Hyaluronic Acid as a New Biomarker to Differentiate Acute Kidney Injury From Chronic Kidney Disease

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Introduction. It may be difficult to differentiate acute kidney injury from chronic kidney disease in patients with no past medical reports of kidney function. This study aimed to investigate the role of serum hyaluronic acid (HA), which is known as a marker of fibrosis, in differential diagnosis of kidney failure.
Materials and Methods. A total of 90 patients (52 women and 38 mne) admitted to our renal unit with uremia for the first time were included. Serum HA level was measured. The diagnostic role of the test was investigated using the receiver operator curve curves.
Results. The mean age of the patients was 54.6 ± 17.9 years. The diagnosis was chronic kidney disease (CKD) in 41.1%, acute kidney injury (AKI) in 48.9%, AKI on CKD in 6.7% (3 died without a diagnosis). The mean serum HA was significantly higher in the CKD group (146.1 ± 119.3 ng/mL) than the AKI group (68.9 ± 69.1 ng/mL; $P < .001$). Serum HA significantly correlated with proteinuria (r = 0.717, $P < .001$) and serum albumin level (r = - 0.599, $P < .001$) in the CKD group only. Serum HA cutoff level of 61 ng/dL had a sensitivity of 82% and specificity of 67% for differential diagnosis of AKI and CKD.
Conclusions. Serum HA level may be used as tool to differentiate AKI from CKD. Further larger studies are warranted to clarify the definite the role of this marker.

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INTRODUCTION

Accurately determining acute or chronic nature of kidney failure is frequently needed in clinical practice in patients presenting with uremia for the first time, especially in developing countries without a good medical recording system. Distinguishing acute from chronic kidney disease (CKD) in clinical practice can be quite difficult for patients presenting with uremia for the first time whose kidney function 3 months prior is unknown. The presence of findings consistent with CKD makes the diagnosis easier. However, when the kidney size is normal (diabetes mellitus, multiple myeloma, polycystic kidney, hydronephrosis, renal vein thrombosis, and tumor infiltration),[1] it is difficult to distinguish acute kidney disease and CKD. Patient's history, examination and routine biochemical tests, itching, long-standing hypertension, uremic neuropathy, anemia, hypocalcemia, hyperphosphatemia are findings consistent with CKD but still with low sensitivity and specificity.[2]

Among the many growth factors related to glomerular damage, transforming growth factor-1 shows the most powerful fibrogenic effects. Transforming growth factor-1 enhances the synthesis of matrix proteins. Extracellular matrix markers amend to both qualitative and quantitative changes during fibrosis. Potential markers of fibrosis include glycosaminoglycans and extracellular matrix glycoproteins, matrix synthesis and enzymes related to degradation, and collagen synthesis and enzymes related to

degradation. One of the direct markers of fibrosis is hyaluronic acid (HA), which has been shown to be elevated in CKD.[3-8] We aimed to make the distinction between acute kidney injury (AKI) and CKD in patients presenting with advanced uremia, by investigating the serum levels of HA.

MATERIAL AND METHODS

Patients

A total of 90 newly diagnosed uremic patients hospitalized with a diagnosis of AKI or CKD at Dicle University School of Medicine were enrolled in the study. Patients on dialysis programs, patients with hepatic fibrosis, rheumatoid arthritis, amyloidosis or any disease known to increase hyaluronic acid, and those younger than 18 years of age were excluded. The etiology of AKI was almost all renal origin because easily reversible prerenal azotemia and urinary obstruction cases were not included.

Acute kidney injury was defined according to the risk, injury, failure, loss, and end-stage kidney disease (RIFLE) criteria and was of any RIFLE severity that reverses in less than 24 hours after replacement of intravenous fluids or relief of obstruction. Acute kidney injury on CKD was defined according the RIFLE in those with known CKD. A diagnosis of CKD was defined according to diagnostic criteria of the National Kidney Foundation Kidney Disease Outcomes Quality study group. The causes of CKD were as follows: hypertension (33%), diabetes mellitus (27%), urological diseases (11%), chronic glomerulonephritis (8%), and unknown and other causes (21%). Only those with CKD stage 5 were included.

