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RELATIONSHIP OF FETUIN - A AND CORONARY ARTERY CALCIFICATION IN HEMODIALYSIS PATIENTS

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ABSTRACT

Extraosseous calcification is an almost inevitable process in patients with end stage renal disease (ESRD). Fetuin-A is a hepatocyte-derived serum protein and potent systemic inhibitor of calcification and a negative acute phase reactant. The study was conducted in the nephrology unit, dialysis department/ Baghdad teaching hospital. A total numbers of 60 patients with end stage renal disease already on maintenance hemodialysis and control 30 cases which have normal kidney function, they were examined for serum Fetuin A and Coronary artery calcium scoring (CACS) were performed by a 64-slice CT scan. The total calcium scores of all patients and control cases were calculated using dedicated software. Fetuin A has been tested by quantitative solid phase enzyme immunometric assay (ELISA) designed for the determination of Fetuin A in human serum, using DiaMetra kit. This case control study that enrolled 60 patients with end stage renal disease already on maintenance hemodialysis were included in the study, 25 males and 35 females. The prevalence of hypertension, diabetes mellitus, anemia and low serum albumin were more among studied patients as compared to control group. There was low serum Fetuin A level in patients with chronic renal failure on maintenance hemodialysis as compared to control group, the difference were statistically significant, ($p<0.0001$). There were also low serum Fetuin A level in studies patients with chronic renal failure on maintenance hemodialysis with higher coronary artery calcium score 11-400 and >400 as compared to control group, the difference were statistically significant, ($p<0.0001$). This is signified that the decrement in serum Fetuin A level lead to more calcification in coronary arteries. Negative relationship between Fetuin-A levels and total coronary artery calcification scores. Fetuin A level is decreased in patients with chronic renal failure with cardiovascular risk factors as male, age older than 55 years, hypertension, diabetes and anemia.

KEYWORDS: End Stage Renal Disease (Esrd), Solid Phase Enzyme Immunometric Assay (Elisa) and Prevalence of Hypertension

INTRODUCTION

Fetuin-A is a hepatocyte-derived serum protein (molecular weight, ~60 kD) and potent systemic inhibitor of calcification and a negative acute phase reactant. Serum concentrations are relatively high with levels in average populations. Fetuin-A is an inhibition of calcification, limiting hydroxyapatite crystal formation.[7] Fetuin-A can exert its effect by various mechanisms[8]:1)Fetuin-A represents an integral part of a highly efficient clearing mechanism for small mineral complexes.[2] 2)Intracellular fetuin-A inhibits apoptosis of vascular smooth muscle cells.[3,4] 3)It inhibits the calcification-inducing effects of transforming growth factor-band bone morphogenetic protein-2.[3,4]Extraosseous

calcification is an almost inevitable process in patients with end stage renal disease (ESRD). Coronary calcium is a sensitive marker of underlying atherosclerotic disease [5,6] and coronary calcification is positively correlated with atherosclerotic plaque burden, both calcified and non-calcified,[7] increased risk of myocardial infarction [8] and of cardiovascular events in renal disease. [9] The coronary artery calcium score has been used for risk stratification in patients with known or suspected coronary artery disease (CAD) [10] and, according to published guidelines, it can help in identifying asymptomatic patients at low-to-intermediate risk, who may benefit from more aggressive risk factor modification.[11]

The goal of our study that to analyze the relationship of Fetuin-A and coronary artery calcification score in patients with chronic renal failure on mainStenance hemodialysis.

RESULTS

This study that enrolled 60 patients with end stage renal disease already on maintenance hemodialysis were included in the study, 25 males and 35 females and their age range from 29 to 70 years old, mean age: 52±12 SD years with male to female ratio 0.7:1. Also the study enrolled 30 control cases 12 males and 18 females and their age range from 30 to 75 years old, mean age: 51±10.5 SD years.

