Original article:

Prevalence of Chronic Kidney Disease in Irakkamam and Addalaichenai DS Divisions of Sri Lanka: An Investigation on Its Association with the Lifestyle Patterns of the Patients

AMM Asmath¹, MMM Najim^{2,5}, Muneeb M Musthafa¹, LF Zisath Shama³, I Farhath Ahamed⁴, UL Abdul Majeed¹.

Abstract

Background: Chronic kidney disease (CKD) has currently become one of the most burning health issues of national concern in Sri Lanka. **Objective:** To assess the lifestyle factors associated with CKD among people of Sri Lanka. **Methods:** A pretested structured questionnaire was administered to collect the data from kidney patients. Lifestyle patterns and other possible contributing factors were investigated between the CKD patients reported in Irakkamam and Addalaichenai Divisional Secretariats Divisions (DSDs) to find out the significant association between both the DSDs. SPSS software was used to perform all the statistical analyses. **Results:** Variables including etiology for CKD, being a farmer, and exposure to direct sunlight showed significant differences (p<0.05) between both the DSDs. Compared to Addalaichenai DSD, the well-known causes of CKD including diabetes and hypertension were not seemed to be a significant factor contributing to CKD in Irakkamam DSD. In contrast, obstruction and inflammation contributed greatly to the majority of cases in Irakkamam DSD. Moreover, CKDu cases were significantly higher in Irakkamam DSD compared to Addalaichenai DSD. **Conclusion:** Proper awareness programs among this region's people have a chance to reduce the emerging issue of CKDu in these areas.

Keywords: Chronic kidney disease (CKD), Chronic kidney disease of unknown etiology (CKDu), Sri Lanka

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Introduction

Chronic Kidney Disease (CKD) is exerting a major burden on the health- care system of Sri Lanka, where diabetes, hypertension, and glomerulonephritis are well-recognized etiologies.¹ In Sri Lanka, roughly 17,000 km² of the total area was affected by CKD with 2.5 million of the human population most of whom reside in rural areas.² Northern Central Province is the region with the highest prevalence of CKD in Sri Lanka, where 4.8 % of the total population mostly the farming community, is affected by kidney failure, especially in the Medawachchiya region.³ Around 3,000-3,500 patients are being treated every year at the Nephrology unit of Kandy teaching hospital, out of which 50-69 % of patients are hailing from the North Central Province.⁴ Some Sri Lankan physicians estimate that 300 to 600 deaths related

- 1. Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka, Sri Lanka
- 2. Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Sri Lanka
- 3. Department of Information and Communications Technology, Faculty of Technology, South Eastern University of Sri Lanka, Sri Lanka
- 4. Medical Officer, Akkaraipattu Base Hospital, Sri Lanka
- 5. Faculty of Agriculture, Universiti Islam Sultan Sharif Ali, Kamus Sinaut, Kampong Sinaut, Tutong TB 1741, Negara Brunei Darussalam.

Correspondence to: Muneeb M. Musthafa, Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka, Sri Lanka. E-mail: muneeb@seu.ac.lk

to CKD occur in hospitals annually. Although home fatalities are rarely recorded in death registers, therefore the actual countrywide number of deaths related to CKD could be as high as 1,400 every year.⁵ In Sri Lanka, hospital admissions for all diseases related to the genitourinary system doubled between 1990 and 2007, with hospital fatalities from such diseases increasing from 2.6% to 9.1% per 100,000 persons.² On the other hand, the prevalence of CKD is spread over the nearest provinces from North Central Province including the North-Western, Eastern, and Uva Provinces, as well as the Central and Northern Provinces.⁶ According to Kafle et al.,⁷ Eastern Province, particularly Ampara District, seems to be an emerging hotspot for CKD. Moreover, 15.3% of people who were affected by CKD are in Ampara district, which was in the fourth place among all the most affected districts in Sri Lanka. According to the results of the preliminary survey conducted, the death rate of CKD in the Ampara district increased dramatically in the last decades. The number of deaths of patients during 2005-2020 (Figure 1) and deaths by gender during 2010-2020 (Figure 2) due to CKD at all ages in Ampara district are shown. The risk of the burden on Sri Lanka's health care system is indicated by the increasing values of CKD patients and deaths each year.