Study Design

This was a single-center prospective observational study. Serum HA, urea, creatinine, albumin, calcium, phosphorus, and parathyroid hormone, as well as complete blood count were studied in patients with uremia. Urinalysis was studied in all patients. Protein amount in 24-hour urine was measured. All patients underwent renal ultrasonography. Hyaluronic acid serum levels were measured with HA Corgenix Test Kit (Lot: HAE-131, Corgenix Inc, CO, USA). Normal serum levels were zero to 100 ng/mL.

Statistical Analysis

All continuous variables were expressed as mean ± standard deviation, and frequencies were expressed as percentages. The mean values for AKI and CKD groups were compared by the Student *t* test. The Mann-Whitney U test was used to compare means with nonhomogeneous distribution. The Pearson correlation test was applied to the associations between HA, proteinuria, and serum albumin levels and the amount of proteinuria in 24-hour urine. The diagnostic accuracy of serum HA value was investigated with the receiver operator curve analysis. Sensitivity and specificity values were calculated for some HA threshold values. A *P* value less than .05 was considered significant.

RESULTS

The mean age of the patients was 54.6 ± 17.9 years. A total of 52 patients (57.8%) were men and 38 were women (42.2%). The study included 37 CKD patients (41.1%), 44 AKI patients (48.9%), and 6 with AKI on CKD (6.7%). Patients who died were not considered for statistical evaluation.

The mean serum HA levels were 68.9 ± 69.1 ng/mL in the AKI patients and 146.1 ± 119.3 ng/mL in the CKD patients ($P < .001$; Table and Figure 1). The mean serum HA level was 179 ± 195 ng/mL in the CKD patients developing AKI. The mean serum HA level was 349 ± 128 ng/mL in 3 patients who died; we were not able make a final diagnosis of AKI or CKD in those. The amount of daily proteinuria was 0.7 ± 1.2 g in AKI patients and 3.5 ± 3.6 g in CKD patients ($P < .001$; Table). No significant difference was detected between the two groups in terms of age or serum levels of urea, creatinine, hemoglobin, calcium, phosphate, parathyroid hormone, and albumin (Table).

The area under the receiver operator curve designed for the role of HA in the differential diagnosis of AKI and CKD was 0.773 (Figure 2). When the HA threshold was defined as 61 ng/mL, the sensitivity of the test was 67% and specificity was 82%. Twenty-five of 37 patients with AKI had low levels and 36 of 44 patients had HA levels higher than 61 ng/mL.

There was a significant correlation between serum HA levels and amount of 24-hour urine proteinuria ($r = 0.716$, $P < .001$; Figure 3). However, in subgroup analysis, we found that the correlation was significant only in those with CKD ($r = 0.717$, $P < .001$), and not in those with AKI ($r = 0.022$, $P > .05$; Figure 3). When graphically analyzing the

Characteristics of 81 Patients With Kidney Function Impairment

Characteristics	Acute Kidney Injury (n = 37)	Chronic Kidney Disease (n = 44)	*P*
Hyaluronic acid, ng/mL	68.9 ± 69.1	146.1 ± 119.3	< .001
Sex			
Male	13	22	
Female	24	22	> .05
Age, y	53.8 ± 15.0	56.4 ± 19	> .05
Urea, mg/dL	184.4 ± 87.0	196.0 ± 77.4	> .05
Creatinine, mg/dL	6.9 ± 3.0	8.1 ± 4.4	> .05
Hemoglobin, g/dL	10.5 ± 1.6	9.3 ± 1.9	> .05
Calcium, mg/dL	8.2 ± 1.1	7.8 ± 1.2	> .05
Phosphate, mg/dL	5.4 ± 2.0	5.8 ± 2.4	> .05
Parathyroid hormone, pg/mL	159.4 ± 104.7	366.7 ± 477.0	> .05
Albumin, g/dL	2.8 ± 0.3	2.4 ± 0.7	> .05
Proteinuria, g/24 hour	0.7 ± 1.2	3.5 ± 3.6	.001
Hemodialysis treatment, %	59.4	63.6	> .05

