

Urinary Citrate: A view in Chronic Renal Failure

SANTHOSH KUMAR.N^{1*}

SUBHAS CHANDRAPPA. M¹

BHEEMASEN. R¹

BALU MAHENDRAN. K²

Department of Biochemistry¹,
Shridevi Institute of Medical
Sciences & Research Hospital,
Tumkur, Karnataka, India
Division of Biochemistry², Rajah
Muthiah Medical College and
Hospital, Annamalai University,
Annamalai Nagar, Tamil Nadu,
India.

Corresponding Authors:

*Santhosh Kumar. N,
Asst. Professor, Department of
Biochemistry, Shridevi Institute of
Medical Sciences & Research
Hospital, Tumkur, Karnataka, India.
Email:mahendran.kanumuru@gm
ail.com.

Abstract:

Aim & Objective: To evaluate the 24 hour urinary citrate levels in chronic renal failure and healthy controls and to define the role of urinary citrates in the chronic renal failures. **Materials and Methods:** The 24 hours urinary citrates, Blood urea, Serum creatinine, Na⁺, K⁺were evaluated in 25 chronic renal failure patients and 25 healthy subjects taken as controls. In both groups participants were on their usual diet. In addition, none of the participant was taking any drugs that could interfere with the citrate excretion. **Results:** The mean 24 hour urinary citrate excretion in patients and healthy controls was 296.3 ± 8.543mg and 323.9 ± 4.304mg respectively. Using previously defined values of normal urinary citrates as more than 320 mg.The difference in 24 hour urinary citrate excretion in all patients and healthy control was statistically significant (<P =0.001). **Conclusion:** There is statistically significant difference in urinary citrate excretion in recurrent renal failures and healthy controls. Uniformly low citrate excretion in patients indicates that low citrate levels may be a feature seen in predisposing factor for renal failure.

Keywords: Urinary citrate, NPN substances, serum electrolytes, chronic renal failure (CRF), Kidney stones

INTRODUCTION:

Citrate (2-hydroxy-1, 2, 3-propanetricar-boxylic acid) is a weak tricarboxylic acid& an alkalinizing agent [1,2].Intracellular citrate is the result of both dietary intakes (eg; citrus foods)[3] and endogenesis as intermediate in Krebs' cycle, by citrate synthase which catalyzes the condensation of oxaloacetate with acetyl CoA to form citrate [4]. But ATP citrate lyase activity serves as important role in the hypocitraturia of metabolic acidosis, this cytosolic enzyme cleaves citrate to oxaloacetate and acetyl coA and is present in kidney tissue [5].

Citrate is reabsorbed in the renal proximal tubule by a sodium-coupled transporter, the Na⁺ / dicarboxylate co-transporter, with broad substrate specificity for Krebs' cycle intermediates [6,7].

The rate of intracellular metabolism of citrate plays a major role in determining the amount of citrate excreted in the urine. Increased excretion is secondary to increased synthesis when citric acid cycle precursors such as malate or succinate are infused and also due to inhibition of citrate metabolism by malonate, maleate, or fluorocitrate is administered[8].

It is evident that effects of acid-base changes are mediated by alteration in the pH gradient across the inner mitochondrial membrane. Metabolic

alkalosis causes cytoplasmic pH and bicarbonate to increase, resulting in a decrease in the mitochondrial pH gradient. This change inhibits the tricarboxylate carrier, slowing entry of citrate into the mitochondrial matrix causing increases in cytoplasm citrate levels, leads to tubular and peritubular citrate uptake are reduced, and citrate clearance increases^[9]. Opposite changes occur in metabolic acidosis. So Change in the mitochondrial pH gradient provides a sensitive mechanism for regulating renal substrate metabolism ^[10].

In recent years, renal handlings of citrate and citrate excretion in the urine have renewed interest due to modern techniques in renal physiology (such as, transport studies in brush border membrane vesicles and perfused proximal tubules) provides insights that excretion of urinary citrate ^[11], (and other organic anions) has been recognized to influence systemic acid-base status, at least in certain species. Citrate is known to inhibit precipitation of calcium oxalate and phosphate and growth of their crystals ^[12, 13].

Tanner et al have recently demonstrated ^[14-16] that citrate salts improve renal function in rats with polycystic kidney disease, mainly due to its alkalinizing effect. The citrate utilized by the kidneys is supplied predominantly by reabsorption of filtered citrate, with peritubular uptake of citrate accounting for the remainder (up to 30 to 40%) of citrate utilized by the kidneys ^[17].

Citrate is thought to be freely filterable at the glomerulus, in humans, 65 to 90% of the filtered citrate is reabsorbed and 10 to 35% is excreted in the urine ^[18, 19]. Pak (1990) has defined normal 24 hour urinary citrates as more than 320 mg for both genders^[20]. Hypocitraturia is defined as urinary citrate excretion lower than 320 mg/day. However, there are some reports of low

urinary citrate output in stone formers (SF) and renal failure as compared with healthy subjects ^[21-23], while other studies found no differences ^[24, 25].

