RESEARCH ARTICLE

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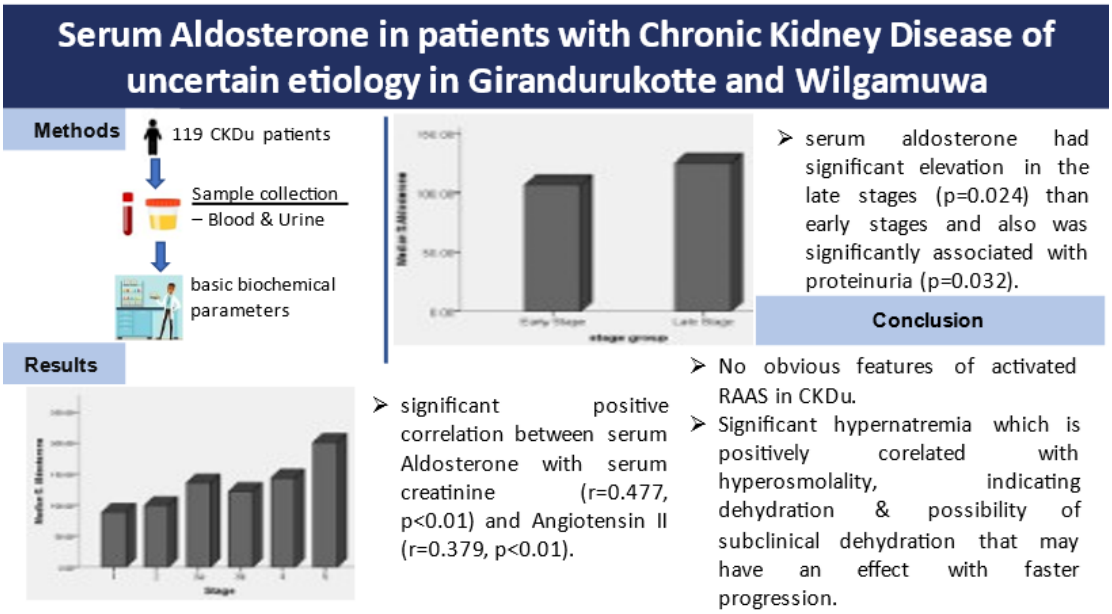

Highlights

- No obvious indications of activated Renin Angiotensin Aldosterone System (RAAS) were found in definite CKDu.
- In the absence of general indications, effects of RAAS blockers are a dilemma.
- Subclinical dehydration may have an effect with faster progression.

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RESEARCH ARTICLE

Serum Aldosterone in patients with Chronic Kidney Disease of uncertain etiology in Girandurukotte and Wilgamuwa, Sri Lanka

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Abstract: The Renin Angiotensin Aldosterone System (RAAS) is the main regulator of body fluid and electrolyte balance in the human body. Features of RAAS activation, such as hypertension and fluid retention, are not apparent in some categories of CKD-like tubular interstitial diseases. The beneficial effects of RAAS blockers are controversial if not activated. This study was conducted to identify the features of RAAS activation in CKDu and to evaluate the representativeness of Aldosterone as a marker of activation. A cross-sectional study was conducted on 119 definite CKDu patients at the renal clinics in Girandurukotte and Wilgamuwa, Sri Lanka. The basic biochemical parameters, serum electrolytes, and osmolality were measured by using serum and urine samples of the participants. Statistical analysis was performed in IBM SPSS statistics version 23. Only 4.4% and 6.7% of study subjects had increased serum Aldosterone and serum Angiotensin II. There was a significant positive correlation between serum Aldosterone with serum creatinine ($r=0.477$, $p<0.01$) and Angiotensin II ($r=0.379$, $p<0.01$). Inversely, it was negatively correlated with eGFR ($r=-0.353$, $p<0.01$). Moreover, serum aldosterone had a significant elevation in the late stages ($p=0.024$) than early stages and was significantly associated with proteinuria ($p=0.032$). Results showed no indications of activated RAAS in patients with the diagnosis of definite CKDu. A significant number of the population had hypernatremia which is positively correlated with hyperosmolality, indicating dehydration. The possibility of subclinical dehydration that may have an effect with faster progression.

