

Impact of water quality on Chronic Kidney Disease of unknown etiology (CKDu) in Thunukkai Division in Mullaitivu District, Sri Lanka

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Abstract

Background

Increase in the number of cases in Chronic Kidney Disease of Unknown etiology (CKDu) in Sri Lanka has become an environmental health issue of national concern. Even though, Northern Province is not identified as a high risk province, there is an increasing trend of CKDu after the end of civil war in the Northern territory.

Methods

The present study was conducted in Thunukkai Division in Mullaitivu District to investigate the socio demographic and clinical pattern of CKDu patients and to evaluate the quality of their water sources. The samples were selected by using stratified purposive random sampling method which represented 29% of total CKDu patients in Thunukkai Division. Pretested structured questionnaire was administered to collect the data from CKDu patients.

Results

Among the patients, 80% of them were male with an age range of 30-80, majority (88%) involved in farming. 26% and 20% of people were engaged in smoking and taking alcohol, respectively. 17% and 43% were suffered with diabetes and hypertension, respectively. Physicochemical characteristics of more than 50% of the water samples recorded higher electric conductivity, salinity, total dissolved solids, total hardness and Na levels compared to drinking water standards in Sri Lanka.

Conclusions

The association between serum creatine excreted by CKDu patients and water quality parameters were determined by using regression model, revealing a significant negative relationship with phosphate and positive relationship with Total Dissolved Solid and arsenic content of the drinking water. Thus, the study suggests, total dissolved solid and arsenic in drinking water have a positive correlation with CKDu in Thunukkai region in the Mullaitivu District.

Background

Chronic Kidney Disease of unknown aetiology (CKDu) is the occurrence of Chronic Kidney Disease (CKD) without a known underlying cause [1]. Since its first report in mid-90's cases of CKDu have been increased tremendously in North Central Province of Sri Lanka [2]. It is estimated that thousands of Sri Lankan people are affected by CKDu, mostly poor families living in remote areas. However, the exact number of CKDu patients and causes of the disease are unknown. Unfortunately, the research studies conducted to date were unable to provide exact cause/s of CKDu. A common conclusion is that the CKDu is caused by multiple factors involving environmental and social impacts [3].

There are several aetiologies, proposed by the researchers of CKDu, including demographic factors of the affecting community [4], quality of their drinking water including hardness [5], agrochemical and heavy metal contaminations [5,6] fluoride level [7], the genetic makeup of vulnerable populations [8], etc. Demographic factors include the socioeconomic characters of a population such as age, sex, occupation etc. Several studies conducted in the Northern central province revealed that the main livelihood of CKDu affected population is farming and the age of the patients is ranged between 30 – 60 with higher prevalence among elderly males over 50 years of age [2,5,9]. Low water consumption during farming activities and dehydration due to the exposure to direct sunlight may have led to renal failure [5].

High groundwater hardness and the occurrence of CKDu are correlated frequently in many CKDu studies in Sri Lanka (8-11). According to World Health Organization (WHO) hard water is mainly caused by the presence of Calcium, Magnesium, Strontium, and Iron together with carbonate, bicarbonate, sulphate and chloride anions [12]. Furthermore, possible correlation between fluoride (F) in drinking water and the prevalence of CKDu was suggested in various instances [10, 11, 13]. According to WHO (2011), Sri Lanka is one of the tropical countries in the world with higher fluoride content, reaching the upper limit value of 0.6 mg/L [12]. In most of the CKDu endemic areas F content exceeds the upper limit value [14]. Maximum permissible contaminant level of Arsenic (As) is 10 µg/L [12] though As contamination in the disease endemic regions exceeded the upper limit [15]. However, Balasooriya et al., [9] and Nanayakkara et al., [8] revealed insignificant levels of As and other trace elements in drinking water of CKDu endemic areas of Sri Lanka.

Algal toxins have also been considered as a suspect of the CKDu [16]. According to WHO, eighteen different types of cyanobacteria are capable of producing toxins under favourable conditions. Among them, fifteen toxic producing cyanobacteria have been identified in Sri Lankan reservoirs and canals. These toxins are identified as hepatotoxic, dermatotoxic, neurotoxic and nephrotoxic compounds causing toxic responses in animals [16].

