

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

Samia H. El-Shishtawy^{1*}, Nevine Sherif¹, Anas Ibrahim¹, Samah Mamdouh², Amna Metwaly³, Ibrahim M. Abd Elazim³, Ahmed Ali M.³ and Osama Mosbah A.¹

1- Nephrology Department, Theodor Bilharz Research Institute, Giza, Egypt, Giza, Egypt

2- Theodor Bilharz Research Institute Biochemistry and Molecular Biology Department, Giza, Egypt

3- Intensive Care Unit Theodor Bilharz Research Institute, Giza, Egypt

*Corresponding author E-mail: samya_shishtawy@yahoo.com

Received: May 3, 2024; Accepted: May 20, 2024; Available Online: May 24, 2024

DOI: 10.21608/AJBS.2024.286311.1089

ABSTRACT

Patients with chronic kidney disease (CKD) exhibit an increased cardiovascular (CV) complications including vascular and valve calcification, coronary artery disease, heart failure, arrhythmias, and sudden cardiac death. The incidence and prevalence of cardiovascular events is significantly higher in patients with early CKD stages compared with the general population, however patients with advanced CKD stages and those on dialysis exhibit a markedly elevated cardiovascular risk. Cardiovascular rather than end-stage kidney disease (CKD stage 5) is the leading cause of death in this high-risk population. CKD causes a systemic, chronic proinflammatory state contributing to vascular and myocardial remodeling resulting in vascular calcification, and vascular senescence as well as myocardial fibrosis and calcification of cardiac valves mainly aortic and mitral valves. In addition, conventional hemodialysis (HD) itself leads to myocardial stress and injury on the already compromised cardiovascular system in uremic patients. Inflammation, low serum fetuin-A levels and high serum ferritin levels are implicated for increase morbidity and mortality in this group of population. So, the current study aims to assess the function of fetuin-A in combination with inflammatory markers; TNF- α , hs-CRP and ferritin in the progression of CV complications in dialysis patients.

A total of (60) chronic hemodialysis patients and (30) healthy volunteers were participated in this study at Theodor Bilharz Research Institute's Nephrology Department. Laboratory parameters were measured; serum Calcium, phosphorus, parathormone, calcium-phosphate, inflammatory markers; Serum levels of fetuin-A, tumor necrosis factor alfa (TNF- α), and highly sensitive C-reactive protein (hs-CRP). The common carotid arteries' intima-media thicknesses (CIMT) and the calcification of the mitral and aortic annuli were performed.

The results indicated that fetuin-A levels showed statistically significant low levels in HD group in contrast to control group ($p < .0001$), while there were statistically significant rise in hs-CRP ($p < .0001$), TNF- α ($p < .0001$), serum ferritin ($p < .0001$) in HD group compared to control group. In addition, there was a negative connection between fetuin-A and CIMT, SBP, DBP, while there was a positive connection between hs-CRP, TNF- α and CIMT.

In conclusion determination of fetuin-A is a useful tool to assess inflammation and CV risk in HD patients.

Keywords: Fetuin-A, hemodialysis, inflammation, vascular calcification, ferritin, C-reactive protein.

INTRODUCTION

The primary reason for morbidity and death for people with end-stage renal disease is still cardiovascular disease (CVD), particularly those dependent on hemodialysis. The heightened risk of cardiovascular complications in this group is not only due to traditional risk factors such as dyslipidemia, hypertension, and diabetes but also to a range of non-traditional factors including vascular calcification, a prevalent and severe complication in hemodialysis patients. Vascular calcification significantly contributes to the stiffening of arterial walls, leading to increased cardiac workload and, subsequently, cardiovascular events (Echefu *et al.*, 2023).

In the search for predictors and mediators of vascular calcification, recent attention has turned towards biomarkers like

Fetuin-A is a glycoprotein predominantly produced in the liver and it has been recognized for its protective role against pathological calcification by stabilizing calcium phosphate salts and inhibiting their deposition in vascular tissues. Studies have found a correlation between reduced levels of fetuin-A and greater rates of vascular calcification and cardiovascular mortality in dialysis patients, indicating a critical function for this protein in the aetiology of cardiovascular issues in this population (Birukov *et al.*, 2022).

Alongside fetuin-A, inflammation emerges as a critical factor in progression of cardiovascular complications. Inflammation markers like hs-CRP and TNF- α , and ferritin are elevated in hemodialysis patients and have been linked to adverse cardiovascular outcomes. These inflammatory markers not only reflect acute phase responses but are also involved in the mechanisms leading to vascular damage and calcification. For instance, elevated levels of hs-CRP and TNF- α have been connected to a rise in

arterial stiffness and calcification, emphasizing their role in cardiovascular pathology (Jairam *et al.*, 2010).

The interplay between fetuin-A deficiency and heightened inflammatory activity presents a potentially synergistic risk for cardiovascular complications in hemodialysis patients. The dual impact of these factors suggests a complex mechanism where the deficiency in calcification inhibitors like fetuin-A, combined with chronic inflammatory states, accelerates vascular pathology, resulting in higher death and morbidity rates (Turkmen *et al.*, 2011; Rudloffm *et al.*, 2022).

Thus, the aim of this investigation was to study the relation of ferritin, hs-CRP, and TNF- α , as well as serum Fetuin-A concentrations, with cardiovascular calcification in chronic renal illness patients who are on routine hemodialysis.