***Keywords*:** Renin angiotensin aldosterone system; Chronic kidney disease of uncertain etiology; Aldosterone; Angiotensin II; Proteinuria

INTRODUCTION

The Renin Angiotensin Aldosterone System (RAAS) is the best-known regulator of blood pressure and the determinant of target organ damage in hypertension. It controls body fluid and electrolyte state through coordinated effects on the heart, blood vessels and kidneys (Heras *et al.,* 2012). Thus, local or tissue-based RAAS is well documented and has been implicated as a key player in the pathogenesis of cardiovascular and renal diseases (Siragy and Carey, 2013). Hence, RAAS blockade has been implicated in reversing the adverse outcome of RAAS activation in hypertension, especially in the presence of concurrent renal or heart diseases. Nevertheless, RAAS activation is protective in dehydration, hypovolemia and hypotension preventing pre- renal Acute Kidney Injury (AKI). Vice versa, the RAAS blockers are, well-known to precipitate AKI in hypovolemia, Chronic Kidney Disease (CKD), sepsis, and in the presence of other nephrotoxins or in the elderly. There is a paucity of research on RAAS in tubular interstitial diseases such as analgesic nephropathy, Dent's disease, Balkan nephropathy and Chronic Kidney Disease of uncertain etiology (CKDu) (Selvarajah *et al*., 2016). CKDu, a recently described tubular interstitial nephropathy, is prevalent in tropical or subtropical countries including pockets of the farming dry zone of Sri Lanka, India, Nicaragua, Costa Rica, Egypt and Tunisia (Weaver *et al.,* 2015). Even though not directly applicable to CKDu Sri Lanka, there are growing evidence to support that hot weather and recurrent dehydration have a causal effect on the onset and progression of CKDu (Jayasumana *et al.,* 2015; Nanayakkara *et al.,* 2019). Intermittent RAAS activation is protective in certain scenarios such as dehydration, hypotension and hypovolemia; hence RAAS blockers have to be used cautiously.

In clinical practice, three drug groups are acting on RAAS: Angiotensin Converting Enzyme inhibitors (ACEi), Angiotensin Receptor Blockers (ARBs) and finally, mineralocorticoid receptor antagonists (MRA). ACEi or ARBs have been recommended as the first line antihypertensives in CKD and cardiovascular diseases based on enormous evidence (Becker *et al.,* 2012; Bavishi *et al.,* 2016). Nonetheless, RAAS blockade is not always beneficial in CKD, especially the dual blockade (ACEI and ARS), triple (ACEI ARS and Aldosterone antagonists), or complete blockade (ACEI ARS, Aldosterone antagonists and Renin inhibitors) despite favorable effects on proteinuria (Casas *et al.,* 2015; Makani *et al.,* 2013). Along with, there are contradictory evidences for the use of aldosterone antagonist or direct renin blockers for anti-proteinuric effect in some subgroups of CKD (Furumatsu *et al.,* 2008; Tuttle *et al.,* 2005; Pimenta and Oparil,

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2009; Parving *et al.,* 2009). It has been shown a greater potential for faster deterioration of renal function with the use of RAAS blockers in elderly patients with advanced CKD (Turgut *et al*., 2014). In the same line, though not directly applicable to CKDu, discontinuation of RAAS blockers in elderly (mean age 73.3 years) at stages 4 and 5 CKD has demonstrated an improvement of Glomerular Filtration Rate (GFR) from 16.38 to 26.6 ml/min over 12 months (Ahmed *et al*., 2010). These findings suggest RAAS expression is not similar in all types of CKDs and RAAS blockade is not equally beneficial in across the line of CKD. More harmful effects were observed when stop these drugs even in advanced CKD (Bhandari *et al*., 2016; Mukoyama and Kuwabara, 2022). According to Bewster and Perazella (2004), renin-angiotensin II-aldosterone system contributes to renal dysfunction (Bewster and Perazella., 2004). Some studies showed that the renin-angiotensin-aldosterone system also causes proteinuria through novel effects on renal nephrin expression (Benigni *et al*., 2001; Bonnet *et al*., 2001).

