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Comparison of Serum Fetuin A Levels and Biochemical Parameters In Patients With and Without Calcium Oxalate Stones: A Cross Sectional Study

Neha Martin Honnalli[1], Usha Adiga[2*], Sachidananda Adiga[3], Rajeev T. P[4]

[1] Research Scholar, Department of Biochemistry, KS Hegde Medical Academy, Nitte-Deemed to be University, Mangalore, Karnataka, India
[2] Professor, Department of Biochemistry, KS Hegde Medical Academy, Nitte-Deemed to be University, Mangalore, Karnataka, India
[3] Professor, Department of Pharmacology, KS Hegde Medical Academy, Nitte-Deemed to be University, Mangalore, Karnataka, India
[4] Professor and Head, Department of Urology, KS Hegde Medical Academy, Nitte-Deemed to be University, Mangalore, Karnataka, India
Email: ushachidu@yahoo. com

Abstract

Introduction: Renal stone formation is a multi step process. Identifying molecules and metabolic alterations that affect this process may provide an opportunity to intervene for preventing stone formation. Fetuin A is the one that is least explored. Objective of the study was to compare serum fetuin A levels and other relevant biochemical parameters in patients with and without urinary oxalate stones.
Methodology: The cross-sectional study recruited fifty each subjects with and without urinary oxalate stones as cases and control respectively. Their serum samples were used to assay serum fetuin A by ELISA and other biochemical parameters by semi-autoanalyzer. Statistical analysis was carried out using SPSS 23.
Results: Serum fetuin A was insignificantly lowered($p=0.43$), uric acid significantly lower I cases than controls ($p=0.012$) and creatinine were significantly higher and ($p=0.046$) in cases. eGFR was reduced significantly ($p<0.0001$) in cases suggesting a renoprotective role of fetuin A. Significant positive correlation was observed between fetuin A and calcium levels.
Conclusion: Lowered Fetuin A levels in patients with renal stone disease suggest a protective role of fetuin A. Small sample size is the limitation of the study. However it may be considered as a pilot study and further investigations may be carried out to further explore the role of fetuin A in kidney stone disease.
Keywords: kidney stone, fetuin A, metabolic parameters,kidney function

I. INTRODUCTION

Background

Nephrolithiasis is a complex process involving multiple steps and various contributing factors. Calcium stones are the predominant type of renal stones comprising about 80% of all urinary calculi. Identifying molecules and metabolic alterations that affect this process may provide an opportunity to intervene stone formation. Fetuin A is one such molecule which has been least studied. Fetuin A may be used as a biomarker for predicting calcium oxalate stone formation.

Fetuin-A is a 45-kDa plasma protein consisting of 2 polypeptide chains derived from posttran-scriptional cleavage and is secreted mainly by the liver[1]. It behaves as a negative acute-phase reactant. Additionally, fetuin-A is also a potent inhibitor of vascular calcification and this

activity of the protein is in part due to the formation of the fetuin–mineral complex. It reversibly complexes with calcium and phosphorus and increases their respective serum solubility[2]. In patients on dialysis, in whom serum fetuin-A levels were found to be low, the capacity of serum to inhibit calcium–phosphate product precipitation was significantly impaired, and it was shown that patients on dialysis with low fetuin-A levels had coronary or other calcification foci. Further more, reconstitution of serum from these patients with purified fetuin-A to achieve physiological concentrations returned the impaired precipitation inhibition to normal[3]. Price and Lim showed that fetuin-A, by forming complexes with minerals, prevent the precipitation of hydroxyapatite from supersaturated solutions of calcium and phosphate in vitro[4]. Also, it has been documented that in patients with urolithiasis, urinary fetuin-A levels were lower compared with healthy subjects. In the same study, sensitivity of urinary fetuin-A levels in urolithiasis was 97% and specificity was 100%[5].

Serum and urine levels of fetuin A were assayed in stone disorder patients by Arora et al and opined that their levels were low compared to those without renal stones [6]. However contradictory results were reported by other researchers [7,8].

Rationale of the study

Dakshina Kannada and Udupi are the districts situated at the coastal Karnataka, South India. The high incidence and prevalence of renal stone disease in coastal regions could be attributed to the effect of a certain diet peculiar to the coastal area with decreased calcium intake and high intake of animal protein (whether meat, fish or poultry) and oxalate. This results in enteric hyperoxaluria and an increased risk of calcium oxalate stone formation. Moreover, the lower urine volumes, due to the hot, humid and dry climate increased the risk of stone formation in coastal region [9]. Studies are needed to confirm the role of fetuin A in calcium oxalate stone formation.