Figure 1: Number of deaths of CKD patients reported during 2005 - 2020 in Ampara district; Source: Office of the Regional Director of Health Services, Ampara (RDHS)



Figure 2: Number of deaths of CKD patients reported by gender during 2010 - 2020 in Ampara district; Source: Office of the Regional Director of

Health Services, Ampara (RDHS)

Moreover, Dehiattakandiya, Padiyathalawa and Mahaoya District Secretariat Divisions (DSD) were already identified as high-risk areas for CKD among the 20 divisional secretariats in Ampara district. The prevalence of CKD has shown an increase recently at an alarming rate in Irakkamam DSD, where the prevalence of CKD was reported as 0.25 % in the general population. Even though, Irakkamam DSD is not identified as a high-risk DSD, there is an increasing trend of CKD/CKDu recently as per the preliminary survey conducted and no significant studies have been reported on these from the Irakkamam DSD. On the other hand, a similar number of kidney patients were recently reported as same as Irakkamam DSD from the nearby DSD Addalaichenai. Furthermore, the yearly increasing prevalence of CKD and CKDu cases in Irakkamam DSD, and the most significant factors influencing CKDu was unknown. Therefore, to find out the significant associating factors that contributes to this deadly disease, the lifestyle pattern and other possible contributing factors were investigated between the CKD patients reported in Irakkamam DSD with the patients reported in the nearby DSD of Addalaichenai. Therefore, this study aims to assess the factors associated with CKDu in Irakkamam DSD and identify significant factors that contribute to the development of CKD in comparison to the nearest DSD of Addalaichenai.

Methods

Study area: Irakkamam and Addalaichenai DSD are situated in the dry zone of the Eastern Province of Ampara district of Sri Lanka. (Figure 1). Both DSDs are prominent regions for agriculture and cover an area of 83.4 km² and 94.5 km², respectively. Irakkamam DSD is located between the longitude of 81°39'48"E and 81°46'13"E and the latitude of 7°17'50"N and 7°11'36"N while, Addalaichenai DSD lies along the east coast of Sri Lanka and situated between the longitude of 81°46'12"E and 81°51'57"E and the latitude of 7°18'14"N and 7°14'28"N. In Irakkamam DSD, there are currently 19,484 people living, with 5,514 families. Nearly 1755 and 366 individuals are engaged in agricultural practices and freshwater fishing, respectively. Meanwhile, in Addalaichenai DSD currently, 52,743 people live with 15,795 families. Nearly 2,524 and 2003 individuals are engaged in agricultural practices and both marine



Figure 3. Map of Irakkamam and Addalaichenai DSDs

and freshwater fishing, respectively. Male : female ratio is nearly 1:1 in both the DSDs.

Sample selection: To assess the relationship between the reported patients in Irakkamam and Addalaichenai DSDs and associated factors related to CKDu, all the kidney patients in both DSDs (n=93) were selected and assessed using a questionnaire. None were excluded from the study.

Data collection: The questionnaire was prepared including the objectives of this study, consisting of questions related to socio-economic and general information of patients, their employments, medical history of the patients, and lifestyle of the patients, including work, feeding, drinking, and other practices, with the help of experts in the field. A pilot survey was done before the data collection to validate the questionnaire. The questionnaire's flaws were identified, and the necessary changes were made. The finalized questionnaire was used to collect data from all the kidney patients in both the DSDs. The data collection started in February 2021 and was completed by September 2021. The patient's records were collected from Addalaichenai and Irakkamam DSDs, Addalaichenai and Irakkamam Ministry of Health (MOH), and the Regional Director of Health Services Offices (RDHS) in Ampara. The data on patient details were collected using the questionnaire, and the information on the medical conditions of the patients were collected from the clinical records of the patients with the help of the doctor.

Data Analysis: Data were analyzed using the statistical software SPSS (version 26.0) with a non-parametric test using the collected qualitative data based on the responses to the questions. Descriptive analysis, chi-square test, and Binary Logistic Regression model were used to analyze the data. Association between the reported patients in Addalaichenai and Irakkamam DSD were identified by the chi-squared test at a p=0.05 significant level. Further, forward stepwise logistic regression analysis was performed to identify the confounding risk factors for CKDu.