PATIENTS AND METHODS

Study design and population:

This cross-sectional research was executed at the Nephrology Department of Theodor Bilharz Research Institute. A total of 90 subjects were participated in this investigation and they split into two groups: Group I (hemodialysis patients, HD group) included 60 patients with end-stage renal disease (ESRD) on regular HD, and the control group included 30 healthy subjects. Participation in this study was voluntary, with all subjects providing informed consent. **The inclusion criteria** consisted of participants aged 18 years or older who had been going through consistent HD for a minimum of 3 months.

Exclusion Criteria: Cases were excluded from the study if they had recent histories of trauma, acute coronary injury, malignancy, acute hepatitis, acute infection, or if informed consent could not be obtained.

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

Clinical and Laboratory Assessment

All participants underwent a clinical examination that measured height, weight, blood pressure, and BMI. Cardiac evaluation was performed using echocardiography. Measurements of albumin, alkaline phosphatase, creatinine, urea, uric acid, haemoglobin, Phosphorus, calcium, and intact parathyroid hormone (iPTH) were among the laboratory evaluations. An ELISA kit was employed to ascertain levels of fetuin-A. The levels of hs-CRP were established quantitatively, with a reference result of 0.5 mg/dl considered normal. Additional tests included erythrocyte sedimentation rate, ferritin levels, and lipid profile.

Vascular and Valve Assessments

Vascular calcification was assessed via Doppler sonography of both right and left carotid arteries. The intima-media thickness (CIMT) of carotid artery was measured at the higher, middle, and lower levels on both sides, and the average value was used. An enhanced CIMT was defined as having a measurement of 0.8 mm or more on either side. Valve calcification, including calcification of mitral or aortic valves or of the mitral annulus (MAC), was identified through two-dimensional echocardiography. For this study, cardiovascular system calcification was defined to include calcification of the arteries and/or valves.

Ethical considerations:

The Research Ethics Committee approved the study before it could be completed. Written informed consent was given by each patient before they were enrolled. Their permission to take part in the study and for the data to be published was clearly stated in the consent form, which also guaranteed their privacy and confidentiality. Declaration of Helsinki, World Medical Association's code of ethics for human subjects' research, has been followed in the conduct of this work.

Statistical analysis

The statistical analysis was executed employing SPSS 15.0. To establish whether or not our variables follow a normal distribution, we will utilize Shapiro-Wilk test. The mean and SD of normally distributed continuous variables were reported. In the absence of a normal distribution, the median was utilized. Percentages describing the categorized variables were provided. To determine the significance of the disparities among the groups, we used Student's t-test. To contrast categorical variables, the Fisher's exact test was employed. If the probability level is less than 0.05, it was thought of significant.

RESULTS

No significant variations were detected between the two groups regarding age ($p=0.131$), BMI ($p=0.428$), and gender ($p=0.878$) (Table 1).

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

	Group 1 (No.=60)		Group 2(No.=30)		P value
	Mean	SD	Mean	SD	
Age (Years)	51.80	10.923	48.33	9.718	0.131
BMI (Kg/m²)	24.67	4.221	25.47	4.607	0.428
Gender					0.878
Male	37 (61.7%)		18 (60)		
Female	23 (38.3%)		12 (40%)		

BMI; Body mass index. Statistically significant at $p<0.05$ was considered significant.

Table (2) shows statistically significant higher levels of serum P, Ca and Ca x P products and increases in serum iPTH, serum creatinine, urea, uric acid, total cholesterol, and triglyceride in the HD group in contrast to control group ($p < .0001$). Patients with HD show statistically significant low serum albumin levels in

contrast to those of healthy controls ($p < .0001$). The HD group had significantly greater levels of ESR, hs-CRP and ferritin and TNF- α in contrast to the healthy control group ($p < .0001$). Serum Fetuin-A levels were statistically significant lessened in HD group compared to those in the control group ($p < .0001$).

Table 2. Comparison between groups regarding clinical and laboratory data

	Group 1 (No.=60)		Group 2(No.=30)		
	Mean	SD	Mean	SD	P value
Ca	8.290	.7865	9.583	.571	<.0001
P	6.08	1.08	4.02	.427	<.0001
Ca x P	50.033	8.012	38.475	4.214	<.0001
iPTH	669.63	230.906	33.80	6.008	<.0001
Alb	3.635	.4668	4.777	.3884	<.0001
ALP	176.05	50.523	89.77	19.134	<.0001
Creatinine	7.2323	1.13530	.8660	.14562	<.0001
Urea	148.075	22.4140	30.033	7.4161	<.0001
UA	8.340	1.4509	4.803	.8079	<.0001
ESR	53.77	25.302	8.67	2.670	<.0001
CRP	22.50	16.755	6.00	.000	<.0001
Ferritin	501.82	140.845	138.93	60.506	<.0001
Cholesterol	232.85	42.593	123.27	23.929	<.0001
TGS	218.68	62.818	106.63	21.274	<.0001
HB%	9.370	.9800	12.830	.7013	<.0001
Fetuin-A	.5027	.40332	2.2877	.54651	<.0001
TNF-α	45.96	22.64	35.62	21.61	0.039

Ca: calcium, P: phosphorus, iPTH, intact parathyroid hormone, UA; uric acid, Alb.: albumin, Creat: creatinine Chol: Cholesterol. ESR: erythrocyte sedimentation rate, ALP; alkaline phosphatase, CRP: C-reactive protein, Hb: hemoglobin, TNF- α ; tumor necrosis factor alfa. Statistically significant at $p < 0.05$ was considered significant.