Some CKDs such as CKDu are, less likely to have hypertension, fluid retention and proteinuria, especially in earlier stages of the disease (Jayasumana *et al.,* 2017). Further, cardiovascular disease, proteinuria or compelling indications for RAAS blockers are not common among CKDu, as in CKD (Wijewickrama *et al.,* 2019; Hettiarachchi *et al.,* 2021). More importantly, CKDu is characteristically reported in tropical countries where excessive dehydration, hypovolemia, hypotension and pre-renal AKI are expected with strenuous regular work in hot weather. In this background, the intermittent activation of RAAS, may be protective in CKD/ CKDu, challenging the regular use of ACE/ARBs for the prevention of progression. There is no single biomarker to evaluate the RAAS activity in CKD. Beside serum Aldosterone levels reported to be high in more than 90% of CKD (Hene *et al.,* 1982). In this study, we evaluated the representativeness of serum Aldosterone as an indicator of RAAS activity.

MATERIALS AND METHODS

All definite (biopsy-proven) CKDu cases (Supplementary Table 01) in two renal clinics in Girandurukotte and Wilgamuwa (endemic areas) (119: male 97, female 22) were enrolled after informed written consent from June 2015 to February 2017. The ethical clearance was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Peradeniya (2016/EC/28). A structured questionnaire collected demographic and medical information (age, gender, body mass index [BMI]. Blood samples (10 ml) were collected from peripheral veins into K-EDTA tubes, plain tubes and Na-citrated tubes in the morning. Then serum was separated by centrifugation at 3000rpm for 10 minutes. At the same time spot urine samples were collected from all the recruited cases into empty, sterile polypropylene urine containers. Then all the serum and collected urine samples were processed for routine biochemical parameters on the same day of the sample collection at, the medical laboratory, Teaching Hospital, Kandy. Basic biochemical parameters and serum electrolytes were measured using Indiko plus Analyzer (Thermo Scientific ™, Finland) and Electrolyte machine (BioCare BIOLYTE 2000, Taiwan), respectively. Serum and urine osmolality were measured using an osmometer (OsmoTECH^{R} 3320, Norwood). Serum Aldosterone and Angiotensin II were analyzed using Chemiluminescence Immunoassay Analyzer (MAGLUMI 600, Chennai). Proteinuria was detected by using 3% sulfosalicylic acid. Stage 1, 2, and 3a are considered as early stages and stages 3b, 4 and 5 are considered as late stage. Finally, the estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation (Levey *et al.,* 2009).

All analyses were performed in IBM SPSS statistics version 23. Continuous variables were reported as means (mean ± SD), whereas categorical variables were expressed as the number and the proportions. Pearsons' correlation was used to measure the correlation between the parameters. Blood pressure, serum electrolytes, serum osmolality, urinary osmolality

RESULTS

One hundred and nineteen (119; 97 males and 22 females) out of 132 definite CKDu cases were responded and enrolled in to the study. The demographic data are shown in Table 1.

Most of the patients (72.3%) were between, 40 to 60 years while the majority were male (81.5%). In the sample, 54.6% of patients were in the early stage (stage 1, 2, 3a) while 45.4% were in the late stage (stage 3b, 4, 5). Only 3.4% of patients had high diastolic blood pressure while 15.4% had high systolic blood pressure. Moreover, the majority of the patients (73.9%) were reported with absence of proteinuria. Among the study subjects, 39.5% were on ACE inhibitors and 10.1% of them were on ARBs. Only 4.2% had shown coronary artery disease.

The clinical characteristics of the study subjects are shown in Table 2. Among them, the average values of serum creatinine, and serum osmolality were increased than the reference range. Only 4.4% and 6.7% of study subjects had increased serum levels of Aldosterone and Angiotensin II. Among the study subjects, 47.5% had normal serum osmolality while 3.4% had low serum molality. And also, 62.7% had normal urine osmolality while 35.6% had low urine osmolality.