Results

The results of the present study revealed that both Irakkamam and Addalaichenai DSDs are not identified as high-risk DSDs, and seem to show a quick surge in CKD/CKDu. These localities are becoming emerging hotspots of CKD/ CKDu because there is an increasing trend of the prevalence of the disease with cases related deaths increasing annually. In Irakkamam DSD 2, 4, and 5 deaths were reported in 2019, 2020, and 2021 respectively but in Addalaichenai DSD 2, 7, and 2 deaths were reported in 2020, 2021, and 2022 respectively. Non-communicable diseases including diabetics (25.5%) and hypertension (20.9%) played a major role in developing CKD in Addalaichenai DSD but in Irakkamam DSD, the majority (28%) of kidney patients' etiology is unknown (Table 1). However, obstruction and inflammation account for 20% and 18% of all kidney patients' causative factors, respectively. It further implies that these non-communicable diseases have little influence on the development of CKD in Irakkamam DSD compared to Addalaichenai DSD. Moreover, etiologies of both DSDs show significant difference (p<0.05) between them. In Irakkamam DSD, CKD was more prevalent among males, where the male: female ratio for CKD is nearly 3:2 but CKD has the same prevalence among the males and females in Addalaichenai DSD, where male: female ratio for CKD is nearly 1:1 (Table 2). No significant difference (p=0.229) was observed in terms of gender between both the DSDs. The most vulnerable age group for CKD in Addalaichenai DSD is 40-60, comprising to 41.9 % of the total affected population but, CKD is more prevalent among the younger generation in Irakkamam DSD where the majority (34%) of the CKD patients were below the age of 40. Nearly 34% of the CKD patients were farmers in Irakkamam DSD, while only 7% of the patients were farmers in Addalaichenai DSD. Comparably housewives were nearly the same numbers among the affected population in both the DSDs. Moreover, in Addalaichenai DSD, 23.3% of the CKD patients were businessmen. However, the main occupation of the CKD patients showed a significant difference (p=0.006) between both the DSDs. The incidences of CKD were more prevalent among those who have married and studied below Ordinary Level (O/L) in both DSDs. The unemployment rate among CKD patients is higher compared to the general population and they normally lose their job after the development of this deadly disease because their body condition become unfit for work and they have to spend more time on clinical and dialysis purpose. Therefore, in Irakkamam and Addalaichenai DSD, 60% and 74.4% of the affected population were unemployed, respectively. The majority of the CKD patients in both DSDs have low personal monthly income, however comparably majority (65.2%) of the CKD patients from Addalaichenai DSD have below Rs. 10, 000 (27.35 USD) as their income. Compared to Irakkamam DSD, majority of the affected populations were females in

Addalaichenai DSD. Females in these areas usually do not want to work due to cultural preferences. All CKD patients received Rs. 5,000 per month (13.68 USD) as a government allowance to help them meet their basic needs.

Table 1: Etiology of kidney patients	in
Irakkamam and Addalaichenai DSD	

Etiology	Percentage of patients in Irakkamam DSD (n=50)	Percentage of patients in Addalaichenai DSD (n=43)
Diabetics	5 (10%)	11 (25.5%)
Hypertension	3 (6%)	9 (20.9%)
Both diabetics and hypertension	2 (4%)	6 (13.9%)
Glomerular disease	-	1 (2.3%)
Obstruction/ stones	10 (20%)	4 (9.3%)
Inflammation/ Infection	9 (18%)	5 (11.6%)
Birth defect	5 (10%)	4 (9.3%)
Acute Kidney Injury (AKI)	2 (4%)	1 (2.3%)
Leptospirosis	-	1 (2.3%)
Unknown	14 (28%)	1 (2.3%)

Table 2: Demographic data of kidney patients in
Irakkamam and Addalaichenai DSD

_	Irakl DSD	kamam (n=50)	Adda DSD	laichenai (n=43)	
Variables	n	%	n	%	P value
Gender					0.229
Male	31	62	22	51.2	
Female	19	38	21	48.8	
Age					0.717
<40	17	34	12	27.9	
40-60	15	30	18	41.9	
61-75	16	32	10	23.3	
>75	2	4	3	6.9	
Main					0.006
Farmer	17	34	3	7	
House wife	13	26	15	34.9	
Driver	4	8	-	-	
Businessman	2	4	10	23.3	
students	6	12	6	14	
others	8	16	9	20.9	
Current					0.570
employment					0.570
Unemployed	30	60	32	74.4	
students	6	12	3	9.3	
employed	14	28	7	16.3	
Education					0.183
Below O/L	48	96	38	88.4	

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	Irakkamam DSD (n=50)		Addalaichenai DSD (n=43)		
Variables	n	%	n	%	P value
O/L	1	2	5	11.6	
A/L	1	2	-	-	
Monthly					
personal					0.052
income					
< Rs.10, 000	22	44	28	65.2	
Rs.10, 001- 15.000	4	8	8	18.6	
Rs.15, 001-20	7	14	3	7	
Rs.20, 001- 25,000	9	18	2	4.7	
>Rs.25, 000	8	16	2	4.7	-

