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ORIGINAL ARTICLE

Serum fetuin-A as a predictive biomarker of chronic kidney disease

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ABSTRACT

Keywords: Fetuin-A, chronic kidney disease, natural calcium antagonist, hemodialysis.

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Background: Chronic kidney disease (CKD) is a worldwide public health problem. Fetuin A is a natural calcium antagonist. Both human and animal studies have shown that low circulating fetuin A level are associated with vascular calcification and may be an independent risk factor for premature death in CKD patients. **Objective:** The aim of this study was to evaluate the effect of serum fetuin-A in the pathogenesis of chronic kidney disease and its correlation with routine renal biomarkers.**Patients and methods:** The study population was divided into 3 groups. Group I: Pre-dialysis group: on conservative treatment. Group II (CKD): on regular hemodialysis, 3 sessions/week for more than 9 months. Group III (Control group).**Results:** There was significant negative correlation between blood urea, serum creatinine versus serum fetuin-A in conservative group with arterial stiffness. There was significant positive correlation between eGFR and serum fetuin-A in conservative group with arterial stiffness, while there was no significant correlation between serum fetuin-A and Hb, HcT, platelets, WBCS, FBS, Na^{+}, K^{+}, $PO4^{-2}$, serum uric acid, total bilirubin, ALT, AST, ALP, serum albumin and prothrombin time. There was significant negative correlation between serum Ca^{+2} and serum fetuin-A. There was significant negative correlation between total cholesterol as well as triglycerides versus serum fetuin A.**Conclusion:** serum fetuin-A level significantly reduced in hemodialysis patients, significantly correlated with the disease duration, and negatively correlated with serum Ca, T. cholesterol, and T.G.

INTRODUCTION:

Chronic kidney disease (CKD) is a syndrome defined as persistent alterations in kidney structure, function or both with implications for the health of the individual [1]. CKD results from a large number of systemic diseases that damage the kidney or from disorders that are intrinsic to the kidney. A glomerular filtration rate (GFR) persistently below 60 mL/minute/1.73 m2, which is below the level of kidney function expected to occur with aging, defines clinically significant CKD [2]. Earlier identification CKD with biomarkers that can also predict CKD progression would help to initiate nephron-protective interventions [3]. Fetuin-A is a circulating serum glycoprotein with a molecular mass of approximately 60 KDa. Like serum albumin, fetuin-A is predominantly liver-derived (>95%). Extrahepatic fetuin-A expression may occur in kidney, the choroid plexus and in all major organs during fetal development [4]. Fetuin-A stabilizes colloidal nanoscopic complexes with Ca and P and prevents crystal growth by shielding mechanisms; the complex formed is termed "calciprotein particles" because they share functional and structural similarities to lipoprotein particles like low-density lipoproteins. Fetuin-A is responsible for about 50% of the precipitation- inhibitory effect in serum and extracellular fluids [5]. Vascular smooth muscle cells do not produce fetuin-A suggesting that fetuin-A is taken up from the surrounding medium in a process probably mediated via annexin II-binding at the cell membrane [6]. Serum fetuin-A levels were lower in patients on hemodialysis when compared with control populations and were inversely correlated with CRP levels confirming fetuin-A regulation as part of a negative acute-phase response. Fetuin-A deficiency was identified as an inflammation-associated risk predictor of all cause and cardiovascular mortality [7]. In addition, fetuin-A deficiency was also strongly correlated with hypoalbuminemia and appeared to be a more potent predictor of mortality than elevated CRP levels. Fetuin-A deficiency thus seems to be a key element of the malnutrition inflammation-atherosclerosis syndrome exacerbated by genetic susceptibility [8]. Fetuin-A replacement, which could be envisioned as either plasma or (recombinant) protein infusion, will potentially, not be feasible owing to the concentrations that need to reach the extracellular space. From point of view, the focus concerning therapeutic approaches should be laid on the understanding of the regulation of hepatic fetuin-A synthesis and secretion [9]. Anti-inflammatory treatment strategies (e.g., tumor necrosis factor-a [TNF-o:] antagonism) may have potential to counteract suppression of fetuin-A production [10]. So that, this work was designed to evaluate the effect of serum fetuin A in the pathogenesis of CKD & its relation with routine renal biomarkers.

PATIENTS AND METHODS

Ethical consideration:

This study was approved by Aswan University Hospital ethical committee (367/4/19) and a written concept was taken from all included subjects.

