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# Role of α 2- Heremans and Schmid glycoproteins (α 2-HSG) as a threshold concept of calcification in patients with Chronic Kidney Disease

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***ABSTRACT**--Fetuin A (FA, AHSG or α 2-HS glycoprotein) is a glycoprotein mainly produced by liver. FA can inhibit the de novo formation and precipitation of the apatite precursor mineral (namely calcium phosphate). Therefore, FA can prevent undesirable calcification in the circulation without inhibiting bone mineralization.Cardiovascular disease (CVD) in chronic kidney disease (CKD) is explained in part by traditional cardiovascular risk factors; by uremia-specific factors, abnormalities of mineral metabolism, and the vascular calcification process. Lower serum concentrations of FA was independently associated with risk of cardiovascular. The main aims of the study were to follow the role of FA in the progression of renal disease which promoted cardiovascular disease. Improving the understanding of the mechanisms of action might undoubtedly lead to well therapies to combat the destructive effects in CKD.The present work included a cross sectional study for a group of 80 subject patients with end stage chronic kidney disease were selected from hemodialysis unit at the Nephrology Department, Al Hussein Teaching medical city. Blood samples were collected from dialysis unit from fistule pre heparinized, pre dialysed patients. Enzyme Link Immunosorbat Assay system (ELISA) was performed using Sandawich method to measure the concentrations of serum Human Fetuin A and Parathyriod hormone (PTH). Significant differences in continuous variables among the parameters were confirmed through analytical statistical tests.Associations of characteristic parameters with serum Fetuin A levels were shown high significant difference specially with Creatinine, Urea, K, increased levels of these parameters were significantly associated with decreased FA levels (P<0.001), while ; in spite of the normal range of (Na, Ca, P and Albumin) levels, there was a significantly associated with decreased FA levels (P<0.001). Analysis of correlation illustrated that FA has a positive relationship with Hb, serum Albumin levels and serum K levels (P<0.001), while inverse and significant correlations of FA was manifested with serum Urea, Creatinine , Phosphorous l, Na, Ca levels, all have (P<0.001).In CKD patients, FA levels were decreased. Serum concentrations of FA were decreased in patients with end stage renal disease on dialysis, and lower serum concentrations were independently associated with risk of cardiovascular (vascular calcification) which increase the mortality in this population, fetuin A was strongly independent prognostic factor of the severity of CVD.*

***Key words**—Fetuin, CKD, CVD.*

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# I. INTRODUCTION

The kidney is a structurally complex organ that has evolved to carry out a number of important functions: excretion of the waste products of metabolism, regulation of body water and salt, maintenance of acid balance, and secretion of a variety of hormones and prostaglandins.

Acute kidney injury refers to abrupt onset of renal dysfunction characterized by an acute increase in serum creatinine often associated with oliguria or anuria (decreased or no urine flow). It can result from glomerular injury (such as rapidly progressive Glomerular Nephritis (GN), interstitial injury, vascular injury (such as thrombotic microangiopathy), or acute tubular epithelial cell injury.

Chronic kidney disease results from progressive scarring in the kidney of any cause. It is characterized by various metabolic and electrolyte abnormalities such as hyperphosphatemia, dyslipidemia, and metabolic acidosis. However, it is often asymptomatic until the most advanced stages, when symptoms of uremia develop.

• End-stage renal disease (ESRD) is irreversible loss of renal function requiring dialysis or transplantation typically due to severe progressive scarring in the kidney from any cause. As progressive renal damage destroys more and more nephrons, adaptive mechanisms are initated that try to maintain renal function. Among such adaptive mechanisms, glomerular hyperfiltration tends to compensate for decreased glomerular filtration rate resulting from the loss of nephrons. An increase in the rate of excretion of solutes per nephron via increased plasma concentrations (for creatinine), decreased tubular reabsorption (for sodium, phosphate, and calcium), or increased tubular secretion (for potassium and hydrogen ions) helps to maintain homeostasis until the late stages of chronic kidney disease. The rate of functional decline varies based on the original disease; however, renal function often deteriorates progressively even when the original insult is controlled. Uncontrolled hypertension, regardless of the etiology, results in more rapid renal functional decline. (1).

