Originalinvestigations/commentaries

The Relationship between serum Fetuin-A levels and Cardiovascular Risk Factors in Hemodialysis Patients

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Abstract. Fetuin-A is a circulating inhibitor of calcium deposition in the vasculature and may be involved in the pathogenesis of cardiovascular disease. Low plasma fetuin-A level is independently associated with increased risk for cardiovascular disease mortality among patients on regular hemodialysis. Aim: This research was conducted into the correlation between vascular and valvular calcification in hemodialysis patients and serum Fetuin-A levels. Methods: A total of 69 chronic hemodialysis patients and 20 healthy volunteers participated in the study at Theodor Bilharz Research Institute's Nephrology Department. Calcium, phosphorus, parathormone, and Fetuin-A concentrations in the blood were measured. The presence of plaques in carotid Doppler sonography or an intima-media thickness (IMT) of 0.8 mm were used as diagnostic criteria for vascular calcification. In two-dimensional echocardiography, calcification of the mitral annulus was included in the category of cardiac valvular calcification. Cardiovascular calcification was defined as the presence of either disease or both (CVC). Results: Serum fetuin levels proved to be markedly lower in HD patients than in healthy controls. In addition, we proved a negative correlation between serum fetuin level and IMT as well as serum phosphate, Ca×P product, iPTH, CRP, systolic and diastolic blood pressure. Conclusion: Our findings suggest that fetuin-A deficiency is a predictor of vascular disease in patients on regular hemodialysis.

Keywords: Human fetuin-A, end stage renal disease, cardiovascular risk factors, vascular calcification, hemodialysis

Introduction

Dialysis patients have a greater mortality rate than age- and sex-matched members of the general population due to cardiovascular disorders, a major contributor to diseases and fatalities among these patients [1]. Hypertension, dyslipidemia, diabetes mellitus, and smoking are the basic risk factors for cardiovascular events in individuals with end stage renal disease (ESRD). Although these factors play a role in a significant death rate among these individuals, they cannot account for all of them, suggesting the presence of other risk factors, the most significant of which is cardiovascular calcification (CVC) [2]. The most prevalent type of extra-osseous calcification in ESRD patients is cardiovascular calcification (CVC) [3]. It is linked to both conventional and non-conventional predisposing factors, such as calcium, phosphorus, PTH, and vitamin D levels that are abnormal [4]. Patients with ESRD are more likely to develop CVC due to the high incidence of these risk factors [5].

As a putative inhibitor of calcium phosphate deposition , fetuin-A (a2-Heremans-Schmid glycoprotein) is mostly synthesized in the liver [6]. It controls multiple cellular processes, including apoptosis, vesicle calcification, and phagocytosis, that contribute to vascular smooth muscle calcification (VSMC) [7]. It controls release of vesicle content and forms calciprotein particles by interacting with calcium and phosphorus. This inhibits precipitation of hydroxyapatite in vessels and therefore controls vascular calcification early on [6]. Studies have linked low levels of Fetuin-A with coronary calcification, prevalence of carotid plaques, valve calcification, and mortality in ESRD patients [8, 9]. The level of Fetuin-A in patients on regular hemodialysis is lower than that in the healthy population. Serum Fetuin-A levels tend to drop in people with chronic kidney disease (CKD) or who are on hemodialysis, however not all studies have found a correlation between this and increased vascular calcification [10, 11].

Hemodialysis patients who have a high Fetuin-A level have been shown to have a lower possibility of passing away from cardiovascular causes [12]. Some

additional research, however has failed to confirm this. Hence, it appears that more research is needed to fully understand the correlation between Fetuin-A levels and CVC in dialysis patients [11, 13].

Aim of the study:

Our primary aim was to examine whether or not fetuin A was related to valve calcification, carotid intima medium thickness (CMT), and biochemical parameters as risk factors towards valvular, vascular, and atherosclerotic disease in patients with (HD).

Patients and methods

Theodor Bilharz Research Institute played host to this cross-sectional investigation. There was a total of 69 ESRD patients receiving routine hemodialysis, as well as 20 age- and sex-matched healthy controls. Participation in this study was voluntary, and all subjects provided informed permission. To qualify, participants needed to be 18 or older and engaged in consistent HD for a minimum of 3 months.

Exclusion criteria

Serum inflammatory indicators were considered an excluding criterion, as were recent histories of trauma, acute coronary injury, malignancy, acute infection, and acute hepatitis. Failure to obtain informed consent was also a factor in excluding people from the study.