II. OBJECTIVES

1. To compare serum fetuin A levels in patients with and without urinary oxalate stones.
2. To compare other biochemical parameters like serum calcium ,phosphorus, uric acid ,creatinine and albumin levels in patients with and without renal stones
3. To find the correlation of serum fetuin A levels with the other biochemical parameters in kidney stone patients

III.METHODS

The prospective cross-sectional study was conducted in Central research laboratory KSHEMA in collaboration with Department of Urology, Justice K S Hegde charitable Hospital, Mangalore.

Kidney stone samples were obtained either after extracorporeal shock wave lithotripsy or surgery for treatment. Calculi analysis was done using chemical methods. Only patients with calcium oxalate stones will be included in the study. Fifty patients in the age group of 18-65yrs of either gender, with normal eGFR with the calcium oxalate kidney stone confirmed by qualitative tests were included as cases.

Patients with uric acid / cysteine stones diagnosed by qualitative tests or those with primary hyperparathyroidism diagnosed by investigation were excluded.

Fifty healthy subjects of either gender, in the age group of 18-65 yrs, who are not having urinary stone by Ultrasonography with normal GFR were recruited as control group.

IV. LABORATORY INVESTIGATIONS:

Five ml plain blood was collected by aseptic precautions for metabolic evaluation. Serum calcium, phosphorus, uric acid, creatinine, albumin were assayed by using semi automated chemistry analyzer, and serum levels of fetuin A was assayed by ELISA.

V. STATISTICAL ANALYSIS:

Statistical analysis was done using SPSS version 23 software. Metabolic parameters will be compared between cases & controls using Mann Whitney U test for non parametric variables and

unpaired t test for parametric variables. Correlations were carried out using Spearmann's correlation . ROC was constructed to assess the diagnostic accuracy of fetuin A. P value less than 0. 05 was considered statistically significant.

VI. RESULTS

Demographic profile of the subjects is depicted in table 1.

Serum Fetuin A levels were insignificantly lowered in cases compared to controls (table 1). Serum uric acid were significantly lower in cases and creatinine were significantly higher in patients with kidney stones compared to controls. However significantly lowered albumin levels and eGFR values were noted in cases (table 1).

A correlation analysis was performed between serum fetuin A levels and other biochemical parameters. A significant positive correlation was observed between Fetuin-A level and calcium (r=0. 377, p=0. 012)(Fig 1). In contrast, eGFR and Phosphorous exhibits a negative correlation,were not statistically significant (fig 2,fig 4). Other statistical correlations were statistically insignificant (fig 2-5).

ROC curve analysis,the cut-off of 75ng/ml fetuin-A level was able to identify patients with kidney stone (sensitivity 75%; specificity 53%) with the area under the curve being 0. 542 (fig 6).

Table 1: Demographic data And Biochemical Parameters in case and control groups

Parameters	Control group	Case group	P value
Age	33. 7±10. 47	46±13. 87	**<0. 01***
Gender(Male/Female)	34(68%)/16(32%)	18(36%)/32(64%)	-
Uric acid (mg/ dl)	7(4. 8-11. 9)	4. 6(2. 3-8. 8)	**0. 012***
Creatinine(mg/ dl)	0. 81(0. 66-0. 98)	1(0. 7-1. 41)	**0. 046***
Calcium(mg/ dl)	9. 3±1. 84	8. 45±3. 81	0. 146
Albumin(mg/ dl)	2. 76±1. 211	1. 92±1. 159	**0. 007****
Phosphorus(mg/ dl)	5. 4(4. 5-6. 3)	4. 8(4. 2-5. 7)	0. 181
eGFR	122. 9(96. 6-208. 1)	78. 2(53. 8-105)	**<0. 0001*****
Fetuin A(ng/ml)	40. 09±14. 56	37. 54±16. 53	0. 43

** significant*

*** highly significant*

**** very highly significant*

Correlation of Fetuin-A level with biochemical Parameters in Patient groups:

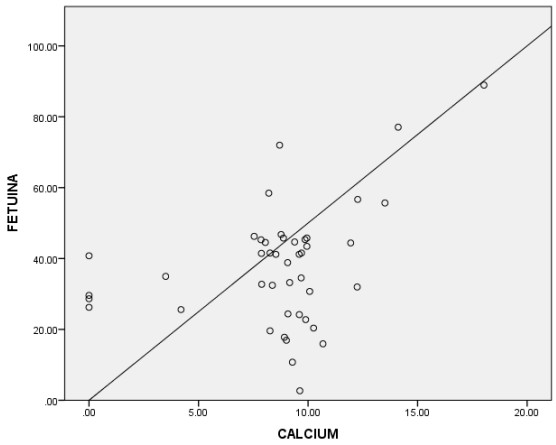

Fig 1:Correlation between Calcium and Fetuin-A (r =0. 377 P=0. 012)

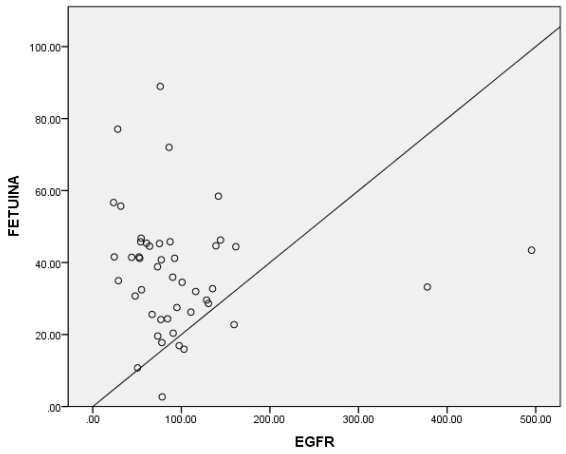

Fig 2: Correlation between eGFR and Fetuin-A (r = -0. 070 P=0. 661)

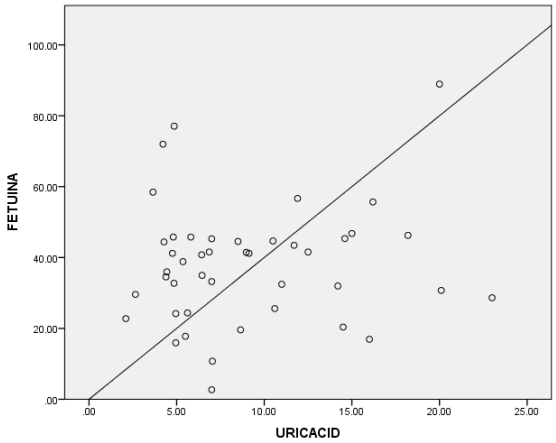

Fig 3: Correlation between Uric acid and Fetuin-A(r = 0. 126, p =0. 413)

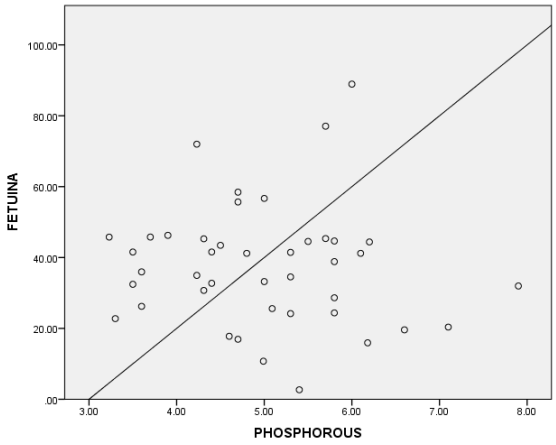

Fig 4: Correlation between Phosphorous and Fetuin-A (r = -0. 070, p =0. 661)

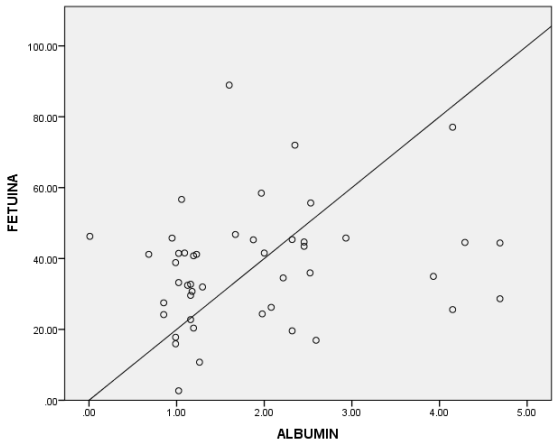

Fig 5: Correlation between Albumin and Fetuin-A (r =0. 203,p =0. 177)