Table 1: Demographic Characteristics of Patients and Control Groups

Parameter	Patients N=60 (%)*	Control N=30(%)*	P€
Male	25(41.7)	12(40)	
Female	35(58.3)	18(60)	0.88
Age≤ 55 yr	6(10)	18(60)	
Age>55yr	54(90)	13(43.3)	<0.0001
Hypertension	55(91.7)	20(66.7)	0.003
Diabetes mellitus	25(41.7)	13(43.3)	1
Anemia	58(96.7)	16(53.3)	<0.0001
Low serum albumin	55(91.7)	0(0)	0.001
Hypercholesterolemia	15(25)	28(93.3)	<0.0001
Hypertriglyceridemia	25(41.7)	30(100)	<0.0001
Low serum Fetuin A	45(75)	1(3.3)	<0.0001

€ Significant using Pearson chi-square test at 0.05 level of significance.

***** The percentage in result tables were considered for column rather than row.

Table 1 shows that there were statistically significant difference among studied patients with older than 55 years as compared with younger or equal to 55 years. There were no statistically significant difference among studied patients between males and females as compared to control group. Also it shows that hypertension and anemia were more among studied patients as compared to control group, the difference were statistically significant. The hypercholesterolemia and hypertriglyceridemia were higher in the control group than in the studied patients, the difference was statistically significant. Diabetes showed no significant difference between case and control groups, as shown in table1.

Table 2: Distribution of Coronary Calcium Calcification Score in Studied Patients with Chronic Renal Failure and Fetuin A Level

Calcium Score	Low Fetuin A N=46 (%)*	Normal sFetuin A N=44(%)*	P€
0-10	6(13)	24(54.5)	
11-400	25(54.3)	7(15.9)	

Table 2 Contd.			
>400	15(32.6)	13(29.5)	
			<0.0001

€ Significant using Pearson chi-square test at 0.05 level of significance.

Table 3: Serum Fetuin A Level Distribution between Case and Control Groups

Variables	Patients N=60(%)*	Control N=30(%)*	P€
Low Fetuin A	45(75)	1(3.3)	
Normal Fetuin A	15(25)	29(96.7)	
			<0.0001

€ Significant using Pearson chi-square test at 0.05 level of significance.

Table 4: Risk Factors and Chronic Kidney Disease Complications Distribution and Serum Fetuin A Level

Parameter	Low Fetuin A N=46 (%)*	Normal Fetuin A N=44(%)*	P€
Male	26(56.5)	11(25)	
Female	20(43.5)	33(75)	0.02
Age≤ 55 yr Age>55yr	7(15.2) 39(84.8)	16(36.4) 28(36.6)	 0.021
Hypertension	46(100)	29(65.9)	<0.0001
Diabetes mellitus	26(56.5)	12(27.3)	0.005
Anemia	46(100)	28(63.6)	<0.0001
Low serum albumin	46(100)	39(88.6)	0.019
Hypercholesterolemia Hypertriglyceridemia	16(34.8) 36(78.3)	27(61.4) 29(65.9)	0.012 0.19

€ Significant using Pearson chi-square test at 0.05 level of significance.

***** The percentage in result tables were considered for column rather than row.

The hypertension, anemia, hypocalcaemia, hyperphosphatemia, high calcium phosphate product and high parathyroid hormone were more among studied patients with end stage renal disease already on maintenance hemodialysis as compared to control group, the difference were statistically significant, (p=0.003, p<0.0001, p<0.0001, p<0.0001, p<0.0001, p<0.0001 respectively).

DISCUSSIONS

This case control study that enrolled 60 patients with end stage renal disease already on maintenance hemodialysis, 25 males and 35 females and their age range from 15 to 70 years old, with male to female ratio 0.7:1. Control cases that enrolled 30 cases were considered from medical and cardiologic outpatients clinic (12 males& 18 females), with consideration to be matched for age, gender and to cancel the effect of confounding factors, not suffering from renal failure.

There were statistically significant difference among studied patients with older than 55 years as compared with younger or equal to 55 years, (p<0.0001). There were no statistically significant difference among studied patients between males and females as compared to control group, (p=0.88).