It was obvious from data in Table (3) there was a statistically significant increase in carotid intimal medial thickness (CMT) when contrasting HD patients to healthy controls ($p < .0001$) and there was a statistically significant rise in both systolic (SBP) and diastolic blood

pressure (DBP) in HD group in contrast to healthy group ($p < .0001$).

Study of valve calcification in HD group showed that 46 (76.7%) of HD patients showed aortic calcification and 13 (21.7%) showed mitral calcification as shown in Figure (1).

Table 3. Carotid intimal thickness (CMT), SBP and DBP of HD patients and healthy subjects.

Item	Group 1 (No.=60)		Group 2(No.=30)		P value
	Mean	SD	Mean	SD	
CMT	.9683	.30448	.6767	.35881	<.0001
SBP	136.75	20.888	121.83	7.484	<.0001
DBP	84.28	11.504	74.93	7.398	<.0001

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

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

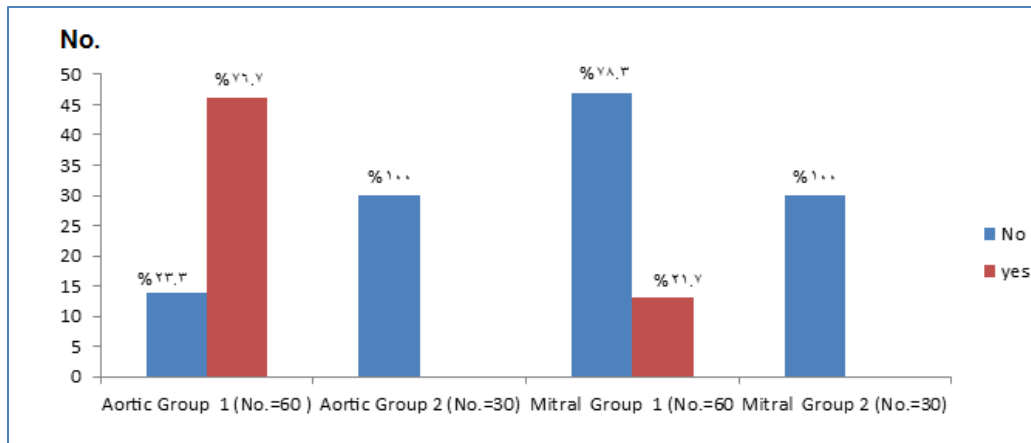


Fig. 1: Aortic and mitral valves calcification in both groups

There were negative connection between serum fetuin-A and Ca x P ($p=.0001$), iPTH ($p<.000$), alkaline phosphatase ($p=.0001$), serum urea, serum creatinine ($p<.000$), ESR ($p=.0001$), hs-CRP

($p <.0001$), ferritin ($p<.0001$), TNF- α , CIMT cholesterol ($p=.0001$), TG ($p<.0001$), both SBP and DBP ($p<.001$) ($p=.0001$) respectively, and carotid intimal medial thickness (CIMT) ($p<.0001$) (Table 4).

Table 4: Connection between Fetuin-A level and different clinical and laboratory parameters in the investigated patients (n=60).

Variable	R	P value
Ca	.674**	< 0.0001
P	-.761	< 0.0001
Ca x P	-.635**	< 0.0001
iPTH	-.820**	< 0.0001
ALP	-.680**	< 0.0001
Urea	-.861**	< 0.0001
Creat	-.878**	< 0.0001
ESR	-.819**	< 0.0001
hs-CRP	-.578**	< 0.0001
Ferritin	-.760**	< 0.0001
Choles	-.791**	< 0.0001
TGs	-.668**	< 0.0001
Alb	.801**	< 0.0001
SBP	-.340**	< 0.001
DBP	-.321**	< 0.002
TNF- α	-.208*	< 0.049
(CIMT)	-.373**	< 0.0001

Ca; calcium, P; phosphorus, iPTH; intact parathormone, ALP; alkaline phosphatase, Creat; creatinine, ESR, erythrocytic sedimentation rate. hs-CRP; C-reactive protein, Choles; cholesterol, TGs; triglycerides, Alb; albumin, TNF- α ; tumor necrosis factor alfa, CIMT; carotid intimal medial thickness, , SBP; systolic blood pressure, DBP; diastolic blood pressure.

It was obvious from Table (5) that there was negative connection between serum hs-CRP and serum Ca and albumin levels ($P < .0001$) and positive connection between hs-CRP and serum phosphorus

levels, iPTH, ALP, creat., urae, uric acid, ESR, ferritin, cholesterol, triglyceride, and hemoglobin ($P < 0.0001$) positive connection between hs-CRP and CIMT (P value 0.005).