Study population:

This study included 40 patients with chronic kidney disease attending Hemodialysis Unit and Outpatient Clinics of Medical Department of Aswan University Hospital, Egypt in addition to 10 healthy control individuals. The study population was divided into three groups. Group I (CKD patients on conservative therapy): Pre-dialysis group included twenty CKD patients (stage 3 and 4) on conservative treatment (11males and 9 females). Group II (CKD on regular hemodialysis therapy): Included twenty CKD patients on regular H.D, 3 sessions/ week for more than 9 months (12 males and 8 females). Group III (Control group): Included 10 healthy individuals (5males and

5 females) with exclusion criteria like congenital renal disease patients, and Pregnancy. All the studied groups were subjected to the following: History taking including (Age, sex duration of disease), History of any previous renal disease and Dialysis history (date of starting HD, duration of HD, number of sessions per week and duration of session). Followed by thorough physical examination and Laboratory Investigations.

The routine Laboratory Investigations:

The routine lab data were included in the study as Complete Blood Count (CBC): Done by Sysmex KX-21 automated hematology analyzer (Sysmex corporation, Japan), Kidney function tests (Urea & Creatinine) using the open system autoanalyzer synchronCX5 (Beckman, USA). Estimated creatinine clearance (eCCr) using Cockcroft-Gault equation: eCCr (inml/min) = [(140– age in years) x weight in Kg] ÷ (72xserum creatinine in mg/dl) x (0.85if female). Serum electrolytes: Serum calcium & phosphorus levels using the open system auto analyzer synchron CX5 (Beckman, USA). Serum sodium and potassium levels using the open system auto analyzer synchron CX5 (Beckman, USA). Fasting blood glucose and 2 hours post prandial blood glucose levels. Estimation of lipid profile (including total cholesterol and triglycerides) using the open system autoanalyzer synchron CX5 (Beckman, USA), Liver function tests (total bilirubin, serum albumin, prothrombin time, serum transaminases and alkaline phosphatase) using heopen system auto analyzer synchron CX5 (Beckman, USA).

Special investigations:

Serum fetuin –A, measured by ELISA.

Methods

A-Samples:

From each subject, under complete aseptic technique, 5ml venous blood samples were collected. Samples were allowed to clot, then were centrifuged at1000 xg for10 minutes within one hour after collection. A part of the separated serum is aliquoted and stored frozen at - 20^{O}C for subsequent determination of serum fetuin-A.

B-Assessment of serum fetuin–A:

The kit assay Human Fetuin-A level in the sample, use purified Human Fetuin-A antibody to coat microtitre plate wells, make solid –phase antibody, then add Fetuin-A to wells, combined fetuin-A antibody which with enzyme labeled, become antibody-antigen enzyme-antibody complex, after washing completely, add substrate, substrate becomes blue color. At HRP enzyme-catalyzed, reaction was terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Human Fetu-A in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Statistical Analysis:

The results were tabulated and statistically analyzed using standard computer program using MICROSOFT EXCEL 2016 and SPSS V.21 program for MICROSOFT.

RESULTS:

Clinical characteristics of the studied subjects:

The Clinical characteristics of the studied subjects were illustrated in table (1). There was significant increase as regard age of patients in conservative group compared to other groups (f=4.2) (p <0.05). In addition, there was significant decrease of weight of patients in haemodialysis group compared to other groups. (f= 11.9) (P <0.01). While, there was highly significant increase of disease duration of patients in conservative group compared to H.D. group (f= 26.5), (p <0.001). Whereas, there was significant increase of the numbers of hypertensive patients in H.D. group patients compared to other groups (f=11.9), (p<0.01).

Laboratory characteristics of the studied subjects regarding CBC:

Laboratory characteristics of the studied subjects regarding CBC was illustrated in table (2). There was significant decrease as regard Hb among the hemodialysis group (f= 78.3) (P < 0.001). Also, there was significant decrease as regard HCT % among the HD group (f = 86.2) (P < 0.001). While, there was significant increase as regard WBCS in the hemodialysis group (f = 7.24) (p < 0.01).

Laboratory characteristics of the studied subjects regarding Renal function:

The Laboratory characteristics of the studied subjects regarding renal function were illustrated in table (3). There was significant increase as regard blood urea in the hemodialysis group (f = 31.3) (p < 0.001). Also, there was significant decrease as regard serum creatinine in the hemodialysis group (f= 424.8) (p < 0.001). While, there was significant increase as regard eGFR in the control group (f = 436.7) (p < 0.001). Whereas, there was significant increase of serum potassium (K^{+}) in the hemodialysis group (f= 11.69) (p < 0.001). And also, there was significant increase as regard serum calcium (Ca^{+2}) in the control group (f = 58.98) (p < 0.001). While, there was significant increase as regard serum phosphorus in the hemodialysis group (f = 42.6) (p < 0.001).