Table 3 shows the Pearson correlation of biochemical investigations with serum Aldosterone. Serum Aldosterone was significantly positively correlated with serum creatinine, Angiotensin II, potassium, calcium, bicarbonate and urea while it was significantly negatively correlated with eGFR and Hemoglobin.

Figure 1 shows the significance of serum Aldosterone with the different categories of the study subjects according to the independent-sample median test where significance was considered as $p<0.05$. Serum Aldosterone was significantly increased with the stages of the CKDu ($p= 0.044$), in the late stages compared to the early stage of CKDu ($p= 0.024$) and also with the proteinuria ($p= 0.032$).

Table 1: Demographic and clinical characteristics of the study subjects (n=119)

Patients' characteristics	Frequency	Percentage (%)	Mean ± SD	Range	Reference Range
Age (years)	119		51 ± 9	19 - 76	NA
20-40	12	10.1			
40-60	86	72.3			
60-88	21	17.6			
Gender	119		NA	NA	NA
Male	97	81.5			
Female	22	18.5			
Current disease stage	119		NA	NA	≥45ml/min/1.73m^2 <45ml/min/1.73m^2
Early stage (1,2,3a)	65	54.6			
Late stage (3b,4,5)	54	45.4			
Systolic BP (mmHg)	119		123.7 ± 13	80 - 160	
Low	60	51.3			<129
Normal	39	33.3			129 – 139
High	18	15.4			≥ 140
Diastolic BP (mmHg)	119		77.8 ± 7.2	60 - 100	
Low	04	3.4			< 69
Normal	101	86.3			69 – 89
High	12	3.4			≥90
Proteinuria			NA	NA	
Present	31	26.1			+, ++,> ++
Not present	88	73.9			Nil or trace
ACEI/ARBs			NA	NA	NA
on ACEI/ARBs	58	48.7			
Not on ACEI/ARBs	61	51.3			
Presence of CAD	05	4.2	NA	NA	NA

SD, standard deviation; BP, blood pressure; CAD, coronary artery disease; NA, not applicable

Table 2: Biochemical investigations of CKDu patients (n=119).

Patients' characteristics	Frequency **(%)**	Mean ± SD	Range	Reference Range
Serum creatinine (µmol/L)	119	178.2 ± 122.2	(38.9- 809.9)	M < 113 F < 96
Normal	40 (33.6)			
Low	-			
High	79 (66.4)			
[a]Serum Aldosterone (pg/mL)	114	138.9 ± 85.5	(36.7- 591.9)	70 - 300
Normal	93 (81.6)			
Low	16 (14)			
High	5 (4.4)			
[b]Serum angiotensin II (pg/mL)	105	80.1 ± 72.3	(26.7- 647.8)	50 - 120
Normal	69 (65.7)			
Low	29 (27.6)			
High	7 (6.7)			
[c]Serum osmolality (mOsm/Kg)	118	300.2 ± 23.8	(230 – 413)	275 - 295
Normal	56 (47.5)			

Low	4 (3.4)			
High	58 (49.1)			
[c]Urine osmolality (mOsm/Kg)	118	396.4 ± 181.5	(99 – 985)	300 - 900
Normal	74 (62.7)			
Low	42 (35.6)			
High	2 (1.7)			
Serum Sodium (mmol/L)	119	140.4 ± 6.3	(128 – 156)	136 - 145
Normal	64 (53.8)			
Low	28 (23.5)			
High	27 (22.7)			
Serum Potassium (mmol/L)	119	4.5 ± 0.6	(3.1 – 6.0)	3.5 – 5.1
Normal	98 (82.4)			
Low	5 (4.2)			
High	16 (13.4)			

SD, standard deviation; a n=114, b n=105, c n=118

Table 3: Correlation between blood pressure, renal functions, electrolytes, urine osmolality, serum osmolality and Angiotensin II with the serum Aldosterone