Table 5. Correlation between hs-CRP and other parameters (n=60).

variables	R	P value	variables	R	P value
Ca	-.533*	< 0.0001	ESR	.716**	< 0.0001
P	.711*	< 0.0001	Ferritin	.700**	< 0.0001
iPTH	.676**	< 0.0001	TG	.625**	< 0.0001
Alb	-.686*	< 0.0001	Hb%	-.633**	< 0.0001
ALP	.556**	< 0.0001	Fetuin	-.578**	< 0.0001
Creat	.609**	< 0.0001	Ca x P	.617**	< 0.0001
Urea	.609**	< 0.0001	CIMT	.295**	0.005
Uric acid	.701**	< 0.0001	EF	-.311**	0.003

Ca; calcium, P; phosphorous, iPTH; intact parathormone, ALP; alkaline phosphatase, Creat; creatinine, ESR, erythrocytic sedimentation rate. hs-CRP; C-reactive protein, Choles; cholesterol, ALB; albumin, TNF- α ; tumor necrosis factor, EF; ejection fraction, CIMT; carotid intimal medial thickness, CIMT: carotid intimal thickness, SBP; systolic blood pressure, DBP; diastolic blood pressure.

As shown in Table (6) there were positive connection between ferritin and systolic (p value < 0.0001) and DBP (p value 0.037), serum P (p value < 0.0001), iPTH (p value < 0.0001), albumin (p value < 0.0001), ALP (p value < 0.000), serum urea (p value < 0.000), serum creatinine (p value < 0.0001,

serum uric acid (p value < 0.0001), ESR (p value < 0.000), CRP (p value < 0.0001), CIMT (p value < 0.0001), Cholesterol (p value 0.0001) and TG (p value 0.0001), and negative correlation between ferritin (p value < 0.0001) and serum Ca (p value < 0.0001) and albumin (p value < 0.0001).

Table 6. Correlation between Ferritin and other parameters (n=60).

Variables	R	P value	Variables	R	P value
SBP	.375**	< 0.0001	Urea	.860**	< 0.0001
DBP	.390**	0.037	Uric acid	.834**	< 0.0001
Ca	-.638**	< 0.0001	ESR	.749**	< 0.0001
P	.767**	< 0.0001	hs-CRP	.700**	< 0.0001
iPTH	.859**	< 0.0001	Cholest	.842**	< 0.0001
Alb	-.794**	< 0.0001	TGs	.775**	< 0.0001
ALP	.771**	< 0.0001	Fetuin-A	-.760**	< 0.0001
Creat	.881**	< 0.0001	Ca x P	.650**	< 0.0001

Ca; calcium, P; phosphorous, iPTH; intact parathormone, ALP; alkaline phosphatase, Creat; creatinine, ESR, erythrocytic sedimentation rate. hs-CRP; C-reactive protein, Choles; cholesterol, TGs; triglycerides, Alb; albumin, TNF- α ; tumor necrosis factor alfa, CIMT; carotid intimal medial thickness, SBP; systolic blood pressure, DBP; diastolic blood pressure.

There were negative connection between TNF- α and hemoglobin levels (p value 0.047) in HD group and positive

correlation with CIMT (p value < 0.0001) as shown in Table (7).

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

Table 7. Correlation between TNF- α and other parameters (n=60).

	Hb%	FETUIN	CIMT
R	-.210*	-.208*	.530**
P value	0.047	0.049	< 0.0001

TNF- α ; tumor necrosis factor alfa, Hb; hemoglobin, CIMT; carotid intimal medial thickness.

DISCUSSION

In the current investigation, 60 HD patients were enrolled under regular HD and 30 subjects as healthy control, and there were a highly statistically significant serum fetuin-A low levels in dialysis group in contrast to control one ($p < .0001$), other studies showed the same results (Mazzaferro *et al.*, 2007; Cozzolino *et al.*, 2006). The mechanism of working fetuin-A to inhibit vascular calcification is due to its strong binding to calcium phosphate, which in turn buffers serum calcium phosphate and may prevent calcification. Soft tissue calcification inhibitors, especially fetuin-A, are lacking in those suffering from chronic renal impairment, particularly those receiving long-term dialysis (Schafer *et al.*, 2003). In HD patients, there was a negative connection ($p = .000$) between serum fetuin-A and carotid intima-media thickness (CIMT). A prior study found a connection between fetuin-A and ESRD patients' coronary and valvular calcification (Larik, 2023).

On the other hand, Ulutas *et al.* (2018) examined 93 ESRD patients and found no connection between the level of vascular calcification and serum fetuin-A. Low serum fetuin A may be an independent risk factor for early death in CKD patients, and observational studies suggest that people with slightly raised serum fetuin A levels may have a survival benefit over those with reduced levels (Uyar *et al.*, 2015). Moreover, it has been demonstrated that fetuin-A in vitro is a strong inhibitor of the calcification process and that fetuin-A loss in mice tends to exacerbate vascular calcification (Kanbay *et al.*, 2010). Vascular calcification may begin early in the course of CKD, before