Laboratory characteristics of the studied subjects regarding Lipid profile:

The laboratory characteristics of the studied subjects regarding lipid profile were illustrated in table (4). There was significant increase as regard total cholesterol in the hemodialysis group (f = 9.5) (p < 0.001). Also, there was significant increase as regard triglycerides in the hemodialysis group (f = 7.4) (p < 0.01). While there was significant increase as regard LDL in the haemodialysis group (f = 7.4) (p < 0.01). Whereas, there was significant increase as regard HDL in the control group (f = 10.4) (p < 0.001). Also, there was significant increase as regard Alkaline phosphatase in the conservative group (f = 10.6) (p < 0.001). While, there was significant decrease as regard serum albumin in the haemodialysis group (f = 5.4) (p < 0.01).

Laboratory characteristics of the studied subjects regarding Liver function:

The laboratory characteristics of the studied subjects regarding liver function were illustrated in table (5). There was no significant difference as regard platelets, FBS, 2h PPBS, Na^{+}, serum uric, total bilirubin, AST, ALT, and PT.

Laboratory characteristics of the studied subjects regarding Fetuin – A.

Laboratory characteristics of the studied subjects regarding fetuin – A were illustrated in table (6). There was significant decrease as regard serum fetuin-A in the hemodialysis group (f = 10.8), ($p < 0.001$).

Number and distribution percent of subjects with arterial stiffness as estimated by S. fetuin A in all studied groups.

The number and distribution percent of subjects with arterial stiffness as estimated by S. fetuin A in all studied groups were illustrated in table (7). There was non-significant increase as regard number and percentage of distribution of subjects with abnormal serum fetuin A ≤ 475 ng/dl in the hemodialysis group (f = 7.29), ($p < 0.001$).

Clinical characteristic of patients (GI & GII) with arterial stiffness as estimated by s. fetuin-A level.

Clinical characteristic of patients (GI & GII) with arterial stiffness as estimated by serum fetuin-A level were illustrated in table (8). There was significant increase of number of patients with hypertension in the hemodialysis group with (T= 6.2) (p= 0.013). Also, that there was significant increase as regard disease duration in the conservative group with arterial stiffness as estimated by serum fetuin-A level with (T= 6.5) (P< 0.001). While, there was no significant difference as regard age, sex, weigh, diabetes, SBP, DBP among patient with arterial stiffness as estimated by serum fetuin-A level in both groups.

Correlation coefficient between serum fetuin –A and laboratory characteristic in conservative group with and without arterial stiffness.

Correlation coefficient between serum fetuin–A and laboratory characteristic in hemodialysis group (II) with arterial stiffness were illustrated in table (9). There was significant negative correlation between blood urea and serum fetuin-A in conservative group with arterial stiffness (r= 0.726) (P=0.001). In addition, there was significant negative correlation between serum creatinine and serum fetuin-A in conservative group with arterial stiffness with (r= 0.726) (P=0.001). While, there was significant positive correlation between eGFR and serum fetuin-A in conservative group with arterial stiffness (r=0.791) (p< 0.001). Whereas there was no significant correlation between serum fetuin-A and Hb, HcT, platelets, WBCS, FBS, Na^{+} , K^{+} , Ca^{+2} , PO4-2 , serum uric acid, T. cholesterol, triglycerides, total bilirubin, ALT, AST, ALP, serum albumin and prothrombin time.

Correlation coefficient between serum fetuin–A and laboratory characteristic in hemodialysis group (II) with arterial stiffness.

Correlation coefficient between serum fetuin–A and laboratory characteristic in hemodialysis group (II) with arterial stiffness were illustrated in table (10). There was significant negative correlation between serum Ca^{+2} and serum fetuin-A with (r= -0.47), (P= 0.042). Also, there was significant negative correlation between total cholesterol and serum fetuin-A (r= - 0.655), (P Whereas, there was no significant correlation between serum fetuin-A and Hb, HCT, platelets, WBCS, FBS, 2hrpBS, blood urea, Serum creatinine, eGFR, Na^{+}, K^{+}, Ca^{+2}, Po4, serum uric acid, T. Bilirubin, ALT, AST, ALP, serum albumin and prothrombin time.