These results were consistent with Turkmen K. et al[12] study who showed that patient older than 55 years had high risk of chronic renal failure,statistically significance (p=0.001). Also the same study[12] showed no statistical difference between male and female. These could be explained by the aging is a slow, inflammatory biologic process that affects

many organs, of which the kidney is one of the main targets. Aging is associated with a decline in renal function coincident with a progressive loss of nephrons, with glomerular and tubulointerstitial scarring. These changes begin in the fourth decade of life and accelerate between the fifth and sixth decades, resulting in alterations in glomerular and tubular function, systemic hemodynamics, and body homeostasis. [13]

The hypertension, anemia, hypocalcaemia, hyperphosphatemia, high calcium phosphate product and high parathyroid hormone were more among studied patients with end stage renal disease already on maintenance hemodialysis as compared to control group, the difference were statistically significant, ($p=0.003$, $p<0.0001$, $p<0.0001$, $p<0.0001$, $p<0.0001$, $p<0.0001$ respectively).

Hypertension is common in chronic kidney disease patients could be explained primarily by volume dependent, hyperactive renin-angiotensin system, hyperactivity of the sympathetic nervous system. This consists with Peco et al [14] study.

Renal anemia is also common in chronic kidney disease patients could be explained primarily by It is mostly due to erythropoietin deficiency, inhibition of erythropoiesis by uremic solutes, and reduction in red blood cell life span. This consistent with Ansari et al [15] study.

The hypocalcaemia, hyperphosphatemia, high calcium phosphate product and high parathyroid hormone which were related to the uremic state affect the skeleton and result in the complex disorders of bone known as renal osteodystrophy. Total serum calcium tends to decrease during the course of CKD as a result of phosphate retention and decreased production of 1,25-dihydroxyvitamin D (calcitriol) from the kidney, decreased intestinal calcium absorption, and skeletal resistance to the calcemic action of PTH, but the levels of free calcium remain within the normal range in most patients as a result of compensatory hyperparathyroidism. Because calcium is a major regulator of PTH secretion, persistent hypocalcemia is a powerful stimulus for the development of hyperparathyroidism and also contributes to parathyroid growth. This consistent with Valkovsky et al [15] study.

The low serum Fetuin A level in patients with chronic renal failure on maintenance hemodialysis as compared to control group, the difference were statistically significant, ($p<0.0001$). This could be explained by Fetuin-A concentration in systemic circulation may be reduced during inflammation in hemodialysis patients meaning that it is a negative acute phase protein.

The low serum Fetuin A level in studies patients with chronic renal failure on maintenance hemodialysis with higher coronary artery calcium score 11-400 and >400 as compared to control group, the difference were statistically significant, ($p<0.0001$). This is signified that the decrement in serum Fetuin A level lead to more calcification in coronary arteries. These results were consistent with Turkmen K. et al[12] study with statistically significant ($p= <0.001$) and other studies. [17, 18] This signified that Fetuin-A may be inhibitor of calcification and a negative acute phase reactant.[7]

There were decreased serum Fetuin A level in studies patients who were male, age older than 55 years, hypertension, diabetes, anemia, hypocalcaemia, hyperphosphatemia, high calcium phosphate product, hypercholesterolemia, high parathyroid hormone, and low serum albumin as compared to normal level of serum Fetuin A, the difference were statistically significant. This is signified that the decrement in serum Fetuin A level more in some cardiovascular risk factors and chronic kidney disease complications. These results were consistent with other studies. [12, 17, 18]

These could be explained by following:

- Age is the most powerful independent risk factor for atherosclerosis. Pre-menopausal women have lower rates of disease than men, although this sex difference disappears after the menopause.[19]
- The incidence of atherosclerosis increases as BP rises, and this excess risk is related to both systolic and diastolic BP, as well as pulse pressure. Antihypertensive therapy reduces cardiovascular mortality, stroke and heart failure. [19]
- Diabetes mellitus: This is a potent risk factor for all forms of atherosclerosis and is often associated with diffuse disease that is difficult to treat. Insulin resistance (normal glucose homeostasis with high levels of insulin) is associated with obesity and physical inactivity, and is a risk factor for coronary artery disease. [19]
- The risk raises of ischemic heart disease with increasing serum cholesterol concentrations and forming atheroma which become lipid-laden macrophages or foam cells lead to atherosclerotic plaque that will remain asymptomatic until it becomes large enough to obstruct arterial flow. [19]
- The physiologic consequences of long-standing anemia are an increase in cardiac output and a reduction in peripheral vascular resistance. Anemia is a risk factor for the development of left ventricular hypertrophy in CKD patients and thought to exacerbate left ventricular dilation. Sustained correction of anemia in CKD patients results in a reversal of most of these cardiovascular abnormalities, with the notable exception of left ventricular dilation. Once the left ventricle is stretched beyond the limits of its elasticity, correction of anemia cannot reverse this.[20]
- The abnormalities in mineral metabolism in uremia that predispose to vascular and soft tissue calcification, but no single abnormality is sufficient to predict the development of this disorder. Elevated levels of parathyroid hormone (PTH) have been associated with an increased risk of vascular and soft tissue calcification. A perturbation of the calcium and phosphate homeostasis most probably underlies the positive association. Insufficient activation or expression of inhibitors of calcification should also be considered in the pathogenesis. Inhibitors of vascular calcification include Fetuin-A may be decline with inflammation. Probable risk factors include low serum albumin concentrations, the use of calcium salts and vitamin D analogues lead to increased risk of vascular and soft tissue calcification. [21]