Serum Fetuin A (FA) is an important regulator of extracellular matrix mineralization. FA plays a critical role in the formation and stabilization of high molecular weight colloidal protein–mineral complexes known as calciprotein particles. Inflammation appears to be associated with mineral stress even in the absence of renal dysfunction. Patients with calcific uraemic arteriolopathy on haemodialysis have very high serum FA reduction ratios, suggesting that this measurement may have a prognostic/diagnostic role in this condition, since FA is a magic inhibitor highly related to be protective against calcification. (2).

Importance of study and the Knowledge gap were regarding the Calcification which is a common problem in many physiologic and pathologic conditions, i.e., aging, diabetes, dyslipidemia, genetic diseases, and diseases with disturbances of calcium metabolism. In chronic kidney disease, vascular calcification is even more common, develops early, and contributes to the markedly increased cardiovascular risk in such a particular population. This study was design to increasing knowledge about calcification. FA is one of the inhibitors of calcification whose level is lowered in patients with CKD. The aim of this work was to study the association between fetuin-A level in end stage patients with CKD.

The extent of atherosclerosis and arteriosclerosis is undoubtedly exceedingly high in patients with CKD and the consequences (cardiovascular events) represent a major clinical problem in these patients. Experimental

findings confirmed an acceleration of atherosclerosis, which seems to starts very early in the course of CKD and is characterized by marked medial and intimal calcification. The types of calcification can occur independent of each other with media calcification already being present in early stages of CKD and show some differences in pathogenesis and clinical outcome. Although in advanced stages of CKD different types of calcification may often be present (3).

Therefore, as a knowledge gap, further understanding of the mechanism by which vascular calcification occurs should offer a potential hope of developing therapeutic strategies to arrest this process. Also it might be a good idea to look for some relation between FA level and other marked biochemical changes in serum patients of end stage CKD. Lastly, a full understanding of the principle and roles of the potential circulating inhibitors (FA) might result in novel strategies aimed at the prevention and/or reversal of the life-limiting calcifying vasculopathies seen in CKD patients.

This work was aimed to examine the levels of FA as a marker related to a higher risk of cardiovascular diseases in chronic kidney disease which might result from ectopic calcification. Also the study was studied the relation and association of FA levels and other abnormalities of minerals such as Calcium and Phosphorus.

## II. MATERIALS AND METHODS

The present work included a cross sectional study for a group of 80 subject patients with end stage chronic kidney disease were selected from nephrology wards, outpatients clinics and the hemodialysis unit at the Nephrology Department, Al Hussein Teaching medical city. History of family, blood pressure, smokeing state, weights and heights were taken from each patient. For relationship purposes, patients were divided into diabetic/ Non diabetics, Hypertensive/Non hypertensive, smoker / non smokers, duration of dialysis and grouped by age. Enzyme Link Immunosorbat Assay system ( ELISA) was performed using Sandawich method to measure the concentrations of serum Human Fetuin A (4) data of quantitation renal function were collected directly form weekly routine tests of pre-dialysed patients.

## III. RESULT AND DISCUSSION

Serum FA measurement was performed on sera of 80 symptomatic end stage chronic kidney disease cases. All adult patients were ongoing dialysis session three times a week. Pateints have presented hemodialysis unit at the Nephrology Department, Al Hussein Teaching medical city. The demographic and clinical characteristics were listed in Table (1).

**Table 1:** Patients demographic and clinical characteristics..

| *Parameters* | | *Mean* |
|---|---|---|
| *Demographics* | *Age* | *52 (years)* |
| | *Gender (Male/Female)* | *(47/33)* |

| | | |
|---|---|---|
| *Medical history* | *Hypertension ( Yes/ No)* | *(62/18)* |
| | *Diabetics Mellitus II (Yes/ No)* | *(35/45)* |
| | *Smoking (Yes/ No)* | *(22/56)* |
| *Materials of Dialysis Membrane* | *Membrane area(m2)* | *1.7* |
| | *Wall thickness µm* | *50* |
| | *Polyflux* | *170H* |
| | *Inner diameter µm* | *215* |