All participants in the clinical examination had their height, weight, and body mass index measured, as well as their blood pressure. ECHO was utilized to perform cardiac evaluation. We determined the levels of calcium, phosphorus, intact PTH (iPTH), albumin, alkaline phosphatase, creatinine, urea, uric acid, and hemoglobin. The link between these factors and fetuin-A levels was examined. Using the quantitative CRP method, a reference result of 0.5 mg/dl was deemed normal. The erythrocyte sedimentation rate, ferritin, and lipid profile were determined. For fetuin-A measurement, an enzyme-linked immunosorbent test (ELISA) kit was utilized.

Doppler sonography of the carotid arteries (both right and left) was used to check for vascular calcification. The intima-media thickness (IMT) of the carotid artery was measured at the higher, middle, and lower levels on both sides and the average was used. An enhanced IMT was defined as having a measurement of 0.8 mm or more on either side [12]. Valvular calcification was defined as the development of calcification of the mitral or aortic valves or of the mitral annulus (MAC) as visualized by two-dimensional echocardiography. For the purposes of this definition, calcification of the cardiovascular system included calcification of the arteries and/or valves (CVC).

Statistical analysis

The statistical analysis will be performed using SPSS 15.0. To establish whether or not our variables follow a normal distribution, we'll utilize the Shapiro-Wilk test. The mean and standard deviation of normally distributed continuous variables will be reported. In the absence of a normal distribution, the median will be utilized. The percentages describing the sorted variables will be provided. In order to determine the significance of the disparities among the groups, we will use Student's t-test. Fischer's exact test will be used to compare continuous variables. If the probability level is less than 0.05, it is considered significant.

Results

Sixty-nine people with HD and twenty controls participated in the study. Table 1 shows that the groups did not differ from one another in terms of age, gender, or body mass index.

Table 1. Demographic and clinical data of the HD patients and Healthy subjects

	HD patients N=69	Healthy subjects N=20	P value
Age	52.51 ± 10.59	38.20 ± 9.77	0.759
Gender Male Female	 36 (52.2%) 33 (47.8%)	 10 (50%) 10 (50%)	
BMI (kg/m^2)	24.77 ± 4.39	25.55 ± 4.33	0.887

BMI: Body mass index

Higher levels of serum phosphorus and Ca x P product were seen in the HD group in contrast with the control group, statistically significant increase in serum PTH, serum creatinine, urea, total cholesterol, and triglyceride was observed in the HD group compared to the control group. In comparison to the control group, those in the HD group had significantly lower serum calcium levels (p= 0.0001). Patients with HD had similar serum albumin levels as those of healthy controls.

The hemodialysis group had significantly greater ESR, CRP and ferritin levels in comparison to the healthy control group. Serum fetuin levels were lower in the HD group than those in the control group (p<0.0001). (Table 2).

Table 2. Comparison of Biochemical Parameters in HD and Control Groups

Item	**HD patients** **N=69**	**Healthy subjects** **N=20**	**P value**
Ca	8.35± 0.84	9.62 ± 0.56	0.0001
P	5.89± 1.29	4.02 ± 0.43	0.0001
Ca × P	48.56± 9.16	38.61 ± 4.01	0.0001
PTH	602.74± 252.98	33.50 ± 6.47	0.0001
Alb.	3.64 ± 0.47	4.74 ± 0.38	0.112
Creat	7.15 ± 1.13	0/85 ±0.15	0.0001
Urea	145.80 ± 23.29	30.20 ± 7.54	0.0001
Chol	223.09 ± 46.97	123.60 ± 22.43	0.003
Triglyceride	216.74 ± 68.79	106.35 ± 21.64	0.0001
ESR	51.64 ± 24.84	8.70 ± 2.54	0.0001
CRP	22.36 ± 17.05	<6	0.0001
Ferritin	462.70 ± 70	144.95 ± 63.10	0.0001
Hb%	9.36 ± 1.01	12.98 ± 0.73	0.0001
Fetuin-A	0.50 ± 0.40	2.31 ± 0.55	0.0001

Ca: calcium, P: phosphorus, PTH, parathyroid hormone, Alb.: albumin, Creat: creatinine Chol: Cholesterol. ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Hb: hemoglobin. Statistically significant at p<0.05 was considered significant

The highest SBP and DBP values were found in the HD group versus the control group (P value <0.001 and <0.034 respectively). There is a statistically significant increase in carotid intimal thickness when contrasting HD patients to healthy controls as shown in table 3.