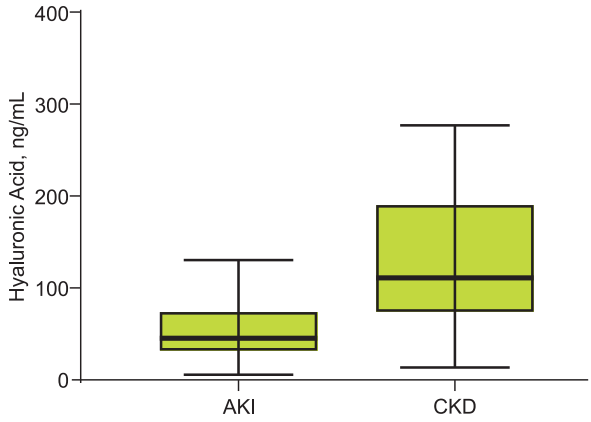

Figure 1. Boxplot demonstrating serum hyaluronic acid levels in patients with acute kidney injury (AKI) and chronic kidney injury (CKD).

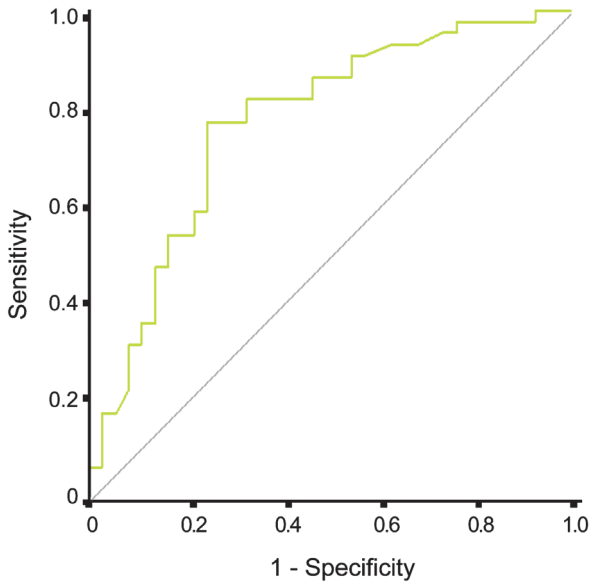

Figure 2. Receiver operator curve analysis of hyaluronic acid levels in acute kidney injury (AKI) versus chronic kidney injury (CKD).

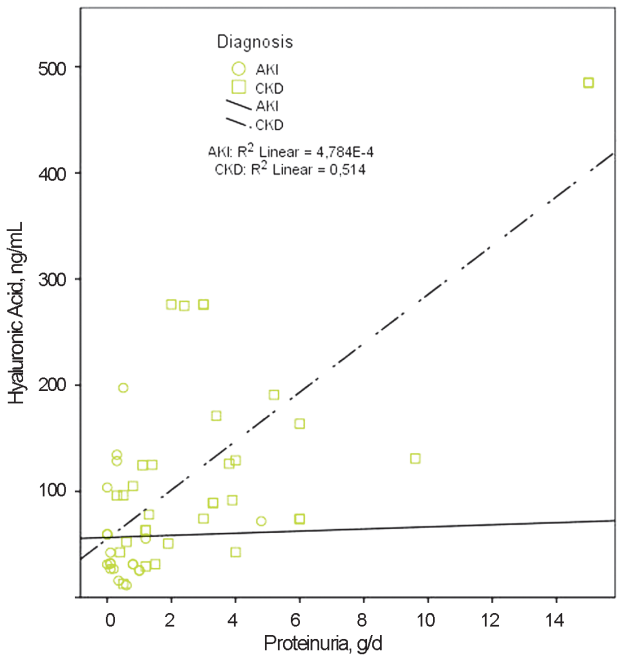

Figure 3. Linear relationship between serum hyaluronic acid levels and 24-hour proteinuria.

deviation between serum albumin level and serum HA levels, it was noted that the deviation increased with the decrease of albumin values. There was a significant negative correlation between serum HA level and serum albumin level (r = -0.599, $P < .001$) in only the CKD group.

DISCUSSION

The differentiation between AKI and CKD in patients presenting with uremia for the first time with unknown kidney function in the past 3 months and normal imaging may be difficult in clinical practice.