Medical history of CKD patients: Medical history of the CKD patients showed that in both DSDs, the majority of the patients were affected by noncommunicable diseases including diabetes and hypertension (Table 3). However, in Irakkamam DSD, 66 % of the patients have a history of noncommunicable diseases, but in Addalaichenai DSD 79.1 % of the CKD patients were having noncommunicable diseases. Moreover, among those who have a history of diabetics and hypertension, nearly 41.17 % of patients got their CKD after the development of both diseases, and only 31.8 % and 25.9 % of CKD patients have been suffering from diabetes and hypertension for more than ten years in Irakkamam DSD. On the other hand, in Addalaichenai DSD, nearly 14.7 % of the patients were diagnosed with hypertension and diabetics secondarily, only after they developed CKD, and 47 % and 38.2 % of the CKD patients had diabetes and hypertension for more than ten years. Therefore, compared with Addalaichenai DSD, in Irakkamam these non-communicable diseases have little influence on the development of CKD. In both DSDs, higher number of patients got their CKD recently, with nearly 42 % of the patients diagnosed with CKD within 3.5 years. In Addalaichenai 11.6 % of CKD patients have a family history of CKD, but in Irakkamam DSD, 18 % of them have a family history of CKD. However, a family history of CKD showed a nonsignificant relationship (p=1.000) between both DSDs. Compared with Addalaichenai DSD, the majority (16%) of the CKD patients in Irakkamam DSD have experienced a snake bite, but this didn't show any significant differences (p=0.052) between both the DSDs. The majority of the CKD patients in both the DSDs have a healthy weight however 10 % and 13.9 % of the patients are obese in Irakkamam and Addalaichenai DSD, respectively.

Irakkamam and Addalaichenai DSD						
Variahles	Irakkamam DSD (n=50)		Addalaichenai DSD (n=43)		P value	
variabics	n	%	n	%		
Affected years by kidney disease					0.779	
less than 3.5	21	42	18	41.9		
3.5 to 6	14	28	10	23.3		
More than 6	15	30	15	34.8		
Family history of CKD					1.000	
Yes	9	18	5	11.6		
No	41	82	38	88.4		
Snake bite					0.052	
Yes	8	16	1	2.3		
No	42	84	42	97.7		
BMI					0.595	
Under weight	7	14	8	18.6		
Healthy weight	22	44	18	41.9		
Over weight	16	32	11	25.6		
Obese	5	10	6	13.9		
History of diseases					0.229	
Diabetics	22	44	24	55.8		
Hypertension	27	44	27	62.7		
Both diabetics & hypertension	17	34	18	41.8		

Table 3: Medical history of the kidney patients in

Relationship between kidney patients with agrochemicals: The collected data revealed that compared with Addalaichenai DSD, patients from Irakkamam DSD were mostly linked with the agricultural sector. However, paddy farming is predominant among CKD patients from Addalaichenai DSD while in Irakkamam DSD patients were commonly engaged in both paddy and sugar cane farming. In Irakkamam DSD, 26 % of CKD patients have usually been exposed to agrochemicals and the majority of them (12 %) have reported exposure to agrochemicals for more than 10 years (Table 4), but in Addalaichenai DSD, only 18.6 % of the CKD patients have been exposure to agrochemicals and 9.3 % of them have reported exposure to agrochemicals more than 10 years. However, exposure to agrochemicals including fertilizer, pesticides, and weedicides didn't show any significant relation (p=0.473) between patients from both the DSDs.

 Table 4: Relationship between kidney patients

 and handling agrochemicals in both Irakkamam

 and Addalaichenai DSDs

	Irakkamam DSD (n=50)		Addalaichenai DSD (n=43)		
Variables	n	%	n	%	P value
Exposure to agrochemicals					0.473
Yes	13	26	8	18.6	
No	37	74	35	81.4	
Duration of exposure of agro chemicals					0.465
< 5 years	5	10	3	6.9	
5 - 10 years	2	4	1	2.3	
>10 years	6	12	4	9.3	
Frequency of exposure of agro chemicals					0.239
Rarely	3	6	3	6.9	
Some times	1	2	2	4.6	
Often	9	18	3	6.9	