Table 3. Blood pressure and echocardiographic findings of HD patients and Healthy subjects

Item	**HD patients** **N=69**	**Healthy subjects** **N=20**	**P value**
SBP	**136.09** ± 18.67	**121.55** ± 7.88	**0.001**
DBP	**83.55** ± 11.21	**74.47** ± 7.78	**0.034**

CIMT	**1.20** ± 0.97	**0.58** ± 0.29	**0.000**

SBP: systolic blood pressure, DBP: diastolic blood pressure, CIMT: carotid intimal thickness. Statistically significant at $p<0.05$ was considered significant

Study of valvular calcification in HD group showed that 46 (66.7%) of HD patients showed aortic calcification, 14 (20.3%) patients showed aortic and mitral valvular calcification and only 9 (13%) of HD patients were normal as shown in table 4.

Table 4. Valvular calcification in HD patients

Valvular calcification in HD patients N=69 (%)	
Aortic calcification	**46 (66.7)**
Aortic and Mitral calcification	**14 (20.3)**
No valvular calcification	**9 (13%)**

Blood pressure, total cholesterol and triglycerides, as well as serum urea and creatinine, demonstrate a negative linear correlation with fetuin A.

Table 5. Correlation between serum fetuin and other parameters

Variable	r	
Age	-.487**	0.0001
SBP	-.332**	0.002
DBP	-.275**	0.009
Ca	.566**	0.0001
P	-.599**	0.0001
Ca× P	-.474**	0.0001
PTH	-.707**	0.0001
BMI	0.154	0.150
ALB	.756**	0.0001
Creat	-.862**	0.0001
Urea	-.832**	0.000
ESR	-.747**	0.000
CRP	-.512**	0.000
Cholesterol (mg/dl)	-.681**	0.000
TG	-.574**	0.000
CIMT	-.259	0.014

SBP: systolic blood pressure, DBP: diastolic blood pressure, CIMT: carotid intimal thickness
ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Hb: hemoglobin, Ca: calcium, P: phosphorus, PTH, parathormone, Alb.: albumin, Creat: creatinine Chol: Cholesterol

Discussion

Pathogenesis of vascular damage during CKD progression is influenced by many variables, including those that promote and those that inhibit soft tissue calcification. Factors which promote calcification include hypercalcemia, hyperphosphatemia, high level of PTH, low alkaline phosphtase activity, and elevated calcium phosphate product (Ca×P) [14]. In addition to the stimulating factors, inhibitory factors also affect the calcification process. In this study we focus on fetuin-A as an example of inhibitory factor of calcification [15].

Almost one half of the plasma pool of calcification-inhibiting factors is derived from a protein called fetuin-A[16]. This protein strongly binds to calcium phosphate, which in turn buffers serum calcium phosphate and may prevent calcification. Soft tissue calcification inhibitors are lacking in those suffering from chronic renal impairment, particularly those receiving long-term dialysis.

Consistent with many findings, we discovered that the concentration of fetuin-A was considerably reduced in the HD group compared to the control group ($p < 0.0001$) [14, 17-20]. Patients with end-stage renal illness had lower fetuin-A levels, and this may be attributed to their chronic inflammatory state, as fetuin has been found to decrease during inflammation [14]. Research by Dervisoglu et al. [21] demonstrates that inflammation can affect serum fetuin-A levels by showing an inverse relationship between fetuin-A and the proinflammatory cytokines. In adults on HD, the risk of mortality from cardiovascular disease was shown to be higher in people whose fetuin-A levels were low. [14]. Nevertheless, Shroff et al. reported the opposite in children: elevated fetuin-A levels in the blood of children on HD compared to their healthy peers. Increased fetuin-A levels in children on dialysis may be seen as a protective response to proinflammatory and hypercalcemic mediators, as suggested by the research of Shroff et al. The scientists hypothesized that chronic activation of these mediators would weaken the body's adaptive responses and eventually lower levels of naturally occurring inhibitors [22].

Regarding valvular calcification, only 13% of HD patients did not have aortic or mitral calcification in our study, 66.7% had mitral calcification and 20.3% had both mitral and aortic calcification. Patients with HD have a four- to five-fold increased prevalence of calcification of aortic and mitral valves compared to the general population [23, 24]. Patients with and without valvular calcifications showed no discernible variation in serum fetuin, calcium, or phosphorus levels in our study.

The levels of phosphate, iPTH, and Ca×P product were all found to be inversely related to fetuin-A, which lends credence to the idea that insufficient calcification inhibitors may be responsible for undesirable calcification due to the disruption of the balance of Ca and P metabolism. Our results are similar to another study which reported the same in children on regular HD [14]. Caglar et al. showed that fetuin-A levels decreased with a drop in GFR in a sample of nondiabetic patients with various stages of CKD [25]. A similar finding was observed in a study of patients with chronic renal disease in stages 3, 4, and 5 who were not on dialysis (CKD) (a cohort that included both diabetics and non-diabetics) where plasma fetuin-A levels fell over time as renal function deteriorated [26]. Mazzafero et al. found that dialysis patients had lower levels of fetuin-A than those who had undergone a kidney transplant or who were healthy controls [27].