| *Measurments* | *Median* | *95% CI (Lower-Upper)* |
|---|---|---|
| *BMI (Kg/m2)* | *24.41* | *1.40 (23.58-26.39)* |
| *FA (ng/mL)* | *94.22* | *28.90 (131.03-188.90)* |
| *Haemoglubin (g/dL)* | *8.5* | *0.38 (8.22-8.97)* |
| *Albumin (g/L)* | *39* | *1.55 (37.45-40.55)* |
| *Urea (mg/dL)* | *138* | *9.02 (125.96-144.01)* |
| *Creatinin (mg/dL)* | *6.4* | *4.92 (4.18-14.02)* |
| *K (mmol/L)* | *5.1* | *3.31 (3.67-10.28)* |
| *Na (mmol/L)* | *138* | *5.61 (126.77-137.99)* |
| *Ca (mg/dL)* | *2.5* | *0.072 (2.42- 2.56)* |
| *phosphorous (mg/dL)* | *6.1* | *0.36 (5.66-6.37)* |

Chronic kidney disease might result from traditional and non-traditional causes. The traditional include: diabetes mellitus, hypertension and metabolic syndrome while the nontraditional cuase might be chronic inflammation, hyperphosphatemia, hypercaliciemia, hyperkalimeia and hyperhomocysteinemia, increased the oxidative stress and many others. Importantly, the rate of functional decline varies based on the orginal disease (5).

Although, increased serum creatinine was associated with renal disease but, it is often to be asymptomatic until a developed uremia stage. Also, it has poor sensitivity and specificity to detect / grade the severity of acute kidney injury (6).

Therefore, FA might be a good independent prognostic **factor** of the severity of CVD since it was reported that decreased levels of fetuin A occur in all ages and all stages of CKD (5).

***Associations of characteristic parameters with serum Fetuin A levels: Correlation of Age and serum Fetuin A levels:***

The average age of patients dialysis was 52 years (range 10 to 79), figure (1) illustrated the distribution of age which showed no significant difference of FA levels across the patients age groups (p=0.67).

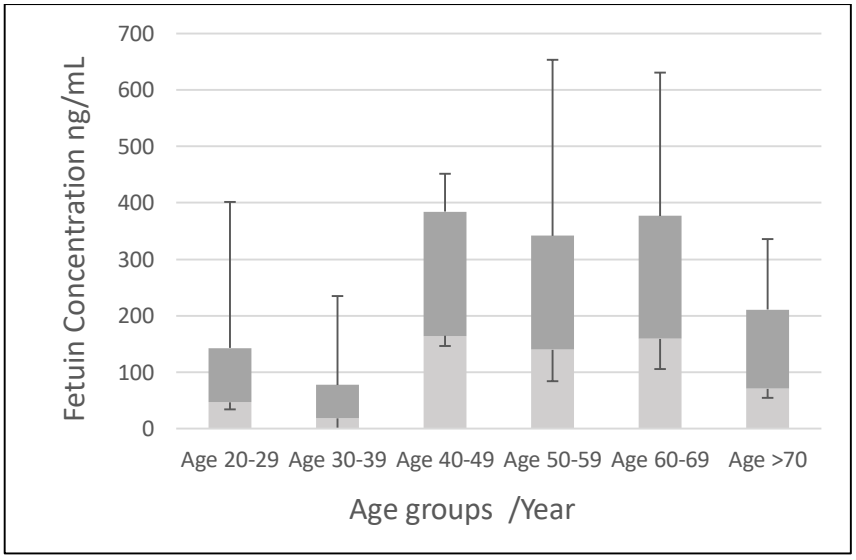


**Figure 1:** Distribution of Fetuin A concentration( ng/ mL) in different age groups patients of CKD.

These results were supported by the founding of (7). Based on their proposed mechanism regarding the general role of FA which included Vascular smooth musule cells (VSMCs) can take up serum FA and pool it in intracellular membrane-bound matrix vesicles. As previously stated, these vesicles are released from VSMCs and become the nidus for mineral nucleation. These released vesicles have abundant FA and abrogate the ability of regular membrane-bound matrix vesicles to form hydroxyapatite crystal. The uptake of FA by VSMCs is also induced by extracellular Calcium but not by extracellular Phosphorus This FA uptake increases the amount of Calcium entering VSMCs and is mediated by annexin Calcium channel activity, facilitating its inhibitory role in VSMC mineralization. Thus, it is not unusual to see CVD in very young hemodialysis patients. (7). Since its independent predictors for extent CVD. (8). Therefore, the study suggested that in a relatively young population with some alterations in renal function and less traditional cardiovascular risk factors a small modifications in serum FA levels might appear early in the course of disease evolution and consider as an independent major predictors for CVD extent since it was confirmed before for young population with mild-to-moderate decreasing in renal function (8).