dialysis begins, and it may worsen gradually, frequently more quickly than in the general population. This could be one explanation for this (Kanbay *et al.*, 2010). In the current study there was a significant negative connection between serum fetuin-A and SBP ($p = .001$) and DBP (.002), $Ca \times P$ ($p = .000$), serum cholesterol ($p = .000$), TG ($p = .000$). Ziłkowska *et al.* (2008) examined the impact of numerous variables, both with and without vascular alterations, that supported the growth of blood vessel calcification, such as lipids, bone turnover, and markers of calcium and P metabolism and demonstrated that the single distinguishing element was fetuin-A. Both aortic valve and mitral valve calcification are more frequently observed as the decline of renal function (Maher *et al.*, 1987). The present study showed that 46 subjects (76.7%) with ESRD had aortic valve calcification and 13 subjects (21.7%) had mitral valve calcification, this can be explained by increasing calcium deposition in cardiac valve apparatus, primarily in the aortic and mitral valves, in dialyzed patients. Zentner *et al.* (2011) found that patients with ESRD had a higher incidence and faster progression of hemodynamically significant aortic valve stenosis in comparison to individuals with normal kidney function. The rationale explains that decreased kidney function creates an ideal environment for the onset and advancement of valve and vascular calcification. This is primarily caused by changes in calcium-phosphate ($Ca \times P$) homeostasis, pathological bone remodelling, inflammation, and decreased calcification inhibitors levels in the systemic and vascular blood, such as fetuin-A (Shuvy *et al.*, 2008). Accompanying the reduction in renal

function and elevated blood phosphorus (P) levels is the development of ectopic calcification in the artery wall. Significant calcium deposition was also reported in several of these studies to be occurring within the valve apparatus (Aikawa *et al.*, 2009).

Clinical data from observational studies of individuals with CKD have demonstrated a correlation between raised carotid intima-media thickness, peripheral artery calcification, and mitral annular calcification when low fetuin-A levels are present (Uyar *et al.* 2015). According to a follow-up research done on 238 ESRD patients receiving peritoneal dialysis, those with low serum fetuin-A levels posed a greater danger of cardiovascular death and a higher prevalence of valvular calcification. Furthermore, independent of renal function, reduced serum fetuin-A levels have also been connected to an accelerated progression of valve disorder (Wang *et al.*, 2005).

Due to an imbalance between antioxidant and anti-inflammatory defences as well as detoxification activities, when renal function diminishes, the kidney is targeted by chronic, ongoing inflammation. As a result, pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) and acute-phase proteins (hs-CRP and fibrinogen) are seen at higher blood concentrations. Vascular calcification might be aided by inflammation in part because it influences circulating factors such as fetuin-A, whose levels may be inhibited by pro-inflammatory cytokines like TNF- α and hs-CRP, intensifying the inflammatory response and perhaps raising mortality (Mihai *et al.*, 2018).

In current research lower fetuin-A serum levels connected significantly to higher parameters of inflammation (ferritin, hs-CRP, ESR TNF- α), which accords with the findings in other studies of dialysis patients (Stenvinke *et al.*, 2005; Hermans *et al.*, 2007).

The pro-inflammatory cytokine TNF- α was discovered to be much greater in dialysis group than in control group ($p=0.039$) in the present investigation. These results are in line with other researchers (Lee *et al.*, 2015). Zhou *et al.* (2021) found that patients with renal failure who receive long-term maintenance hemodialysis (MHD) have lower blood levels of inflammatory markers, comprising hs-CRP, inflammation is still the primary cause of cardiac insufficiency at the beginning of dialysis. Pro-inflammatory cytokines alter the glycocalyx layer and the adhesion molecules on the surface of leukocytes and endothelial cells in the renal vasculature. These changes affect the coagulation system, the function of the endothelial barrier, and the vascular reactivity (Hartzell *et al.*, 2020). This is the process via which inflammation results in CVD. The process of cardiovascular problems in CKD patients is also explained by this modification. Sun *et al.* (2016) originally examined many biomarkers in patients receiving hemodialysis who have advanced renal disease. The findings showed that most inflammatory biomarkers were higher in these individuals, and that these biomarkers are all predictive of the occurrence and outcome of cardiovascular events in these patients.

In the present study there was increasing in inflammatory marker as hs-CRP in HD group in contrast to control group ($p=.000$). Furthermore, we discovered a negative correlation between fetuin-A levels and TNF- α and hs-CRP. In dialysis patients, prior research has shown inverse relationships between fetuin-A levels and pro-inflammatory cytokines like IL-6 and TNF- α and inflammatory markers like hs-CRP (Shroff *et al.*, 2008). Interestingly, Metry *et al.* (2008) found that in HD patients, low levels of fetuin-A only predicted greater mortality when there was raised hs-CRP.

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

It was concluded from the current results that inflammation seems to have a role in calcification process. According to the study of Cottone *et al.* (2010) inflammatory processes are elevated in CKD, even in the initial phases of disease. These inflammatory processes, which are sparked by inflammatory markers like hs-CRP, are connected to endothelial dysfunction (ED) (Caglar *et al.*, 2008). Also, they revealed the opposite results in children where higher blood levels of fetuin-A was detected in HD patients than in their counterparts in good health. The reason given was that greater levels of fetuin-A in dialysis patients could be interpreted as a protective reaction against pro-inflammatory and hypercalcemic mediators, as demonstrated by the analysis of Shroff *et al.* (2008). Long-term stimulation of these variables, according to the scientists, may lessen the body's compensatory mechanisms and lower the concentration of naturally occurring calcification inhibitors.