Parameter	Serum Aldosterone	
	r	P
Systolic BP	-0.083	0.382
Diastolic BP	0.002	0.981
eGFR	-0.353**	<0.01
Creatinine	0.477**	<0.01
S. Angiotensin II	0.379**	<0.01
S. Osmolality	0.068	0.477
U. Osmolality	-0.133	0.159
S. Sodium	0.062	0.510

eGFR, estimated Glomerular Filtration rate; BP, blood pressure; S, serum; U, urine
*Correlation is significant at the 0.05 level (2- tailed)
** Correlation is significant at the 0.01 level (2- tailed)

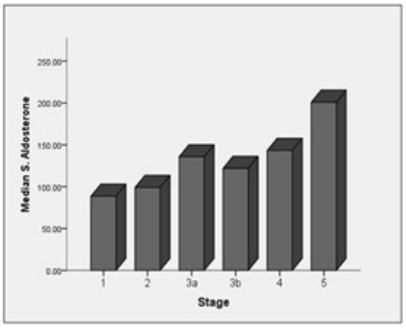

Figure 1-A

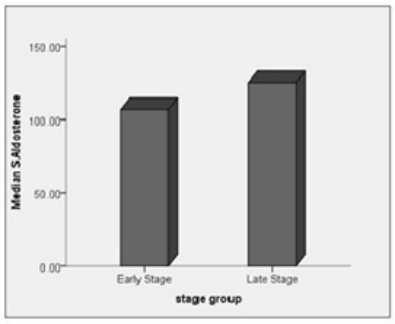

Figure 1-B

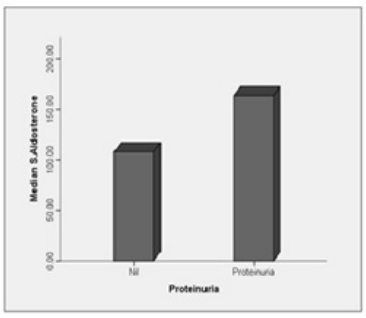

Figure 1-C

Figure 1: Serum Aldosterone (median) among the different categories of the CKDu cases. A. Serum aldosterone (pg/mL, median) among the CKDu stages of the CKDu patients. B. Serum aldosterone (pg/mL, median) between the early stage and the late stage of the CKDu patients. C. Serum aldosterone (pg/mL, median) among the proteinuria and non-proteinuria CKDu patients.

Table 4: Mean comparison between the different markers and the ACEi/ARBs.

	ACEi/ARBs (Mean)									
	Ald	A II	S.osm	U.osm	Na	K	eGFR	U.Pr	SBP	DBP
on	143.4	78.6	301.8	382.5	140.9	4.54	52.8	NA	123.2	77.2
Not on	134.2	81.7	298.8	409.3	139.9	4.46	50.1	NA	124.1	78.5
Sig.	**0.569**	**0.826**	**0.493**	**0.423**	**0.439**	**0.501**	**0.604**	**0.532**	**0.704**	0.316

Ald, Aldosterone; AII, Angiotensin II; S, serum; U, urine; U.Pr, urine protein; NA, not applicable

According to Table 4, Blood pressure, proteinuria, eGFR, Osmolality, sodium, potassium, Aldosterone and Angiotensin II were not significantly different in the patients who were on ACEi/ARBs and not on ACEi/ARBs.

According to the Independent sample- T test, sodium, bicarbonate and urea were significantly different ($p<0.01$) between the high serum osmolality group and the other group (low or normal serum osmolality). Potassium and Phosphorus were significantly different ($p<0.01$) between the high sodium group and the other group (sodium low & normal).

DISCUSSION

In this cross-sectional study, we discovered no indications of activated RAAS in CKDu, an environmental nephropathy in tropical countries. 95% of the population diagnosed with definite CKDu, had their serum Aldosterone levels within normal limits. There were normal serum Angiotensin II levels, normal potassium levels, normal blood pressure and no evidence of cardiovascular disease (CVD) in the majority. All these markers were negatively correlated with eGFR indicating RRAS activation in advanced disease as in CKD (Qian *et al.,* 2018).