## IV. CORRELATION OF GENDER AND SERUM FETUIN A LEVELS

The study consisted of thirty three females (41%) and forty seven males (59%) patients. The FA levels of male patients (mean 162, SD 135.21) was hypothesis to be greater than the FA levels of females patients (mean 157, SD 123.92 ).

In spite of that the mean of FA levels were greater in male patients than females, but, no significant differences were found, P value was (0.42). Similar finding suggested that Estradiol (E2) might affect the synthesis of FA by alteration their levels after estrogen therapy and menopause. (9), (10).

## V. CORRELATION BETWEEN DURATION OF DIALYSIS AND SERUM FETUIN A LEVELS

There was no significant difference in the duration of dialysis among studied patients (duration range <1 to >5 years) (since when the patients first had received dialysis) as shown in figure (2) and the FA levels (p=0.82).

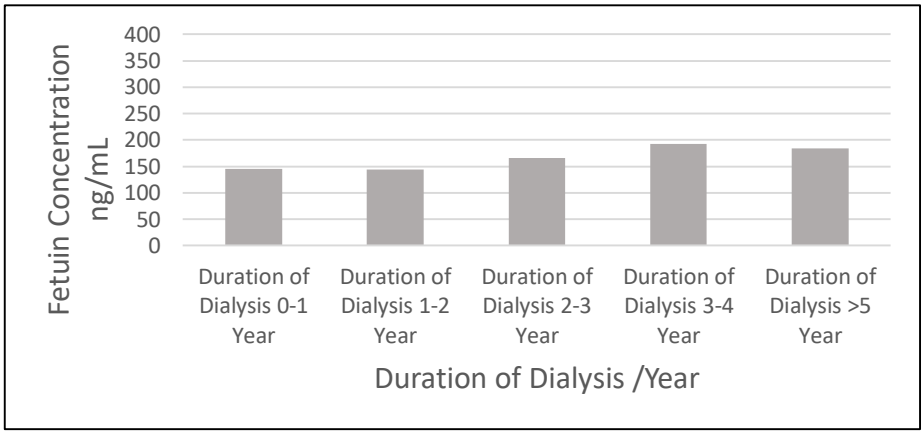


**Figure 2:** Comparison of Fetuin A concentration ( ng/ mL) in Duration of Dialysis groups patients of CKD

Previous studies (10), (11). showed that low level of serum FA were observed relatively in early CKD. This observation indicates that calcification were likely to develop. These results were highly correlated to the significant relationship between serum FA level and endothelial dysfunction .

Also, the effect of duration of dialysis in CKD patients on FA level was based on the net of multiple pathological mechanisms included traditional and non-traditional factors such as chronic inflammation, increased oxidative stress, anemia and malnutrition. (11). Morever, other study reported that the diffeciency of FA was relatively in most dialysis pateints and correlate the most decreased of FA with the high serum calcium and phosphate (12).

## VI. CORRELATION OF BMI AND SERUM FETUIN A LEVELS

Comparetion the mean of FA levels (ng/mL) was shown no significant difference across patients based on weight groups, pateints were underweight (mean 140.39), patients within normal weight (mean 141.55), patients with overweight (mean 179.25) and patients with obese (mean 179.97) (p=0.46) (Figure 3).