CONCISE METHODS

The study was conducted in the nephrology unit, dialysis department/ Baghdad teaching hospital. A hospital based single center case control study were approved by Arab board ethical committees, and the period of data collection was one started from February 2013 to the end of March 2014. Hemodialysis (HD) modality includes conventional 3-4 h HD, two- three times a week with polysulfone dialysers. A 250 ml/min (range 200-300 ml/min) of mean blood flow rate was obtained during dialysis sessions. Dialysate fluid composition includes 140 mmol/l of sodium, 2 mmol/l of potassium, 1.5 mmol/l of calcium, 0.5 mmol/l of magnesium, and 35 mmol/l of bicarbonate.

All recruited patients had their ages, gender and case histories recorded on an already prepared data sheet.

Control cases were considered from medical outpatients clinic (30 cases, 12 males& 18 females), with consideration to be matched for age, gender and to cancel the effect of confounding factors, with normal renal function tests. The sixty patients and thirty control cases were examined for serum Fetuin A and Coronary artery calcium scoring

(CACS) were performed by a 64-slice CT scan (*Brilliance* 64, *Philips Medical* Systems, *Holand*). CACS were calculated by as described by Agatston et al.,[22] calcification was defined as a minimum of two adjacent pixels (>0.52 mm^2) with a density over 130 Hounsfield units. The peak intensity (in Hounsfield unit) and area (in square millimeter) of the individual calcifications were calculated. Scores were obtained by multiplying each area of interest by a factor indicating peak density within the individual area. Image quality and scoring accuracy were assessed by one radiologist who carefully made vessel-by vessel and calcific focus-by-calcific focus inspections of each image. The total calcium scores of all patients were calculated using dedicated software.

Coronary Artery Calcification Score (CACS): In adult population, Agatston calcium scores stratify risk for a cardiovascular event [23] and appear to better predict the risk for future coronary events than age/gender-specific percentile ranking. [24,25]Patients are divided into three groups according to total CACS value with risk stratification for presence of coronary atherosclerosis ;

Group 1: 0-10 Low risk

Group 2: 11-400 Intermediate risk

Group 3: >400 highest risk

Material: Fetuin A has been tested by quantitative solid phase enzyme immunometric assay (ELISA) designed for the determination of Fetuin A in human serum, using DiaMetra kit. Fetuin A is considered normal if the serum level more 45 ng/ml and consider abnormally low serum Fetuin A if the level less than 45 ng/ml.

Statistics: Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20 Statistics) for determination of statistical significance among different variables. A descriptive statistics like mean together with analytic statistics, have been done when appropriate. A p-value of less than 0.05 was considered as significant and calculated by method of Pearson Chi square equation. The percentage in result tables were considered for column rather than row.

CONCLUSIONS

1. Fetuin-A levels is robust marker of vascular calcification in hemodialysis patients.
2. Negative relationship between Fetuin-A levels and total coronary artery calcification scores.
3. Fetuin A level is decreased in hemodialysis patients with cardiovascular risk factors as male , age older than 55 years , hypertension ,diabetes and anemia.
4. Fetuin A level is decreased in hemodialysis patients with hypocalcaemia, hyperphosphatemia, high calcium phosphate product and high parathyroid hormone which were related to the uremic state affect the skeleton and result in renal osteodystrophy.

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DISCLOSURES

There is no disclosure.

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