DISCUSSION:

Chronic kidney disease is a worldwide public health problem and is recognized as a common condition with high prevalence rate of about 13- 15% with the increase prevalence of diabetes mellitus and hypertension [11]. Fetuin A is a natural calcium antagonist and both human and animal studies have shown that low circulating fetuin A levels are associated with vascular calcification. Clinical evidence from observational studies of patients with chronic kidney disease has shown that low fetuin A levels occur concurrently with mitral annular calcification, peripheral arterial calcification and increased carotid intima–media thickness [12]. Observational studies have shown that low serum fetuin A may be an independent risk factor for premature death in CKD patients, and patients with mildly elevated serum fetuin A levels could have a survival advantage over those with lower fetuin A levels [12]. Clinical and epidemiological studies have consistently suggested that vascular calcifications and hence arterial stiffness play an important role in the pathogenesis of cardiovascular disease and are a strong risk factor for increased morbidity and mortality in patients with end-stage renal disease (ESRD) [13]. [14] reported that vascular calcification is common in the study population and is associated with a lower serum fetuin-A level. High or sustained-normal serum fetuin-A levels may have a protective role against the development of vascular calcification in HD patients. [15] demonstrated an inverse association between serum fetuin-A and carotid intima-media thickness in HD patients. It has also been shown that in vitro fetuin-A is a potent inhibitor of the calcification process and that experimental fetuin-A deficiency appears to promote vascular calcification [13]. One possible explanation for this is that the process of vascular calcification may start early during the course of CKD, prior to the start of dialysis, and worsens progressively, often in an accelerated fashion compared with the general population [16]. Disturbances in calcification inhibitors such as fetuin -A appear to play a major role in the pathogenesis and rapid progression of vascular calcification [17]. However, it is notable that compared with calcium and phosphate, the role of fetuin -A in this pathologic process has been the subject of few studies. The pathogenesis of vascular calcification is not well understood, but it is likely to be multifactorial [18]. Fetuin-A stabilizes colloidal nanoscopic complexes with Ca and P and prevents crystal growth by shielding mechanisms, the complex formed is termed "calciprotein Page 9 particles" because they share functional and structural similarities to lipoprotein particles like low density lipoproteins. Fetuin-A is responsible for about 50% of the precipitation- inhibitory effect in serum and extracellular fluids [19]. Calcium phosphate deposition, mainly in the form of carbonate and hydroxyapatite, is the hallmark of vascular calcification and can occur in the blood vessels, myocardium and cardiac valves [20]. The results of this study showed that the number and distribution of hypertensive patients was higher among the H.D group. In a study reported by [21] showed that increased luminal pressure or hypertension also stimulates collagen production which in turn leads to arterial stiffness.The mean value of Hb and HCT was significantly decreased among the HD group patients. In a study reported by [22] showed that in hemodialysis patients, there are 3 mechanisms by which anemia occurs: blood loss in HD patients as the defective blood clotting seen in uraemia makes bleeding more common, Excessive destruction of RBCs (hemolysis). Abnormally low production of red blood cells by the bone marrow due to defective erythropoietin production by the kidney. [23] found that the presence of even mild anaemia with CKD was associated with a synergistic amplification of the risk of cardiovascular events. The mean values of K+, Ca, PO4 are significantly high among the HD group patients. Hyperkalemia is a common problem in dialysis patients [24]. In a study reported by [25] showed that native vitamin D deficiency and secondary hyperparathyroidism are noted early in the course of CKD. Treatment

for secondary hyperparathyroidism was often accompanied by several side effects as cardiovascular disorders; hypercalcemia and increased calcium phosphorus product with vascular and coronary calcification have been documented after excessive calcium containing phosphate binders and/or vitamin D therapy [26]. The mean values of T. cholesterol, T.G. and LDL was significantly higher among the HD group patients than the conservative group patients. In a study reported by [27] showed that the prevalence of hyperlipidemia or dyslipidemia was much higher in HD patients compared to the general population. Total or low-density lipoprotein LDL cholesterol is highest in patients with chronic renal impairment. The mean value of serum fetuin A was significantly lower among the HD group with mean ±SD (376±50) than the conservative group with mean ±SD (489±74). In a study reported by [28] showed that serum fetuin A levels were lower in patients on hemodialysis when compared to control populations this may be due to exposure to high levels of uremic toxins down regulating hepatic fetuin A production, in addition, calcification could consume fetuin A from the circulation. There was no significant statistical difference in age, weight and sex between the two group (I, II) with arterial stiffness. This agrees with a study reported by [29] which reported no significant differences in age, body mass index between the two groups. In a study by [30] showed a significant relationship between CIMT and age which was in concordance with previous studies and indicates the natural progression of atherosclerotic progression with increasing age. The mean values of blood urea and serum creatinine were significantly higher in the HD group patients with arterial stiffness more than the conservative group patients with arterial stiffness.In a study reported by [31] found that more than 10% of their cohort had elevated serum creatinine levels defined as > 1.5 mg/dl in men or > 1.3 mg/dl in women. This study found that mildly elevated creatinine levels were predictive of cardiovascular morbidity and mortality. Elevated creatinine was associated with a high prevalence of cardiovascular risk factors. The mean value of serum fetuin A was lower in the studied HD patients with arterial stiffness than in conservative group with arterial stiffness. As serum fetuin A is a calcification inhibitor its deficiency predisposes to arterial stiffness in HD patients because uraemia suppress fetuin -A level [32]. There was no significant statistical negative correlation between serum fetuin A and blood urea and serum creatinine levels in studied HD group patients with arterial stiffness.In a cross-sectional study by [33] performed in 312 hemodialysis patients, investigating serum fetuin A levels, serum fetuin A levels were lowe in patients on hemodialysis when compared with control populations. The present study revealed a significant statistical negative correlation between serum fetuin A level and serum Ca+2 level in HD patients with arterial stiffness. [32] determined that fetuin A levels were inversely correlated with the calcification burden because calcification could consume fetuin A from the circulation. Also, Significant negative correlation between T. holesterol, T.G and serum fetuin-A was demonstrated in the studied HD group patients with arterial stiffness. This is in contrast to a study by [34] who found that fetuin A was positively associated with truncal obesity and dyslipidemia which are independent of malnutrition and inflammation, it may predict visceral adiposity and dyslipidemia esp T.G in HD patients