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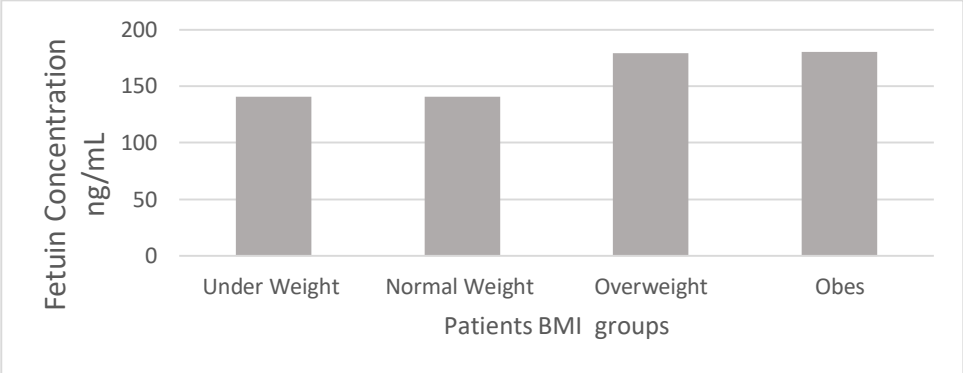


**Figure 3:** Comparison of Fetuin A concentration (ng/ mL) in different BMI groups patients of CKD.

Many studies were shown a inversely (positive) correlation between FA and increased BMI (13), (14) . These studies were observed that obsess CKD patients were benefit of their obesity by increasing the circulation FA levels since (15) confirmed the finding that FA is expressed and secreted by adipose tissue.

## VII. CORRELATION OF MEDICAL HISTORY AND SERUM FETUIN A LEVELS

Table (2) was indicated no significant difference in FA levels across different medical history were taken in the study, the P values were > 0.05.

**Table2:** statistical analysis for the mean concentration of FA across different medical history.

| FA | CKD Patients groups | | | | | |
|---|---|---|---|---|---|---|
| | DM* | Non- DM | HT* | Non-HT | Smoker | Non-smokers |
| Mean | 180.32 | 141.77 | 165.02 | 119.01 | 161.17 | 149.09 |
| P(T<=t) one-tail | 0.09 | | 0.08 | | 0.35 | |

*DM Diabetes Mellitus, HT Hypertensive patients

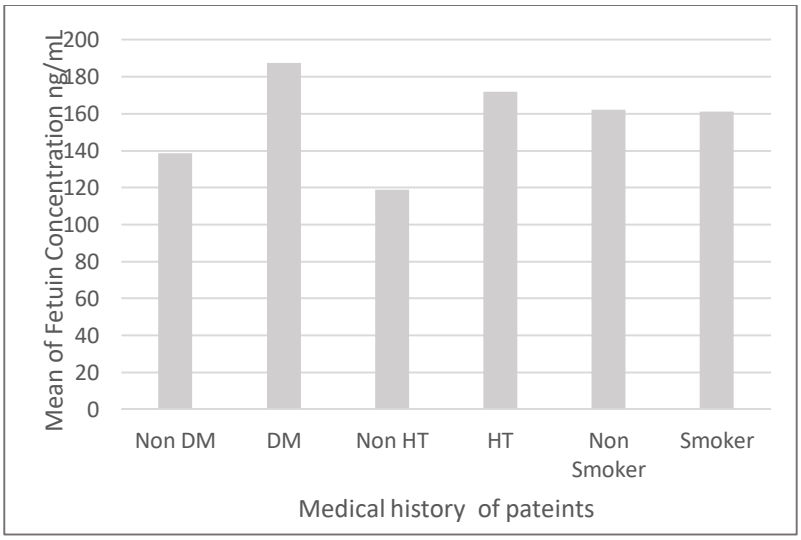


**Figure 4:** Comparison of Fetuin A concentration ( ng/ mL) based on patients medical history groups.

Figure (4) showed the mean of FA increased non singnificantly in DM, HT- CKD pateints compering to the mean of Non- DM, Non- HT CKD (see figure 4) through the role of FA as inhibitor to insulin receptor tyrosine kinase activity by bloking autophosphorylation of tyrosine kinase. (16)

## VIII. CORRELATION OF ELECTROLYES (K, NA) AND SERUM FETUIN A LEVELS

Increased levels of these parameters were significantly associated with decreased FA levels (P<0.001), while ; in spite of the normal range of (Na, Ca and Albumin) levels, there was a significantly associated with decreased FA levels (P<0.001). Simple regression analysis was carried out considering all clinical parameters as a dependent variable to FA levels to demonstrate whethere FA levels has an assessing to predict changes in theses parameters. The correlation coefficient and p value for all measured parameters were listed in the table (3)