CKDu has direct impact on patients' lives including their livelihood activities. As the disease advances, patients become too ill to continue their employment, affecting the economy and wellbeing of the entire family. Due to the severity of the problem, Presidential task force was established in 2015 to take preventive and welfare measures for the benefit of the people in the CKDu endemic areas.

Current distribution data of CKDu show the occurrence of the disease in North Central, North Western, North Eastern, Southern, Eastern and Uva Provinces. Even though, Northern Province is not identified as a high-risk province, CKDu is developing at an alarming rate after the end of civil war in Northern part of this country. It may be due to increased use of agrochemicals and newly emerged industries with unplanned effluent disposal leading to aquatic pollution in natural reservoirs. Northern Province comprises of five districts; Jaffna, Killinochchi, Mullaitivu, Vavuniya and Mannar. Among these Mullaitivu and Vavuniya have been considered "at risk" for the occurrence of CKDu with 09 other districts from North Central, Central and Uva provinces [17]. And Northern province appear to have higher CKD prevalence than Central or Southern provinces [18]. In the present study, Thunukkai of Mullaitivu District

was selected as a scarcely studied area under the perspective of CKDu. Most of the people in Thunukkai are farmers, who carried out farming throughout the year. Majority in Thunukkai Divisional secretariat use shallow dug wells for their daily consumption. They consume water without any treatment in the same way as the people consume water in Anuradhapura district. Thunukkai Divisional Secretariat has many reservoirs. Some GN ("Grama Niladhari"-Public service officer) divisions are named according to the name of reservoirs such as Anichakulam, Thenniyankulam, Kodaikadiyakulam etc. Algal blooms are a common sight in many of these reservoirs.

Objectives of this study were to analyze the socio demographic and clinical pattern of CKDu patients in Thunukkai Division, and to evaluate the water quality parameters, such as heavy metals(As, Cr), nitrate, phosphate, fluoride, hardness, Total Dissolved Solids (TDS), Na, K and electric conductivity of the drinking water sources of the area and to assess possible correlations between water quality parameters and clinical data obtained from CKDu patients, particularly the serum creatinine levels.

Methods

Ethical clearance was obtained from the Ethics Review Committee, Faculty of Medicine, University of Jaffna. Data regarding CKDu patients were gathered during the period of January 2018 to July 2018 from respective Regional Director of Health Service in Northern Province. In Mullaitivu district, 631 cases of both CKD/CKDu were identified in 3 Medical Office of Health (MOH) which are Thunukkai (120 cases), Manthai East (86 cases) and Sampathnuwara (425 cases). Among these Thunukkai was selected for study purpose due to many reasons including, higher CKDu cases (120/631 cases), farming activities conducted throughout the year as the main livelihood of people in the area, disturbances occurred during the civil war in Northern Province, the use of natural ponds in the area for drinking and other purposes.

In Thunukkai Division 120 patients, out of the total population of 10,172 were identified as CKD/CKDu patients by MOH office Mallavi. Sample size (n) was estimated with $n = Z^2 p(1-p) / e^2$, where Z= confidence level at 95% (i.e. 1.96), p= estimated prevalence of the area (120/10,172= 0.0118) and e^2 = margin of error. Even though, the sample size n=18 was statistically defined 35 patients were randomly sampled to cover all the CKDu positive villages of the area.

Sociodemographic data were collected through an interviewer administered questionnaire, filled during the visit to patient's houses. Data include age, gender, occupation of the patient, clinical signs and symptoms of CKDu including serum creatinine level, status of smoking and alcohol consumption as potential habits for the disease progression.

As CKDu patients were distributed in 65% of total villages in Thunukkai Division water samples were collected in these villages during the period of August 2018. A total of 38 water samples; 35 samples from CKDu affected areas (Figure 1, A1-A15, B1-B20) and 3 control samples (Figure 1, C1-C3) were collected. Three control samples were collected from places in Thunukkai, Yokapuram west and Ugilankulam, where there were no records of CKDu patients.