Relationship of behavioral factors with CKD patients: According to the data, in Irakkamam DSD, the majority (32 %) of the patients usually have the practice of working under the sun (Table 5), further habitually they work under the sun for 6 to 8 hours per day and 5 to 7 days per week. But in Addalaichenai DSD, only a few (7 %) patients had this habit. The habit of exposure to direct sunlight was significantly (p=0.037) higher among Irakkamam DSD patients compared to patients from Addalaichenai DSD. In both DSDs, the majority of the patients have a habit of drinking water nearly 2 to 3 liters per day, but compared to Addalaichenai DSD, patients from Irakkamam DSD drink a low amount of water per day, nearly 20 % of the patients consume less than two liters of water per day. However, the habit of drinking water didn't show any significant relationship (p=0.168) between both the DSDs. Based on the data, 26 % of the patients from Irakkamam DSD had the habit of quenching their thirst in farm field by drinking water from an agricultural canal. These types of behaviors result in the ingestion of heavy metals, algal toxins, and inorganic ions, all of which increase the risk of CKD and put them in trouble, but in Addalaichenai DSD, 23.3 % of the patients had this undesirable habit. In both DSDs, habits of alcohol consumption and smoking practices were comparably very low among the patients, but in Irakkamam DSD, 24 % of the patients were daily smokers. No significant (p>0.05) differences on these habits were found between the patients from both the DSDs.

In both DSDs, kidney patients used varieties of water sources for their daily needs. Based on the collected data, the ranking of the mostto-least-used water sources by kidney patients in both DSDs was well, water supplied by the water board, "Poowell" (subterranean water from nearly 2 to 3 feet dug very close to the canal side), reservoir, and tube well, respectively (Figure 4). However, the habit of the use of different water sources didn't show any significant relationship (p=0.211) between patients from both the DSDs. Furthermore, in Irakkamam DSD, nearly 78 % of the kidney patients used well water sources for their daily needs including cooking, drinking, and bathing purposes for more than 15 years, as well as in Addalaichenai DSD nearly 86 % of kidney patients used well water sources for more than 15 years. However, tube wells were rarely used by kidney patients for their day to day purposes in both the DSDs. In Irakkamam DSD, more than 70% of kidney patients usually consumed lake fish, particularly Oreochromis niloticus, at least two days per week, and few consumed marine fish, but in Addalaichenai DSD, more than 60% of the patients consumed marine fish twice a week. Furthermore, either meat or chicken was commonly consumed by most of the patients per week in both DSDs. 14 % of the patients from Irakkamam DSD and 9.3 % of the patients from Addalaichenai DSD habitually consumed freshly harvested rice and vegetables. Moreover, few of the patients from both the DSDs have the habit of consuming star fruits and water-related plantbased food items, including lotus seeds and water lily seed powder.

Table 5: Behavioral factors of kidney patients inboth Irakkamam and Addalaichenai DSDs

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Variables	Irakkamam DSD (n=50)		Addalaichenai DSD (n=43)		Р
variables	n	%	n	%	value
Exposure to direct sunlight					0.037
Rarely	3	6	1	2.3	
Sometimes	9	18	5	11.6	
Often	16	32	7	16.3	
Never	22	44	30	69.8	
Water consumption per day					0.168
<2	10	20	4	9.3	
2 to 3	28	56	27	62.8	
3 to 4	9	18	8	18.6	
>4	3	6	4	9.3	
Habit of drinking water in farm field					0.377
Yes	18	36	10	23.3	
No	32	64	33	76.7	
Alcohol consumption					0.570
Daily	1	2	1	2.3	
Once a week	3	6	3	7	
Once a month	1	2	0	0	
Never	45	90	39	90.7	
Smoking					0.200
Yes	12	24	5	11.6	
No	38	64	38	88.4	

Discussion

Recently the prevalence of CKD increased at an alarming rate in Irakkamam and nearby Addalaichenai DSDs. Even though, both DSDs are not identified as high-risk DSDs, there is an increasing trend of CKD/CKDu recently as per the preliminary survey conducted. Hence, whether there is any correlation between the practices and lifestyle of kidney patients in Addalaichenai and Irakkamam DSD were investigated in detail and a confounding risk factor for CKDu in Irakkamam DSD was identified through this study. Non-communicable diseases including diabetes and hypertension have a higher influence on the development of CKD in Addalaichenai DSD compared to Irakkamam DSD. Moreover, obstruction and inflammation played a major role in developing CKD. Further, a considerable percentage of kidney patients' etiology is unknown in Irakkamam DSD.