Current study is mostly represented by young or middle age male farmers, similar to previous studies on CKDu (Athuraliya *et al.,* 2009; Athuraliya *et al.,* 2011; Jayasekara *et al.,* 2013). CKDu, at least in earlier stages, along with other tubular interstitial diseases, are not proteinuric or hypertensive. In addition, CVD a major cause of mortality in CKD is reported to be less prevalent in CKDu (Hettiarachchi *et al.,* 2021). In the same way, only 25% of the study population had significant proteinuria (one plus or more) and there was very low prevalence of CVD among the participants (1%). In addition, more than 50% of study participants had adequate blood pressure control without even requiring ACEi or ARBS, the first line anti-hypertensive in CKD or CKDu (Athuraliya *et al.,* 2011; Nanayakkara *et al.,* 2014; Wijkstrom, 2018). Along with lower prevalence of hypertension, proteinuria and CVD, only 4.4% and 6.7% of the population had high Aldosterone and Angiotensin levels. In contrast, it has been described that, hypertension, proteinuria, CVD and activated RAAS are common in CKD (Bianchi *et al.,* 2005; Go *et al.,* 2004; Iravanian and Dudley, 2008). Further, serum Aldosterone levels were reported to be high in more than 90% of CKD. However, there was a negative correlation between Aldosterone, Angiotensin II and other disease severity markers with eGFR indicating RAAS activation in advanced CKDu, similar to pattern described in CKD (Qian *et al.,* 2018).

Dehydration and recurrent AKI are commonly discussed risk factors for CKDu which supported by several studies (Johnson and Sanchez-Lozada, 2013; Wesseling *et al.,* 2016). In accordance with, hypernatremia was observed in more than 20% of study population with a positive correlation between serum Sodium and serum osmolality, indicating subclinical dehydration. Theoretically, RAAS activation play a protective role in such scenario by preventing AKI, whereas RAAS blockers could be detrimental. Hence, in the absence of compelling indications like albuminuria, CVD and hypertension, with possible causal relationship to dehydration and recurrent AKI in CKDu, RAAS blockers have to be used cautiously. In accompanying with, a randomized controlled trial reported a faster progression of CKDu in Enalapril group beside significant reduction in proteinuria (Selvarajah and Mendis, 2018). The effect may be similar to that was observed in CKD in elderly population by having faster progression on initiation or paradoxical improvement with the cessation of RAAS blockers (Turgut *et al.,* 2010).

Classically, aldosterone serves to increase sodium reabsorption in the distal convoluted tubule of the kidney in order to maintain circulatory homeostasis and extracellular volume of the human body (Hirsch *et al.,* 2016). Further, Aldosterone induces inflammation, oxidative stress, activation, accelerated fibrosis and enhancement of angiotensin II (Fiebeler *et al.,* 2017; Young, 2018). Hence, scientists have suggested that aldosterone blockade can lead to improve meaningful clinical outcomes in CKD (Toto, 2010). RAAS system is generally activated as a protective response in the presence of hypovolemia and hypotension. In contrast, inappropriate activation of RAAS, have been described in cardiovascular diseases and CKD which associated with adverse outcome. Therefore, features of activated RAAS such as Na and fluid retention, hypertension, commonly manifest in CKD. Interestingly, those features are not common in CKDu and now our results confirm, the poorly activated RAAS in these patients. Interestingly more than 95% of CKDu patients had normal serum aldosterone levels thus a competitive biomarker to evaluate RAAS activity in CKD. Such marker is extremely important in some CKD categories with high pre-renal AKI risk on initiation of RAAS blockers.

CONCLUSION

In this cross-sectional study, we have found that there were no indications of activated RAAS in patients with the diagnosis of definite CKDu. Thus, in the presence

of indications like proteinuria and hypertension, RASS blockers must be considered as first-line treatment. Nevertheless, considering the possible risk of recurrent AKI due to strenuous exertion in hot weather, the use of RAAS blockers has to be cautious. A significant number of the population had hypernatremia which is positively correlated with hyperosmolality, indicating dehydration. Our findings raise the possibility of subclinical dehydration that may affect faster progression.

ACKNOWLEDGEMENT

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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