References

1. Schelling, J.R., 2016: Tubular atrophy in the pathogenesis of chronic kidney disease progression. Pediatric nephrology, **31**(5): p. 693-706.
2. Matsell, D.G., 2019: Congenital Urinary Tract Obstruction—Diagnosis and Management in the Fetus, in Nephrology and Fluid/electrolyte Physiology., Elsevier. p. 391-409.

3. Ju, W., et al., Tissue transcriptome-driven identification of epidermal growth factor as a chronic kidney disease biomarker. Science translational medicine, 2015. **7**(316): p. 316ra193-316ra193.
4. Sato, M., et al., 2015: Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. Liver International,. **35**(3): p. 925-935.
5. Aghagolzadeh, P., et al., 2016: Calcification of vascular smooth muscle cells is induced by secondary calciprotein particles and enhanced by tumor necrosis factor-α. Atherosclerosis,. **251**: p. 404-414.
6. Leopold, J.A., Vascular calcification: mechanisms of vascular smooth muscle cell calcification. Trends in cardiovascular medicine, 2015. **25**(4): p. 267-274.
7. Dai, L., et al., 2017.: End-stage renal disease, inflammation and cardiovascular outcomes. Expanded Hemodialysis, **191**: p. 32-43.
8. Shafiee, M.A., et al., Dermatologic manifestations in end-stage renal disease. Iranian journal of kidney diseases, 2015. **9**(5): p. 339-353.
9. Li, J., et al., Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nature communications*, 2015. **6**(1): p. 1-16.
10. Fasshauer, M. and M. Blüher, Adipokines in health and disease. Trends in *pharmacological sciences*, 2015. **36**(7): p. 461-470.
11. Guérin, A.P., et al., Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrology Dialysis Transplantation*, 2000. **15**(7): p. 1014-1021.
12. Massy, Z.A. and T.B. Drüeke, 2012: Magnesium and outcomes in patients with chronic kidney disease: focus on vascular calcification, atherosclerosis and survival. *Clinical kidney journal*,. **5**(Suppl_1): p. i52-i61.
13. Kanbay, M., et al., 2010: Magnesium in chronic kidney disease: *challenges and opportunities. Blood purification*,. **29**(3): p. 280-292.
14. Okasha, K., A. El Bendary, and A. Mourad, 2010: Evaluation of peripheral vascular calcification and serum magnesium level in a group of Egyptian hemodialysis patients. *Arab Journal of Nephrology and Transplantation*,. **3**(1): p. 11-16.
15. Turgut, F., et al., 2008: Magnesium supplementation helps to improve carotid intima media thickness in patients on hemodialysis. *International urology and nephrology*,. **40**(4): p. 1075.
16. Temmar, M., et al., 2010: Pulse wave velocity and vascular calcification at different stages of chronic kidney disease. *Journal of hypertension*,. **28**(1): p. 163-169.
17. Shroff, R.C., et al., 2010: Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification. *Journal of the American Society of Nephrology*,. **21**(1): p. 103-112.
18. Chen, N.X. and S.M. Moe, 2015: Pathophysiology of vascular calcification. Current osteoporosis reports,. **13**(6): p. 372-380.
19. Uedono, H., et al., 2021: Effects of fetuin-A-containing calciprotein particles on posttranslational modifications of fetuin-A in HepG2 cells. *Scientific reports*,. **11**(1): p. 1-9.
20. Favretto, G., et al., 2021: Uremic endothelial-derived extracellular vesicles: Mechanisms of formation and their role in cell adhesion, cell migration, inflammation, and oxidative stress. *Toxicology Letters*,. **347**: p. 12-22.
21. Sun, Z., 2015: Aging, arterial stiffness, and hypertension. Hypertension,. **65**(2): p. 252-256.