Water samples were collected during the dry season. Before collecting water, the water column was thoroughly stirred with the collecting bucket. If the water surface has floating scum or algae, those were skimmed before collecting the samples. Water was collected over the depth of 10 cm below the water surface. In the case of tube wells, pumps were used to take samples of water. Water samples were collected into cleaned plastic bottles which were then refrigerated at 4-20°C until assaying. Physicochemical parameters such as, turbidity, colour, odour, TDS-total dissolved solids, Salinity, Electrical Conductivity, NO_3^- , PO_4^{3-} , SO_4^{2-} , F^- , Total hardness, Ca^{2+} , Mg^{2+} and Cl^- of the water samples were measured within a week.

Physicochemical properties of the water samples were determined with standard instrumentations, following the standard procedures. The analyses were conducted in the Department of chemistry, University of Jaffna and in the Industrial Technological Institute in Colombo. As and Cd levels were measured with graphite furnace atomic absorption spectrophotometry.

By using Geographic information system (GIS) software (ArcGIS 10.1) hydrogeological mapping was conducted for Nitrate, phosphate, Total hardness, Total dissolved solid, fluoride and Arsenic content in water.

Physicochemical parameters of the test samples were compared with those of the control samples and the Sri Lankan standards for potable water. To assess the correlation between water quality parameters and serum creatinine levels of the CKDu patient's, linear regression, analysis was used. Through this analysis relationships between the target variable (dependent variable) and a set of independent variables (covariates) were quantified. The regression equation estimates a coefficient for each variable. The goal of regression analysis is to generate the line that best fits the observations. However, the best fitted line for the data leaves the least amount of unexplained variation, such as the dispersion of observed points around the line. The following formula describes the linear relationship between dependent and independent variables.

$$Y = \beta_0 + \beta_1 a + \beta_2 b + \beta_3 c + \beta_4 d + \beta_5 e + \beta_6 f + \text{error}$$

Where, dependent variable (Y) is the serum creatinine levels of the CKDu patient. Independent variables; a- nitrate, b-fluoride, c- phosphate, d- Total Dissolved Solid, e- Total hardness, f- Arsenic content in water. Intercept β_0 - is a constant that defines where the linear trend line intercepts the Y-axis.

Coefficient β_1 , β_2 , β_3 , β_4 , β_5 and β_6 : They are constant that represents the rate of change in the dependent variable as a function of changes in the independent variable. It is the slope of the linear line

Error: It represents the unexplained variation in the target variable. It is treated as a random variable that picks up all the variation in Y that is not explained by X.

Results

Demography of the CKDu patients

Age of CKDu patients in Thunukkai Division ranged between 30 and 70 years old. Among the studied patients 24 were under 50-70 years, representing 63% of the total sample (table 1). CKDu was more prevalent among males as the M: F ratio was 4:1. Among them 90% were engaged in agriculture related occupation while the rest were laborers, drivers, or unemployed people.

According to the questionnaire data, alcohol consumption and smoking were identified as disease modifying habits where more than 20% engaged in either smoking or taking alcohol while another 20% involved in both.

Clinical characters among selected patients showed other noncommunicable diseases, such as hypertension (43% prevalence) and diabetes (17% prevalence) with 17% suffering from both the diseases. The disease history of the patients revealed that they were detected with hypertension and diabetics secondarily, only after they developed CKDu. Furthermore, a spotty pigmentation, which was similar to arsenic related keratosis was observed in the palms of three male patients of the study group.