**Table 3:** correlation analysis of the association between FA and the measured parameters.

| Parameters | FA | |
|---|---|---|
| | Correlation coefficient (r) | P(T<=t) two-tail |
| Haemoglubin (g/dL) | 0.12 | 1.67E-16 |
| Albumin (g/L) | 0.04 | 2.07E-12 |
| Urea (mg/dL) | -0.05 | 0.10 |
| Creatinin (mg/dL) | -0.11 | 1.89E-16 |
| K (mmol/L) | 0.16 | 1.03E-16 |
| Na (mmol/L) | -0.31 | 0.07 |

| | | |
|---|---|---|
| Ca (mg/dL) | -0.04 | 2.61E-17 |
| phosphorous (mg/dL) | -0.21 | 7.62E-17 |

Analysis of correlation illustrated that FA has a positive relationship with Hb, serum Albumin levels and serum K levels (P<0.001) .

The concentration **of K** showed a negative correlation with FA level, and Na concentration showed a positive correlation figure (5). These observations seem to be related to the case studied not specific to the FA levels.

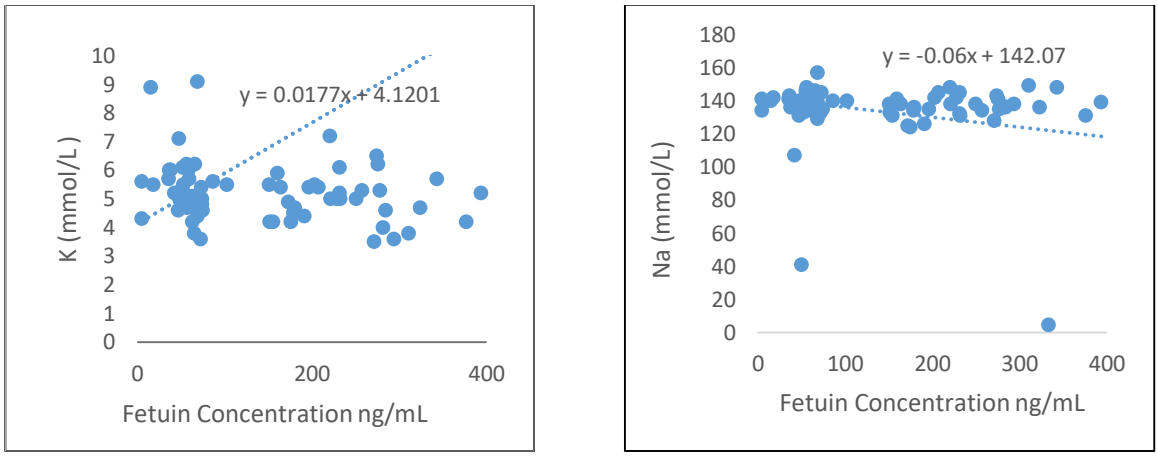


**Figure 5:** Simple correlation of the concentration of electrolytes vs. the concentration of FA (ng/ mL***)***

Potassium (K) and Sodium (Na) are the most abundant intracellular cations. Both ions have involved in membrane function such as a principle cations of extracellular and intracellular fluids. Since kidneys play a central role in the regulation of body fluids and electrolytes, measurments of electrolyes are a routine test for CKD pateints to control inter-dialytic solution and avoiding any further disorders which, in turn lead to serious complications such as hyperkalemia and hypernatremia. Results were showed a normal concencentration of Potassium and Sodium which indicated to the advantage of dialysis as effective therapeutic.

Positive correlation of serum FA was found also with albumin and heamoglubin levels in end stage chronic kidney disease patients as shown in figure (6).