22. Pergola, P.E., et al., 2021: Ziltivekimab for treatment of anemia of inflammation in patients on hemodialysis: results from a phase 1/2 multicenter, randomized, double-blind, placebo-controlled trial. *Journal of the American Society of Nephrology,*. **32**(1): p. 211-222.
23. Eckardt, K.-U., et al., 2021: Safety and efficacy of vadadustat for anemia in patients undergoing dialysis. New England Journal of Medicine,. **384**(17): p. 1601-1612.
24. Toft, G., et al., 2020: Anemia and clinical outcomes in patients with non-dialysis dependent or dialysis dependent severe chronic kidney disease: a Danish population-based study. Journal of nephrology,. **33**(1): p. 147-156.
25. Lu, C.-L., et al., 2018: The emerging role of nutritional vitamin D in secondary hyperparathyroidism in CKD. Nutrients,. **10**(12): p. 1890.
26. Galassi, A., et al., 2021: Current treatment options for secondary hyperparathyroidism in patients with stage 3 to 4 chronic kidney disease and vitamin D deficiency. Expert Opinion on Drug Safety, (just-accepted).
27. de Oliveira Junior, W.V., et al., 2020: Association between dyslipidemia and CCL2 in patients undergoing hemodialysis. Cytokine,. **125**: p. 154858.
28. Zhou, Z., et al., 2019: Circulating fetuin-A and risk of all-cause mortality in patients with chronic kidney disease: A systematic review and meta-analysis. Frontiers in physiology,. **10**: p. 966.
29. Jirak, P., et al., 2019: Clinical implications of fetuin-A. Advances in clinical chemistry,. **89**: p. 79-130.
30. Ossareh, S., A. Alaei, and D. Saedi, 2011: Carotid intima-media thickness in maintenance hemodialysis patients role of cardiovascular risk factor..
31. Fried, L.F., et al., 2003: Renal insufficiency as a predictor of cardiovascular outcomes and mortality in elderly individuals. Journal of the American College of Cardiology,. **41**(8): p. 1364-1372.
32. Benz, K., et al., 2018: Vascular calcification in chronic kidney disease: the role of inflammation. International journal of nephrology,.
33. Ketteler, M., et al., 2003: Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. The Lancet,. **361**(9360): p. 827-833.
34. Ix, J.H., et al., 2006: Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation,. **113**(14): p. 1760-1767.

Tables

Table (1): Clinical characteristics of the studied subjects.

		Groups						F	Sig.
		Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
		Mean±SD	Range	Mean±SD	range	Mean±SD	Range		
AGE years		48.7 ± 9.7	24 - 63	44.9 ± 7.8	28 – 60	39.4 ± 6.4	29- 49	4.2	<0.05*
sex	**Male**	11	55%	13	65%	7	70%	0.8	>0.05
	Female	9	45%	7	35%	3	30%		
WEIGHT kg		80.6 ± 8.8	65 - 95	74.4 ± 10	57 - 95	83.4 ± 7.6	70 -93	3.98	<0.05*
Diabetic		7	35%	6	30%			0.1	>0.05
Hypertensive		9	45%	19	95%			11.9	<0.01*
Disease duration (year)		6 ± 1.5	3 - 9	3.75 ± 1.12	2 - 6			26.5	<0.001*
HD DURATION (year)		. ± .	. - .	4.1±1.7	1.5 – 7				
SBP mmHg		134.50 ± 33	90 - 190	145.5 ± 8.9	130 - 160	126 ± 4.59	120 - 130	2.9	>0.05
DBP mmHg		81.5 ± 19.3	60 - 110	87 ± 9.38	70 - 105	76 ± 5.68	65 -80	2.2	>0.05

* Significant -SBP= Systolic blood pressure -DBP= Diastolic blood pressure.

Table (2): Laboratory characteristics of the studied subjects regarding CBC.