It is known that HA synthesis increases in CKD.[6,7,9] In our study, serum HA levels in patients with CKD were approximately 2-fold higher compared with patients with AKI. This difference was significant. The mean level of HA was higher in both AKI and CKD groups than 41 ± 25 ng/mL which was previously reported from healthy control subjects.[10]

A threshold for HA of 61 ng/dL for differential diagnosis of AKI and CKD yielded a sensitivity of 67% and a specificity of 82%. Hyaluronic acid is a high molecular weight protein composed of N-acetyl glucosamine and glucuronic acid unit. It is an important component of extracelular matrix. Hyaluronic acid synthesis has been shown to increase in liver disease (hepatic fibrosis) and inflammatory disease (rheumatoid arthritis).[11,12] One of the mechanisms that describe the HA increase in hepatic dysfunction is the disorder in HA intake of specific endothelial receptors and disorder of the liver endothelial cell function affecting per sinusoidal lipocytes HA synthesis.[13] The mechanism which increases of serum HA levels in patients with CKD is not fully known. However, it is assumed that uremic toxins also lead to generalized endothelin receptor dysfunction.[14]

Hyaluronic acid also plays a role in the endothelial dysfunction and arteriosclerosis acceleration.[15] In uremic patients, in addition to high endothelial receptor dysfunction, another mechanism for the HA increase is the increase of HA synthesis-stimulating factors. Prostaglandins, cytokines, or both also contribute in the HA synthesis in patients with uremia.[16,17] Some cytokines, especially interleukin-1, interleukin-6, tumor necrosis factor-α, stimulate the synthesis of HA in the connective tissue. In patients with kidney transplantation serum level of HA has been shown to normalize.[18] Limited number of studies are present that show the increase in HA in AKI patients due to HA-CD44 interaction.[19] However, this increase has not been as much as CKD in our study.

An increased HA production by proximal tubular cells, renal fibroblasts, mesangial cells, glomeruli in diabetic kidneys has been reported. Renal HA expression is increased in some chronic disease states, such as diabetes and nephrolithiasis, which in turn have the potential to lead to chronic renal insufficiency.[20] One of many factors that affect the progression of glomeruli damage is proteinuria. Proteinuria activates glomerular cells, tubuloepithelial cells, and interstitial cells. Transforming growth factor-1, which shows strong fibrogenic effect, increases the synthesis of matrix protein that shows.[21] The glomerular and interstitial CD44 and hyaluronic acid expression correlated with proteinuria, and interstitial CD44 and hyaluronic acid expression correlated with creatinine clearance rate. In Sano and coworkers' study, it is shown proteinuria increases the accumulation of HA accumulation.[22] In our study, it was shown that serum HA levels increased as the amount of proteinuria in the 24-hour urine increases. In experimental models, it has been shown that proteinuria leads to T cells and macrophages interstitial infiltration and interstitial fibrosis by increasing extracellular matrix formation.

It has been reported that extensive proteinuria may elicit proinflammatory and profibrotic effects which cause chronic kidney damage.[23] The progression of glomerular or tubulointerstitial fibrosis is an important determinant of the loss of kidney function in any kind of kidney disease.[24] Therefore, we may propose an increased HA in those with high proteinuria and advanced CKD, as was the case in our study.

In a study conducted by Turnkey and colleagues, serum levels of HA above 300 ng/mL indicated poor prognosis.[6] In our study, the mean serum HA levels of the 3 patients who died were found compatibly higher with the previous study. However, is difficult to make evaluation because of the small number of patients in this group.

Serum HA level and prothrombin time have been found to be significant prognostic factors related to the presence of protein-energy malnutrition. It has been speculated that a higher HA level is linked to systemic inflammation, as well as protein malnutrition.[25] A significant negative correlation was noticed between serum HA and serum albumin ($r = -0.599$, $P < .001$) in the CKD group. Although albumin is a well-known negative acute phase reactant, it was lower in our CKD group, which may reflect malnutrition.

This is a pionering study demonstrating the differences of HA in AKI and CFR groups. However, it was conducted at a single institution; consequently, the results may not be directly applicable to other patient populations. The low sample size is another limitation of our study. Urinary HA was increased in postoperative AKI patients.[26] This finding may

also be a clue of blunted diagnostic role of HA for differential diagnosis of uremic patients.

CONCLUSIONS

Serum HA level, which is an indicator of fibrosis, may be a new marker to differentiate AKI from CKD, in patient presenting with uremia whose previous kidney function is unknown, However, further larger studies investigating HA are warranted in patients with AKI and CKD.

CONFLICT OF INTEREST

None declared.

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