CKD was more prevalent among males in both DSDs. However, male prevalence is much higher in Irakkamam DSD compared to Addalaichenai DSD, where male-female ratio for CKD is nearly 3:2 and 1:1 in Irakkamam and Addalaichenai DSDs, respectively. Several studies reported that the prevalence of CKD, disease severity, and mortality rate tend to be higher in males with a faster progression rate than in females due to their unhealthy lifestyles and hormonal variations.^{8,9,10} The higher risk associated with the male sex is probably a consequence of sex-related exposures or behavior including occupation.¹¹ The most vulnerable age group for CKD in Addalaichenai DSD is 40-60 but, CKD is more prevalent among the younger generation in Irakkamam DSD where the majority of the CKD patients were below the age of 40. Some researchers state that the risky age group for CKD is 30-50.12,13,14 Furthermore, Coresh et al. (2007)¹⁵ found that CKD is significantly associated with gender for both men and women and that age is the key determinant related to CKD. However, no significant difference (p>0.05) was observed in term of gender and age between both DSDs.

In comparison to Addalaichenai DSD, patients from Irakkamam DSD were mostly associated with agricultural work. Therefore, a high level of agrochemical exposure was found among Irakkamam DSD patients. However, there was no substantial relationship (p>0.05) between agrochemical exposure and patients from both the DSDs. Several studies state that exposure to agrochemicals including pesticides, fertilizers, and insecticides accelerates the risk for kidney function and which may also be contributing to kidney failure.^{16,17} Moreover, organochlorine pesticides have been found in higher concentrations in patients with CKD.¹⁸

Compared to Addalaichenai DSD, kidney patients from Irakkamam DSD were usually working under the direct sun because the majority of them carried



Figure 4. The rank most to least used of water sources by kidney patients in both DSDs.

out agriculture-related occupations. Moreover, they are frequently subjected to high levels of heat stress-related problems because they needed to work under pressure for a long time in burning sunlight and extremely high temperatures and suffer from dehydration. According to Gobalarajah et al.,¹⁹ low water intake during farming activities, combined with dehydration from direct sunlight exposure, may have resulted in kidney failure. Yaqub²⁰ states that farmers may not be consuming enough water during farming activity due to a bad taste caused by toxins and chemicals in canals and reservoirs. However, the habit of exposure to direct sunlight of patients was significantly (p<0.05) higher among Irakkamam DSD patients compared to patients from Addalaichenai DSD. Compared to Addalaichenai DSD, patients from Irakkamam DSD consume a low amount of water per day. However, a habit of consuming water per day didn't show any significant association (p>0.05) between both the DSDs. Working under the sun for more than six hours per day and consuming less than three liters of water per day were found to significantly increase the risk of contracting CKD.²¹ Smoking and alcohol consumption were both relatively uncommon among patients in both DSDs. However, compared to Addalaichenai DSD, a considerable number of CKD patients were daily smokers in Irakkamam DSD, and there were no other discernible variations in these habits between the two groups of DSD patients. Several studies state that the habit of smoking and alcohol intake respectively, were more closely associated with CKD than were non-alcoholics and nonsmokers.^{22,23} Dug wells served as the commonest

source of drinking and cooking water but tube wells were used not much by CKD patients in both DSDs. According to Yaqub,²⁰ 90% of nephropathy patients in high-prevalence regions are farmers who primarily rely on shallow dug wells for their drinking water. However, water sources used by CKD patients did not show any significant relationship (p>0.05) between both the DSDs.

Conclusion

Addalaichenai and Irakkamam DSDs have a moderate prevalence of CKD with an increasing incidences and it has a high chance of emerging as a hotspot in the future. In this present study, links between CKD patients in both DSDs and factors associated with CKDu in Irakkamam DSD were analyzed. Variables including etiology for CKD, being a farmer, and exposure to direct sunlight showed significant differences between both DSDs. Compared to Addalaichenai DSD, the well-known causes of CKD including diabetes and hypertension were not seemed to be a significant factor contributing to CKD in Irakkamam DSD. In contrast, obstruction, infection/inflammation contributed greatly to the majority of cases in Irakkamam DSD. Moreover, CKDu cases were significantly higher in Irakkamam DSD compared to Addalaichenai DSD.

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Authors' contribution: All authors were equally involved in conception, study design, data collection, statistical analysis, writing, editing, and final approval of the manuscript.

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