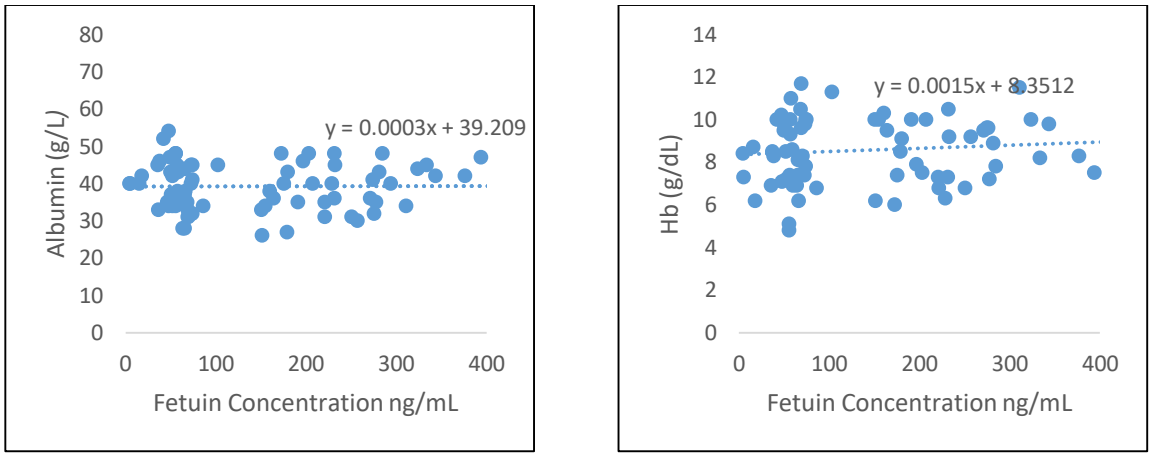


**Figure 6:** Simple regression of the concentration of Hb and Albumin vs. the concentration of FA (ng/ mL).

Serum Albumin levels were within normal range (see figure 8) results were supported by the hypothesis of (17) Which reported that "the extracellular fluid is a metastable system regarding the concentrations of Calcium and phosphate ions, Unlike serum albumin, FA does not preferentially bind ionic Calcium, but rather in the form of apatitic microcrystals, Hydroxyapatite (HAP) would eventually form at most calcification sites because at neutral pH and body temperature it is thermodynamically the most stable of all possible compounds of calcium and phosphate.

On the other hand, there was a significant positive associated of Hb with decreased FA levels (P<0.001) as shown in figure 6 (A). Low level of heamoglubin is one of the common complications of CKD which could relate to the decreasing of erythropoietin due to the renal dysfunction, therefore, all patients showed low level of Hb and a positive associated with FA.

Furthermore, inverse and significant correlations of FA was manifested with serum Phosphorous and Calcium levels, all have (P<0.001) as shown in Figures (7).

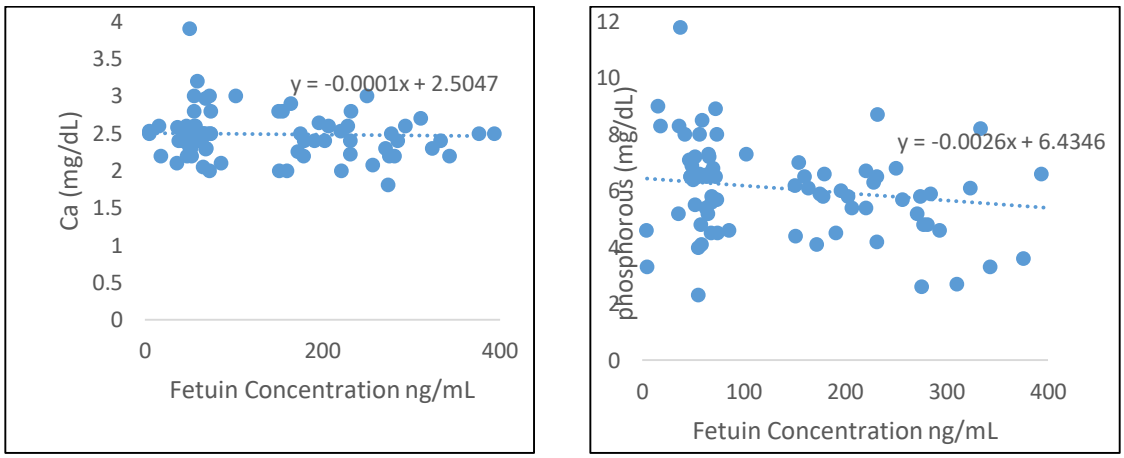


**Figure 7**: Simple regression of the concentration of Calcium mg/dL and Phosphorus mg/dL the concentration of FA (ng/ mL).

