceivable that increased calcium phosphorus product may influence Fetuin-A uptake and subsequent degradation.

Conclusion

It may be concluded that aortic calcification in prevalent HD patients is multifactorial and that aging, longer duration of HD, possibly changes in fetuin A level may be major contributing factors. A large scale study (longitudinal study) with follow up applied on HD patients to assess the factors influencing ACI is required. Further studies on the effect of vitamin D supplementation on serum Fetuin-A level and it's correlation to inflammatory markers as hsCRP should be done.

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