Furthermore, in a model of acute inflammation, it has been demonstrated that the treatment of fetuin-A reduces the generation of tumour necrosis factor. In both the general population and those with ESRD, inflammation is a major risk factor for cardiovascular events and mortality (Carrero and Stenvinkel, 2009). But in HD and peritoneal dialysis patients, a study has not discovered any connection between vascular calcifications and inflammation markers comprising TNF- α , hs-CRP, and IL-6 (Turkmen *et al.*, 2004). In addition to inflammation-dependent fetuin-A down regulation, other putative factors such as uremic toxins and genetic predisposition may be involved. Benz *et al.* (2018) found that because calcification could remove fetuin-A from the circulation, there was an inverse connection between fetuin-A levels and the load of calcification. Additionally, it

was found that in patients in the HD group who had arterial stiffness, there was a substantial negative connection between serum fetuin-A, T.G., and total cholesterol. In contrast, an investigation carried out by Ix *et al.* (2006) who discovered that truncal obesity and dyslipidemia, which are unrelated to inflammation and malnutrition, are positively correlated with fetuin-A. This implies that fetuin-A may be a predictor of visceral obesity and dyslipidemia, particularly T.G. in HD patients.

In the current research, it was demonstrated a highly statistically significant increase in ferritin levels in HD group in contrast to control group. Kuragano *et al.* (2014) conducted a multicenter, prospective observational research involving 1086 Japanese HD patients and discovered that hyperferritinemia, which is described as serum ferritin levels greater than 100 $\mu\text{g/L}$, is a risk factor for heart disease, hospitalisation, and death. Additionally, they demonstrated a strong correlation between elevated incidence of cardiovascular events and ferritin levels greater than 100 ng/mL. The present findings demonstrated a statistically significant variation in hs-CRP levels between the HD group and the control group. The HD group's levels were higher. Other research showing strong positive relationships between serum ferritin and hs-CRP levels corroborated these findings (Abd El-Hafeez *et al.*, 2019; Elmenyawawi *et al.*, 2017). The body of research has shown that hyperferritinemia is substantially linked to a bad prognosis, which includes a greater mortality rate in patients with HD and PD. Because ferritin is an acute phase protein, both acute and chronic inflammatory settings cause an increase in its level, regardless of iron status. Ferritin can therefore be utilised as a stand-in marker and as the connection between the state of inflammation and iron accumulation. Elevated ferritin levels are

indicative of inflammation and iron overload, which leads to oxidative damage. Furthermore, prior research has indicated that elevated ferritin levels are linked to a greater chance of infection, mortality, and cerebro-vascular and cardio-vascular disorders in patients receiving hemodialysis (Kuragano *et al.*, 2014).

Conclusion

It was concluded that inflammation and low fetuin-A levels in dialysis patients raise their possibility of CVD. There are no proven ways to alter the amounts of circulating fetuin-A, an essential inhibitor of dystrophic calcification, but raising these levels might be advantageous. Hemodialysis patients frequently experience chronic inflammation, which is indicated by raised levels of pro-inflammatory cytokines and hs-CRP. Chronic inflammation could perhaps lead to the progression of atherosclerosis, and hs-CRP is a valid marker for CVD. Higher cardiovascular and all-causes of mortality are also correlated with higher serum ferritin levels, particularly in the presence of elevated hs-CRP levels. Risk assessment and intervention should start in the initial phases of chronic kidney disease (CKD) since CVD risk variables are elevated even in the initial phases of deteriorating renal function.

Conflict of interest: all authors had no conflicts to declare.

REFERENCES

- Abd El-Hafeez, H.; Al-Alashkar, A.; Mohamed, N. and Mohammed I.Khalil (2019). Association between Neutrophil to Lymphocyte Ratio and Inflammatory Markers in Hemodialysis Patients. *The Egyptian Journal of Hospital Medicine*, 77:5681-9.
- Aikawa, E.; Aikawa, M.; Libby, P., *et al.* (2009). Arterial and aortic valve calcification abolished by elastolytic cathepsin S deficiency in chronic renal disease. *Circulation*, 119:1785-94.
- Birukov, A.; Polemiti, E., Jäger S *et al.* (2022). Fetuin-A and risk of diabetes-related vascular complications: a prospective study. *Cardiovasc Diabetol.*, 21:6.
- Benz, K.; Hilgers, K.; Daniel, C., *et al.* (2018). Vascular Calcification in Chronic Kidney Disease: The Role of Inflammation. *Int. J. Nephrol.*, 2018:4310379.
- Caglar, K.; Yilmaz, M.I.; Saglam, M., *et al.* (2008). Short-term treatment with sevelamer Increases Serum Fetuin-A Concentration and Improves Endothelial Dysfunction in Chronic Kidney Disease Stage 4 Patients. *Clin. J. Am. Soc. Nephrol.*, 3(1):61–68.
- Carrero, J. and Stenvinkel, P. (2009). Persistent inflammation as a catalyst for other risk factors in chronic kidney disease: a hypothesis proposal. *Clin. J. Am. Soc. Nephrol.*, 1:S49-55.
- Cottone, S.; Palermo, A.; Arseno, R., *et al.* (2010). Relationship of fetuin-A with glomerular filtration rate and endothelial dysfunction in moderate-severe chronic kidney disease. *J. Nephrol.*, 23:62-9.
- Cozzolino, M.; Galassi, A.; Biondi, M., *et al.* (2006). Serum fetuin-A levels link inflammation and cardiovascular calcification in hemodialysis patients. *Am. J. Nephrol.*, 26:423-9.
- Echefu, G.; Stowe, I.; Burka, S., *et al.* (2023). Pathophysiological concepts and screening of cardiovascular disease in dialysis patients. *Frontiers in Nephrology*, 3:1198560.
- Elmenyawy, I.; Hassan, A.; Said, A., *et al.* (2017). Relationship Between Hepcidin, Ferritin and C-Reactive Protein in Hemodialysis Patients. *The Egyptian Journal of Hospital Medicine*, 69:1786-93
- Jairam, A.; Das,