Ongoing research has disclosed additional causes of hypocitraturia such as sodium excess, low intestinal alkali absorption, but not primary citrate malabsorption^[26, 27].

This study was aimed to compare the 24 hour urinary citrate excretion in chronic renal failure patients and the normal individuals and to define the role of urinary citrate levels in the chronic renal failure in this region.

On the contrary, several metabolic abnormalities, such as metabolic acidosis, hypokalemia and starving, seem to influence the renal handling of citrate by inducing a decrease in the urinary citrate excretion.

MATERIALS AND METHODS:

Twenty five adult male & female recurrent chronic renal failure patients who underwent treatment in Shridevi Institute of Medical Sciences and Research Hospital, Tumkur were selected as cases for the study group. Twenty five ages matched healthy volunteers with no evidence of chronic renal failure or any positive history of renal stones who agreed to take part in the study were included as controls group. In both groups subjects were on their usual diet and were not taking any regular medications that could interfere with the biochemical results. Patients with diabetes, renal impairment, and documented urinary tract infection, other systemic illness patients were excluded from the study. Both groups detailed history has taken and physical examination is performed. Each study participant received clear verbal and written instructions

about collection of Blood & 24 hour urine sample and was provided with a special container.

Twenty four hour urinary citrate excretion was measured in both groups. All samples were tested in the same laboratory. For the purposes of this study, citrate levels were taken as more than 320 mg/24hr urine^[28]. 24 hr urine was collected in a container with 10ml of 10N Sulfuric acid as preservative ^[29, 30].

After collecting 24hr urine citrate was determined by the quantitative method (Colorimetric kit method), Urine creatinine (Mod. jaffe's Kinetic method), uric acid (uricase/PAP method), urea (Modified Berthelot Method), Serum electrolytes sodium & potassium (ion selective electrode method) by ion selective electrode analyser and routine urine analysis was carried out for all study subjects & controls.

ANALYTICAL METHODS:

Urinary citrate excretion was assayed by the colorimetric method with pentabromoacetone (PBA). Citric acid is oxidized to pentabromoacetone (PBA) by bromine. PBA formed is extracted with ether and reacts with borax buffer solution to form yellow color, which is measured calorimetrically using blue violet filter (or) spectrophotometer at 445nm^[31].

RESULTS:

There were twenty five healthy peoples in control group and twenty five patients in Study group. The mean age of the patients in Study and Control Group was 33 to 57 years respectively. The mean 24 hour urinary citrate values in control Group were 323.9 ± 4.304 while it was 296.3 ± 8.543 in Study group. The mean value of urinary citrate is significantly decreased in study group. Urinary

citrate values in both groups are shown in Fig 1. By applying two samples to *t*-test on citrate values in all participants in both groups, P value was < 0.00 i.e. the difference in citrate values in chronic renal failure and healthy controls was low statistically significant in chronic renal failure patients compare to healthy controls.

NPN values in both groups are shown in Fig 2. By applying *t* tests on NPN values in all participants in both groups, P value was < 0.001 . The mean blood urea, serum creatinine & Uric acid level in the study group was increased (100.6 ± 37.95), (5.817 ± 4.186) & (7.563 ± 1.337) when compared to that of the control groups (26.27 ± 4.143), (0.8967 ± 0.179) & (4.19 ± 0.544). The mean difference was statistically significant. It has been corroborated in various texts that a very high NPN substances level is associated with an azotemic +state, where the kidneys fail to excrete the substance.

Serum electrolytes values in both groups are shown in Fig 3. The mean serum Sodium level in the study group was (131.8 ± 7.69) and the controls was (139.3 ± 3.446) the mean difference was statistically significant. This finding is consistent with texts which describe that patients with renal failure have impaired renal mechanism for conserving Na^+ .

The mean serum potassium level in the study group was (4.387 ± 1.073) and the controls was (4.203 ± 0.423) the mean difference was statistically not significant.

Potassium is freely filtered through glomerulus & is normally totally reabsorbed by PCT. Depending on the nature of pathology or the sites involved, a range of serum K^+ values are noted in kidney disease from hyperkalemia to hypokalemia. Hyperkalemia is a frequent complication of Renal Failure^[32].