	Groups						F	Sig.
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
Hb (gm/dl)	10.2 ± 1.0	8.5 - 12.0	9.8 ± 1.1	7.5 -11.3	14.4 ± .5	13.7 - 15.1	78.3	<0.001*
HCT %	33.1% ± 3.5%	27% - 33%	30.1% ± 1.5%	26.7% - 38.4%	45.5% ± 5%	38.1% - 51.8%	86.2	<0.001*
Platelets	244 ± 43.46	150 - 320	232.5 ± 57.9	150 -350	240 ± 56.96	160 – 350	0.25	>0.05
WBCs	6.72 ± 1.27	5 - 9.2	7.95 ± 1.6	5 - 11	6.07 ± 1.12	4 - 7.8	7.24	<0.01*
FBS (mg/dl)	109 ± 37	65 - 210	106 ± 61	64 - 241	68 ± 4	63 – 77	3.02	>0.05
2hPPBS	158 ± 52	110 - 300	175 ± 75	110 - 330	131 ± 6	122 – 140	1.9	>0.05

*Significant

Table (3): Laboratory characteristics of the studied subjects regarding Renal function.

	Groups						F	Sig.
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
UREA (mg/dl)	88 ± 37	44 - 145	133 ± 38	81 - 214	32 ± 5	25 – 40	31.3	<0.001*
CREAT (mg/dl)	2.66 ± 1.03	1.5 - 4.7	9.5 ± .95	8.10 - 11.90	0.95 ± 0.13	0.70 - 1.10	424.8	<0.001*
eGFR (ml/min)	35.26 ± 14.79	17.44 - 63.3	9.71 ± 0.98	7.80 - 11.60	119.2 ± 4.78	106.9 - 123.3	436.7	<0.001*
Na^+ (mmol/l)	141 ± 3	135 -146	142 ± 3	135 - 146	140 ± 2	137 – 143	1.8	>0.05
K^+ (mmol/l)	4.5 ± .5	3.8 -5.3	4.8 ± .4	4.2 - 5.4	4.1 ± .4	3.5 - 4.8	11.69	<0.001*
Ca^{+2} (mg/dl)	7.4 ± .7	6 -8.4	9.1 ± .4	8.3 - 9.8	9.3 ± .5	8.5 - 10.1	58.98	<0.001*
PO_4 (mg/dl)	5 ± .6	4 - 6.3	5.4 ± .5	4.6 - 6.5	3.5 ± .5	2.9 - 4.1	42.6	<0.001*
S. uric acid (mg/dl)	5.7 ± 1.8	2.8 - 8.1	6.5 ± 1.4	4.6 - 9.1	5.7 ± .7	4.1 - 6.7	1.83	>0.05

*Significant

Table (4): Laboratory characteristics of the studied subjects regarding Lipid profile.

	Groups						F	Sig.
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
T. Choles (mg/dl)	211 ± 24	172 - 250	230 ± 37	180 - 300	181 ± 11	163 - 200	9.5	<0.001*
TG (mg/dl)	148 ± 18	120 -195	154 ± 17	130 - 180	129 ± 12	112 -147	7.4	<0.01*
LDL (mg/dl)	95 ± 22	59 -130	118 ± 24	66 - 149	101 ± 18	78 - 130	5.4	<0.01*
HDL (mg/dl)	43 ± 7	31 -58	44 ± 6	33 - 57	54 ± 6	44 - 63	10.4	<0.001*
T. Bil (mg/dl)	0.9 ± 0.2	.5 -1.2	0.8 ± 0.2	0.5 - 1.2	0.7 ±0 .3	0.4 - 1.2	1.6	>0.05

*Significant

Table (5): Laboratory characteristics of the studied subjects regarding Liver function.

	Groups						F	Sig.
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
ALT (u/L)	19 ± 6	12 -30	21 ± 9	11 - 39	19 ± 5	12 - 30	0.5	>0.05
AST (u/ L)	21 ± 8	12 -40	25 ± 11	12 - 45	18 ± 8	11 - 39	1.8	>0.05
ALP (u/L)	76 ± 21	45 - 110	106 ± 29	45 - 149	113 ± 22	79 - 145	10.6	<0.001*
Alb (g/dl)	3.8 ± .3	3.5 - 4.3	3.7 ± .4	3.0 - 4.5	4.2 ± .5	3.5 - 4.9	5.4	<0.01*
PT (sec)	19 ± 27	11 - 133	12 ± 1	11 - 14	12 ± 1	11 - 13	0.9	>0.05

*Significant

Table (6): Laboratory characteristics of the studied subjects regarding Fetuin – A.

	Group						F	Sig.
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range		
FETA (ng/ml)	489 ± 74	390 - 620	376 ± 50	220 -560	498 ± 58	400 - 580	10.8	<0.001*

*Significant

Table (7): No and % distribution of subjects with arterial stiffness as estimated by S. fetuin A in all studied groups.