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

- R.; Aggarwal, P., *et al.* (2010). Iron status, inflammation and hepcidin in ESRD patients: The confounding role of intravenous iron therapy. *Indian J. Nephrol.*, 20:125-31.
- Hartzell, S.; Bin, S.; Cantarelli, C.; Haverly, M.; Manrique, J.; Angeletti, A., *et al.* (2020). Kidney Failure Associates with T Cell Exhaustion and Imbalanced Follicular Helper T Cells. *Front. Immunol.* 11: 583702..
- Hermans, M.; Brandenburg, V.; Ketteler, M., *et al.* (2007). Association of serum fetuin-A levels with mortality in dialysis patients. *Kidney Int.*, 72:202-7.
- Ix, J.; Shlipak, M.; Brandenburg, V. *et al.* (2006). Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. *Circulation*, 113:1760-7.
- Kanbay, M.; Goldsmith, D.; Uyar, M., *et al.* (2010). Magnesium in chronic kidney disease: challenges and opportunities. *Blood Purif.*, 29:280-92.
- Kuragano, T.; Matsumura, O.; Matsuda, A., *et al.* (2014). Association between hemoglobin variability, serum ferritin levels, and adverse events/mortality in maintenance hemodialysis patients. *Kidney Int.*, 86:845-54.
- Larik, M (2023). Fetuin-A levels in association with calcific aortic valve disease: A meta-analysis. *Atherosclerosis Plus*, 54:27-9.
- Lee, B.; Ahmed, F.; Hamm, L., *et al.* (2015). Association of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease. *BMC Nephrol.*, 16:77.
- Maher, E.R.; Young, G.; Smyth-Walsh, B.m *et al.* (1987). Aortic and mitral valve calcification in patients with end-stage renal disease. *Lancet*, 2: 875–877
- Mazzaferro, S.; Pasquali, M.; Pugliese, F., *et al.* (2007). Serum levels of calcification inhibition proteins and coronary artery calcium score: comparison between transplantation and dialysis. *Am. J. Nephrol.*, 27:75-83.
- Metry, G.; Stenvinkel, P.; Qureshi, A., *et al.* (2008). Low serum fetuin-A concentration predicts poor outcome only in the presence of inflammation in prevalent haemodialysis patients. *Eur. J. Clin. Invest.*, 38:804-11.
- Mihai, S.; Codrici, E.; Popescu, I., *et al.* (2018). Inflammation-Related Mechanisms in Chronic Kidney Disease Prediction, Progression, and Outcome. *J. Immunol. Res.*, 2018:2180373.
- Rudloffm, S.; Jahnhen-Dechent, W. and Huynh-Do, U. (2022). Tissue chaperoning-the expanded functions of fetuin-A beyond inhibition of systemic calcification. *Pflugers Arch.*, 474:949-62.
- Schafer, C.; Heiss, A.; Schwarz, A., *et al.* (2003). The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J. Clin. Invest.*, 112:357-66.
- Shroff, R.; Shah, V.; Hiorns, M., *et al.* (2008). The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. *Nephrol. Dial. Transplant.*, 23:3263-71.
- Shuvy, M.; Abedat, S.; Beerli, R., *et al.* (2008). Uraemic hyperparathyroidism causes a reversible inflammatory process of aortic valve calcification in rats. *Cardiovasc Res*; 79: 492–499 39.
- Stenvinkel, P.; Wang, K.; Qureshi, A., *et al.* (2005). Low fetuin-A levels are

- associated with cardiovascular death: Impact of variations in the gene encoding fetuin. *Kidney Int.*, 67:2383-92.
- Sun, J.; Axelsson, J.; Machowska, A., *et al.* (2016). Biomarkers of Cardiovascular Disease and Mortality Risk in Patients with Advanced CKD. *Clin. J. Am. Soc. Nephrol.*, 11:1163-72.
- Turkmen, K.; Gorgulu, N.; Uysal, M., *et al.* (2011). Fetuin-A, inflammation, and coronary artery calcification in hemodialysis patients. *Indian J. Nephrol.*, 21:90-4.
- Ulutas, O.; Taskapan, M.; Dogan, A., *et al.* (2018). Vascular calcification is not related to serum fetuin-A and osteopontin levels in hemodialysis patients. *Int. Urol. Nephrol.*, 50:137-42.
- Wang, A.; Woo, J.; Lam, C., *et al.* (2005): Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. *Nephrol Dial Transplant.*, 20:1676-85.
- Zentner, D.; Hunt, D.; Chan, W., *et al.* (2011). Prospective evaluation of aortic stenosis in end-stage kidney disease: a more fulminant process? *Nephrol. Dial. Transplant.*, 26:1651-5.
- Zhou, M.; Du, Y.; Wu, Y., *et al.* (2021). Analysis of inflammatory factor levels in serum and risk factors in patients with chronic renal failure undergoing maintenance hemodialysis. *Am. J. Transl. Res.*, 13:6994-7000.
- Ziolkowska, H.; Brzewski, M. and Roszkowska-Blaim, M. (2008). Determinants of the intima-media thickness in children and adolescents with chronic kidney disease. *Pediatr. Nephrol.*, 23:805-11.