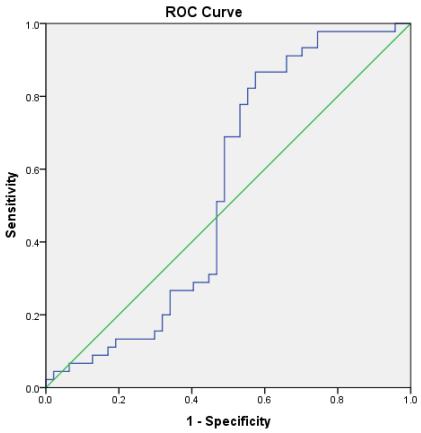

Fig 6: ROC of fetuin A

VII. DISCUSSION

Human fetuin-A, also known as AHSG, is encoded by the AHSG gene, which is located on chromosome 3 (3q27), and it is mainly secreted by hepatocytes and adipose tissues. As a multi-functional circulating glycoprotein, fetuin-A has both pro-inflammatory and anti-inflammatory functions as well as anti-calcifying properties[10].

Reduced serum fetuin A levels observed in renal stone patients in the present study may explain the contribution of fetuin A in stone formation. Reduction in anti-calcifying property may be attributed to stone formation. Our study findings are in accordance with the study by Mehrsai et al [11]. A study by Bassey et al revealed a possible role of fetuin-A in the etiology of declining renal function through mediating body mass index, uric acid, diabetes mellitus, and hypertension via complex causal pathways [12].

Lowered fetuin A levels as well as significantly lowered eGFR in the present study as well as a negative correaltion between fetuin A and eGFR support the renoprotective role of fetuin A.

As an acute phase anti-inflammatory protein, fetuin-A acts as an acute phase reactant in the extra-cellular space to attenuate inflammatory responses. Therefore, in patients with early stages of kidney disease, fetuin-A levels may be normal or slightly elevated. However, when the inflammatory process is prolonged, pro-inflammatory cytokines such as CRP, downregulate or inhibit fetuin-A synthesis, thereby attenuating the protective effect of

fetuin-A [13]. This may explain the low levels of circulating fetuin-A, which is observed in CKD. There are not many studies on serum fetuin A levels in kidney stone disease. However An increased serum fetuin-A concentration correlates with a common form of mild osteogenesis imperfecta whereas decreased levels occur in end-stage renal disease and during acute inflammation [14-16].

Positive correlation of fetuin A with calcium and negative correlation with phosphorus in the present study emphasize its role in stone formation. Furthermore, fetuin-A regulates ectopic calcification that might contribute to cardiovascular mortality [17-19]. In general,fetuin-A inhibits the precipitation of hydroxyapatite from supersaturated solutions of calcium and phosphate *in vitro* [20,21].

Fetuin-A has been proposed as a protective agent through solubilization of calcium phosphate salt and is a determinant of serum phosphate [22]. Also, fetuin-A is a marker of inflammatory as well as nutritional state and changes in fetuin-A levels could be an expression of this condition. Urolithiasis represents another process of unwanted calcification responsible for significant morbidity; >80% of renal stones contain calcium. Urinary factors inhibiting calcification are citrate, glycosaminoglycans, Tamm-Horsfall protein, osteopontin, etc. [23]. Recently, it was identified that exosomal and urine fetuin-A levels increased significantly after cisplatin-induced tubule damage, which occurred before serum creatinine increases. Thus, urinary fetuin-A levels might be a predictive biomarker of structural renal injury [24]. Patients with urolithiasis had lower fetuin-A concentrations independently of other conventional promotors and inhibitors of urine crystallization. Fetuin A-mineral complex in urine saturated with nuclei of crystallization could be the basis of these findings. We consider a better risk prediction of recurrent urolithiasis to be the main advantage of measuring fetuin-A as compared with adopting traditional markers of recurrent urolithiasis.

ROC analysis with low area under the curve and low sensitivity and specificity is not in favor of fetuin A being a marker for the diagnosis of kidney stone disease. Small sample size may be a limitation for this.

VIII. CONCLUSION

Lowered Fetuin A levels in patients with renal stone disease suggest a protective role of fetuin A. Small sample size is the limitation of the study. However it may be considered as a pilot study and further investigations may be carried out to further explore the role of fetuin A in kidney stone disease. Early prediction/detection of urinary oxalate stones helps in life style modification and hence prevention of recurrent stone formation. Early detection and treatment of urinary oxalate stones may help in improving the quality of life of patients.

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