	Group						X^2	p-value
	Conservative (n=20)		Hemodialysis (n=20)		Control (n=10)			
	No.	%	No.	%	No.	%		
Abnormal FETA ≤ 475	11	55.0%	16	80.0%	3	30.0%	2.85	>0.05

***** Significant

Table (8): clinical characteristic of patients (G I & G II) with arterial stiffness as estimated by s. fetuin-A level.

	Group				t-test	Sig.
	Conservative n= (16)		Hemodialysis n = (11)			
	$\overline{X} \pm SD$	Range	$\overline{X} \pm SD$	Range		
Age /years	49.9±9.7	24.0- 63.0	44.8± 5.8	33.0 -54.0	1.691	0.103
Sex: **- Male**	8	50.0%	7	63.6%	0.491	0.484
- Female	8	50.0%	4	36.4%		
Weight/ kg	79.8 ± 9.5	65.0 - 95.0	74.4 ± 10.0	57.0 - 95.0	0.744	.466
Diabetic	6	37.5%	3	27.3%	0.307	.580
Hypertensive	7	43.8%	10	90.9%	6.217	.013*
Disease duration	6.08±1.38	4.00 - 9.00	3.09 ± .83	2.00 -5.00	6.520	<0.001**

* Significant ** highly significant

Table (9): Correlation coefficient between serum fetuin –A and lab. characteristic in conservative group with and without arterial stiffness.

serum fetuin –A lab. Characteristic	Conservative (G=I)			
	Without stiffness (n= 4)		With stiffness (n= 16)	
	r	Sig	R	Sig. (2-tailed)
Hb (g/dl)	0.44	0.71	0.07	0.80
HCT (%)	0.33	0.79	0.17	0.51
Platelets	0.19	0.88	-0.16	0.54
WBCs	0.85	0.35	0.10	0.69
FBS (mg/dl)	0.89	0.31	-0.31	0.23
2hr PPBS (mg/dl)	0.79	0.42	-0.39	0.12
UREA (mg/dl)	0.74	0.47	-0.73	0.001*
CREAT (mg/dl)	0.62	0.58	-0.81	<0.001*
eGFR (ml/min)	-.25	0.84	0.79	<0.001*
Na^+ (mmol/L)	1.00	<0.001*	0.45	0.07
K^+ (mmol/L)	0.98	0.12	-0.37	0.15
Ca^{+2} (mg/dl)	0.87	0.33	-0.25	0.34
PO_4 (mg/dl)	-0.60	0.59	-0.27	0.30
S. uric acid (mg/dl)	-0.14	0.91	0.23	0.38
T. CHOLEST (mg/dl)	-0.66	0.54	-0.02	0.94
T.G. (mg/dl)	-0.95	0.19	-0.12	0.66
T.BIL (mg/dl)	0.50	0.67	-0.08	0.78
ALT (u/L)	-0.87	0.33	0.09	0.73
AST (u/L)	-1.00	0.03*	-0.06	0.82
ALP (u/L)	0.89	0.30	-0.26	0.31
ALB (g/L)	-0.69	0.51	-0.17	0.53
Pt (sec)	0	1	-0.14	0.59

* Significant

Table (10): Correlation coefficient between serum fetuin–A and lab. characteristic in hemodialysis group (II) with arterial stiffness.

serum fetuin –A lab. characteristic	H.D. (G II) (no=20) With arterial stiffness	
	R	Sig. (2-tailed)
Hb (g/dl)	0.24	0.33
HCT (%)	-0.21	0.38
Platelets	0.13	0.59
WBCs	-0.22	0.37
FBS (mg/dl)	-0.02	0.93
2hr PPBS (mg/dl)	-0.06	0.81
UREA (mg/dl)	-0.40	0.09
CREAT (mg/dl)	-0.38	0.11
eGFR (ml/min)	0.02	0.95
Na^+ (mmol/L)	-0.29	0.23
K^+ (mmol/L)	-0.07	0.77
Ca^{+2} (mg/dl)	-0.47	0.04*
PO_4 (mg/dl)	0.17	0.49
S.uric acid (mg/dl)	0.18	0.46
T. CHOLEST (mg/dl)	-0.66	0.002*
TG (mg/dl)	-0.51	0.03*
TBIL (mg/dl)	-0.26	0.29
ALT (u/L)	-0.41	0.08
AST (u/L)	-0.44	0.06
ALP (u/L)	0.41	0.08
ALB (g/L)	-0.16	0.51
PT (sec)	-0.17	0.50

* Significant