Similar to albumin, Fetuin-A levels decrease with inflammation, being a negative acute phase reactant [28]. It is well established that inflammatory mechanisms contribute to atherosclerosis development. To avoid the formation of blood calcium-phosphate crystals and their sedimentation in soft tissues, fetuin forms calciprotein particles (CPPs) in plasma with calcium and phosphorus [29]. Furthermore, it affects insulin resistance by modulating insulin receptor function and decreasing excessive production of proinflammatory cytokines [30].

In addition to limiting calcification, fetuin-A inhibits the function of transforming growth factor (TGFβ) and macrophages, reducing the release of proinflammatory cytokines [26]. Our results revealed a positive correlation between serum albumin and fetuin-A levels. This was in consistency with Wang et al. (2005) in their study on peritoneal dialysis patients as they proved a positive correlation between higher albumin and serum fetuin-A levels [9]. We also detected an inverse correlation between serum fetuin-A and CRP. This was in agreement with Wang et al. (2005) in their study on peritoneal dialysis patients [9] and also in agreement with Oikawa et al. (2007) in their study on hemodialysis patients [8]. Dialysis patients were found to have a negative correlation between fetuin-A levels and inflammatory mediators such as hs-CRP [14, 22] and proinflammatory cytokines such as IL-6 and TNF-α [31]. Metry et al. noticed that decreased fetuin-A levels only enhanced mortality in HD patients with elevated CRP [32]. These findings suggest that inflammation enhances calcification.

Coen et al. found a correlation between fetuin-A and prealbumin, and they found that CRP levels were highest in patients with the lowest fetuin-A tertile. According to their findings, fetuin-A defficiency may contribute to atherosclerosis by increasing vascular

calcification and inflammation in the body [19]. Malnutrition, independent of the etiology of inflammation, may be connected to reduced fetuin-A levels. While there is some evidence for a link between fetuin-A deficiency and inflammation and malnutrition in CKD patients, the vast majority of available data come from ESRD patients. [4].

In addition, there is a statistically significant inverse association between serum Fetuin-A and cholesterol and triglycerides in our investigation, which is relatable to the findings of Amani et al [28] .

Serum fetuin-A was found to have an inverse relationship with both systolic and diastolic blood pressure in our investigation ($P<0.002$ and <0.009) respectively. Makulska et al reported similar results which may be explained by arterial stiffness and atherosclerosis [14].

Ziokowska et al. looked into the role of multiple factors responsible for blood vessel calcification and found that in children with different stages of CKD fetuin-A was the only discriminating factor. Factors they studied included markers of calcium and phosphorus metabolism, bone turnover, and lipids [30].

Ultrasound evaluation of CIMT is considered a low-cost, easy, reproducible, noninvasive rationale for diagnosing atherosclerosis [33]. While coronary angiograms can reveal lesions within the artery lumen, measuring CIMT allows for a look at atherosclerosis progression prior to the onset of anatomic stenosis while the problem is still confined to the arterial wall.

Many studies reported an association between CIMT and coronary and valvular calcifications [22, 25] and that coronary events and fatalities are both more likely to occur when CIMT is high. [34].

CIMT is an independent prognostic factor of cardiovascular mortality in dialysis patients with end-stage renal disease. In our investigation, a negative connection was observed between fetuin-A and CIMT. Many pieces of evidence indicate that fetuin-A protects against vascular calcification and stiffness. The presence of coronary artery calcifications, CIMT, and pulse wave acceleration is inversely correlated with fetuin-A levels, as shown in both animal models and clinical trials in patients with CKD [33].

With its anti-inflammatory and calcification-inhibiting properties, Fetuin-A may have a pivotal function in controlling atherosclerosis. Researchers have found that calcification in the vascular structures of people with ESRD is an integral part of the atherosclerotic process. The severity of calcification was positively related to atherosclerotic mortality beginning at a young age. Vascular calcification was shown to be higher among 30–65-year-olds with CKD in relation to the general population as discovered by Kramer et al. [34] Activation of osteogenesis in the arterial wall, absence of calcification inhibitory factors such as fetuin-A, rapid bone turnover, and abnormalities in mineral metabolism can all explain the process of vascular calcification [33].

Conclusion

Reducing mortality and morbidity in CKD primarily depends on reducing the risk of cardiovascular events. Pre-recognition of cardiovascular risk factors is important, so large-scale studies on vascular calcification inhibitors are needed. Future studies are needed to determine whether measurement of plasma fetuin-A will be useful as a CVD risk stratification tool.

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