Table 1: Mean \pm SD in urinary citrates, NPN substances & serum electrolytes in control group & study groups:

PARAMETERS	Control group	Study group	P- value
	mean &Std. Dev	mean &Std. Dev	
Urinary Citrate (mg/24hr urine)	323.9 \pm 4.304	296.3 \pm 8.543	< 0.001
Blood Urea (mg/dl)	26.27 \pm 4.143	100.6 \pm 37.95	<0.001
Serum creatinine (mg/dl)	0.8967 \pm 0.179	5.817 \pm 4.186	<0.001
Serum uric acid (mg/dl)	4.19 \pm 0.544	7.563 \pm 1.337	< 0.001
Serum Sodium (mEq/L)	139.3 \pm 3.446	131.8 \pm 7.69	< 0.001
Serum potassium (mEq/L)	4.203 \pm 0.423	4.387 \pm 1.073	< 0.388

Fig 1: Urinary citrate values in both groups

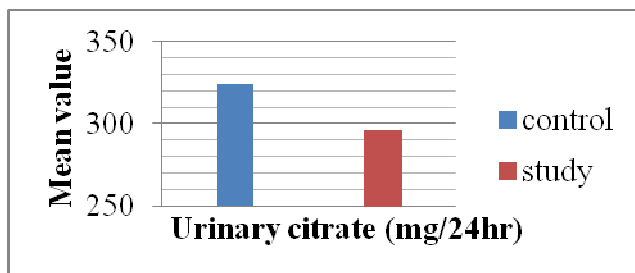


Fig 2: NPN values in both groups

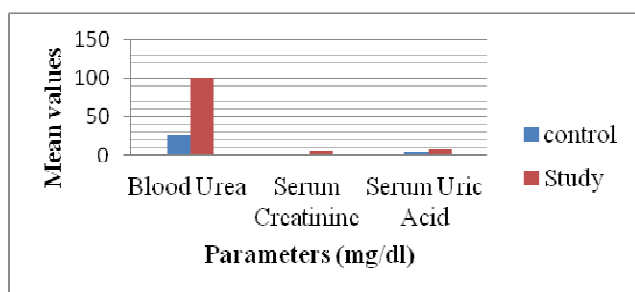
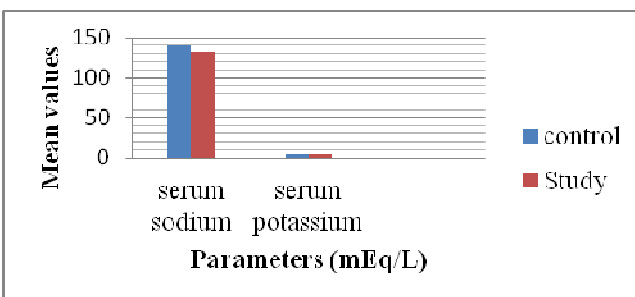


Fig 3: Mean \pm SD in Serum Electrolytes in control group & study groups



CONCLUSION:

Chronic Renal failure (CRF) is a worldwide health problem. According to WHO Global Burden of Disease Project, it ranks as the 12th leading cause

of death. It is estimated that approximately one lakh new patients develop ESRD in India annually. Change in lifestyle leading to obesity, hypertension, and diabetes all contribute to increased risk of CRF.

As there is steep rise in cases of Diabetes and Hypertension there is increase as well as in the cases of CRF which virtually end in ESRD, which is causing a great burden for the country in terms of morbidity & mortality.

As glomerular filtration rate (GFR) decreases, there is a stepwise decrease in the amount of citrate that is filtered; however, in the early stages of CRF, the increased fractional excretion of citrate prevents an abrupt decline in urinary citrate, such that overt hypocitraturia is not usually observed until advanced stages of CRF [32]. Urinary citrate excretion depends upon the urinary volume, calcium, magnesium excretion and GI - alkali load. High meat intake increases the urinary excretion of calcium, oxalate, and uric acid and decreases urinary pH and citrate excretion. The use of high-protein, low-carbohydrate diets for weight loss has led to concern about increased risk of stone formation, as these diets have been shown to be associated with decreased urinary citrate and pH levels and increased urine calcium and sodium levels in the induction and maintenance phases [32].

Hypocitraturia enhances urine calcium salt supersaturation and reduces calcium crystallization inhibition, increasing the risk of calcium nephrolithiasis. It also may play a role in uric acid solubility and uric acid stone formation. The hypocitraturia of indeterminate causes or idiopathic hypocitraturia may be secondary to intrinsic renal defects (dysfunction of the sodium-citrate co-transport or disorder red intracellular citrate regulation etc), in appropriate intestinal citrate or alkali absorption, or a normal physiologic response to animal protein- rich diets.

The major outcomes of CRF, as well as ARF to some extent, regardless of specific diagnosis, (i.e. type of kidney disease), include progression to kidney failure and complications from decreased kidney function. Increasing evidence shows that early detection followed by treatment often can delay or prevent some of these adverse outcomes.

Our study was aimed to evaluate urinary citrates, non protein nitrogenous substances in the serum & serum electrolytes in cases of CRF. Mean urinary citrates decreased significantly when compared with controls. It was observed that mean Blood Urea, Serum creatinine & Serum uric acid were significantly increased when compared with controls. The mean serum Sodium levels decreased and Serum potassium level increases significantly when compared with controls.

Early diagnosis and treatment as well as regular monitoring of these cases will help in decreasing the morbidity and delaying the fatality.

Overall there was a major difference between the two groups in urinary citrate as risk factors that predispose to stone formation in chronic renal failure patients.

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