Calcium and phosphate are the principle ions involved in the deposition of mineral in the human body. Inhibitors of mineralisation are essential for the prevention of ectopic mineral precipitation and deposition.

On the other hand, a good theory for the biomimetic nucleation of calcium phosphate was reported by (18). The theory was answered the question about how the body could regulate the process of crystal growth in bone and prevent the formation of crystal comples in the extraosseous tissues particularly in disease conditions. Figure (9) was clearly showed the formation of both ions complex based on the adapted mechanism proposed by Habraken et al. (19)

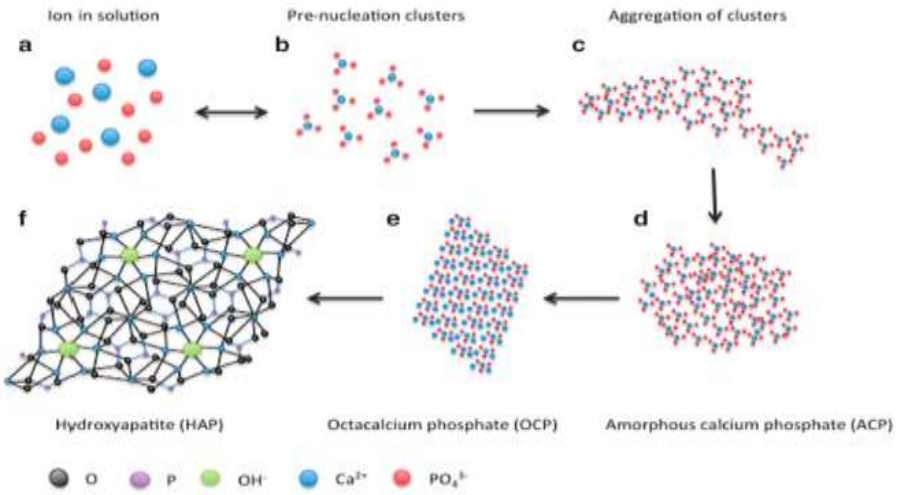


**Figure 9:** Proposed pathway of hydroxyapatite formation. Adapted from Habraken et al.

Generally, (CKD) is correlated with fatal cardiovascular consequences in part due to the extraosseous calcification of soft tissues particularly arteries, capillaries, and cardiac valves. In vivo, many experimental studies were suggested that this problem could result from the imbalance of hyperphosphatemia and increasing levels of stored intracellular and /or high circulation Ca-$PO_4$ complex. Such increasing might increase the consumption of the local inhibitors such as FA (11).

Statistical analysis of the results demonstrated that the mean values of both ions (Calcium, Phosphorus) were within the normal range (2.5, 6.0 mg/dL) but, the regression coefficient between Fetuin A and the concentration of (Calcium, Phosphorus) ions showed highly signifigant negative relationship with p value < 0.001 as illustrated in (Figure 9). The absence of Fetuin in patients with CKD could increase the susceptibility of increasing the morbidity due to the calcification.

Most area investigations are looking for further circulation proteins might have a role in the regulation of calcification either by homeostasis of the Calcium, Phosphorus levels or by directly maintenance the vascular cell functions.

Albumin, is one of the modulate Proteins that might be a good candidate to control the crystal formation by two ways:

Reducing the binding of free Calcium ions to the acidic amino acid residues. However, this process is relatively in-sufficient to prevent crystal precipitation based on the early fact that report due to the abundance, albumin accounts to has a half percent of the mineralisation inhibitory activity in serum (20).

## IX. CONCLUSION

In CKD patients, FA values were decreased as renal function declines. Serum concentrations of FA are further depressed in patients with ESRD on dialysis, and lower serum concentrations were independently associated with risk of cardiovascular and all-cause mortality in this population, FA remained an independent prognostic factor of coronary arterary disease CAD severity.

Purefied or recommbanent FA might be a good choice to develop treatments that might prevent or slow the progression of this process.

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