The relation between Fetuin-A and inflammatory markers in cardiovascular calcification in hemodialysis dependent patient

العلاقة بين فيتوين أ وعلامات الالتهاب في تكلس القلب والأوعية الدموية لدى المرضى المعتمدين على غسيل الكلى

سامية حسن الششتاوي ١*، نيفين شريف ١، أنس حسن إبراهيم ١، سماح ممدوح ٢، أمينة أحمد متولي ٣، إبراهيم عبد

العظيم ٣، أحمد علي محمد ٣، أسامة مصباح عبد العظيم ١

١- قسم بحوث الكلى، معهد تيودور بلهارس للأبحاث، الجيزة، مصر

٢- قسم الكيمياء الحيوية والبيولوجيا الجزيئية، معهد تيودور بلهارس للأبحاث، الجيزة، مصر

٣- قسم العناية المركزة بمعهد تيودور بلهارس للأبحاث، الجيزة، مصر

*البريد الإلكتروني للباحث الرئيسي: samya_shishtawy@yahoo.com

المستخلص

في مرض الكلى المزمن (CKD) والخاضعين للغسيل الكلوي الدموي يحدث زيادة في مضاعفات القلب والأوعية الدموية (CV) بما في ذلك تكلس الأوعية الدموية والصمامات، وأمراض الشريان التاجي، وفشل القلب، وعدم انتظام ضربات القلب، والموت القلبي المفاجئ. إن حدوث وانتشار أمراض القلب والأوعية الدموية في كثير من المرضى الذين يعانون من مراحل مرض الكلى المزمن المبكرة مقارنة مع عامة السكان، ولكن المرضى الذين يعانون من مراحل مرض الكلى المزمن المتقدمة وأولئك الذين يخضعون لغسيل الكلى يظهرون خطراً مرتفعاً بشكل ملحوظ على أمراض القلب والأوعية الدموية. يعد مرض القلب والأوعية الدموية وليس مرض الكلى في المرحلة النهائية (CKD المرحلة ٥) هو السبب الرئيسي للوفاة في هذه الفئة المعرضة للخطر الشديد. يتسبب مرض الكلى المزمن في حالة التهابية جهازية مزمنة تساهم في إعادة تشكيل الأوعية الدموية وعضلة القلب مما يؤدي إلى تكلس الأوعية الدموية وشيخوخة الأوعية الدموية وكذلك تليف عضلة القلب وتكلس صمامات القلب بشكل رئيسي الصمامات الأبهرية والتاجي. بالإضافة إلى ذلك، يؤدي غسيل الكلى التقليدي (HD) في حد ذاته إلى إجهاد عضلة القلب وإصابة نظام القلب والأوعية الدموية المتضرر بالفعل لدى هؤلاء المرضى. يؤدي الالتهاب، وانخفاض مستويات المصل فيتوين-A وارتفاع مستويات مصل الفيريتين في زيادة معدلات الإصابة بالأمراض والوفيات في هذه المجموعة من المرضى. لذا، تهدف الدراسة الحالية إلى تقييم وظيفة الفيتوين-A بالاشتراك مع العلامات الالتهابية؛ $TNF-\alpha$ و $hs-CRP$ والفيريتين في تطور مضاعفات السيرة الذاتية لدى مرضى غسيل الكلى. شارك في هذه الدراسة ما مجموعه (٦٠) من مرضى غسيل الكلى المزمن و (٣٠) متطوعاً أصحاء في قسم أمراض الكلى في معهد تيودور بلهارس للأبحاث. تم قياس العلامات المختبرية: مصل الكالسيوم، الفوسفور، الباراثورمون، فوسفات الكالسيوم، علامات الالتهابات؛ مستويات المصل من fetuin-A، وعامل نخر الورم ألفا ($TNF-\alpha$)، والبروتين التفاعلي C شديد الحساسية ($hs-CRP$). تم إجراء سماكة الوسائط الداخلية للشرايين السباتية المشتركة (CIMT) وتكلس الحلقة التاجية والأبهرية.

أشارت النتائج إلى أن مستويات fetuin-A أظهرت مستويات منخفضة ذات دلالة إحصائية في مجموعة HD على عكس مجموعة السيطرة ($p < 0.0001$)، بينما كان هناك ارتفاع معتد به إحصائياً في ($p < 0.0001$)، $hs-CRP$ ، $TNF-\alpha$ ($p < 0.0001$)، $TNF-\alpha$ ($p < 0.0001$)، فيريتين المصل ($p < 0.0001$) في مجموعة HD مقارنة بالمجموعة الضابطة. وبالإضافة إلى ذلك، كان هناك اتصال سلبي بين fetuin-A و CIMT و SBP و DBP، في حين كان هناك اتصال إيجابي بين $hs-CRP$ و $TNF-\alpha$ و CIMT. ويتضح من الدراسة انه يعد تحديد fetuin-A أداة مفيدة لتقييم الالتهاب ومخاطر السيرة الذاتية لدى مرضى HD.