Physicochemical characters of the drinking water

Dug wells and tube wells serve as the major source of drinking water in the study area. Thus, drinking water samples from 31 dug wells and 4 tube wells were collected and analysed for the physicochemical parameters. The results of the analyses were given in the table 2.0. Among the parameters, salinity, TDS and total hardness showed highest deviations with more than 50% of the samples exceeding the relevant values of the Sri Lankan standards, SLS 614:2013 for drinking water (Figure 2.0). Salinity ranged between 0.13-3.66 ppt with an average of 0.69 ppt (standard error of mean, SEM= 0.118), with 50% of the samples exceeding the standard value of 0.5 ppt (Figure 2a). Similarly, in 63% of the samples, TDS content exceeded the standard (400ppm) reporting an average value of 686.63 ppm (SEM= 115) and reaching a maximum of 3570ppm (Figure 2b). Total hardness of the samples ranged between 39.84 -683.26 ppm (SEM= 24.9) with 65% of the samples exceeding the standard (250ppm, Figure 2c).

Among other parameters, turbidity, fluoride, chloride, calcium and As contents showed higher deviations from the SLS 614:2013, with more than 20% samples exceeding the respective limits. Mean turbidity of the drinking water samples was 2.3 NTU (Nephelometric Turbidity Unit) (SEM= 0.745) reporting a range of 0.3-26.5 NTU, all exceeding the mean turbidity of control samples. 10 water samples (29%) exceeded the standard turbidity level (2NTU-SLS 614:2013).

Fluoride content averaged 1.73 ppm (0.1-22.3 ppm) (SEM= 0.693), with 39% samples exceeding the standard of 1ppm. Similarly, chloride content of 31% of the water samples exceeded the standard value of 250 ppm reporting an average of 367.27 ppm (6.95 -2066.53 ppm) (SEM=87.0). Calcium averaged 64.074 ppm (3.98- 193.22ppm) (SEM=7.3) with 21% of the samples reporting higher values than the standard value (100 mg/l) for calcium in drinking water. Even though, detected only in nine samples As content exceeded the standard value (0.01 mg/l) reaching as high as 0.03 ppm in some water samples.

Water quality parameters such as pH, electric conductivity, nitrate, phosphate and magnesium contents showed no substantial deviations, with only 10% of the samples exceeding the SLS 614:2013. pH was 8.18 on the average (SEM=0.07), ranging between 7.45 - 9.12. Only six samples reported more than 8.5 whereas no water sample reported pH below 6.5. The mean value of electrical conductivity was 1416.31 μ s/cm (SEM=216), with the values ranging from 330 μ s/cm - 6690 μ s/cm. 17% of the samples exceeded the standard (750 μ s/cm). Nitrate content of the samples averaged 28.13 mg/l, (SEM=9.01), with values ranging from 0-295 mg/l. Only 15% of the water samples contained higher nitrate levels than the standard value, 50 mg/l. The mean phosphate content was 0.84 ppm (0.06 -4.8 ppm) (SEM=0.109) with 10 % of the samples exceeding the desirable level. Magnesium content reported a mean value of 30.19ppm with 18% of samples reported beyond the standard value.

All the other physicochemical parameters including, Na, K, sulphate contents and alkalinity reported lower levels compared to their respective SL standards. Cadmium was not detected in any of the water samples collected from the sampling area.

According to the data gathered through the questionnaire, the serum creatinine levels of CKDu patients of Thunukkai ranged between 1.31-5.32 mg/dl, reporting an average of 1.906 \pm 0.845 (SEM= 0.143) mg/dl. Serum creatinine of the control samples reported an average, of 0.7 \pm 0.0557 mg/dl (SEM= 0.0321), as illustrated in figure 3, showing a significantly low value (t=8.24, p=0.0001).

Correlation between water quality parameters and CKDu patients

The effect of physicochemical characters of water on the occurrence of CKDu was evaluated by applying the linear regression model, where serum creatinine concentration was treated as the response variable while water quality parameters (nitrate, fluoride, phosphate, etc.) were treated as the explanatory variables. Results were given in the table 3.0 below.

R² value of 0.5109 suggests, that six explanatory variables; fluoride, phosphate, TDS, total hardness and arsenic, together account for about 51.09% of variation in the serum creatine concentration of the CKDu patients. Among these physicochemical parameters, TDS and As showed significantly positive correlation (p<0.05) with the creatinine levels while phosphate showed significantly negative correlation (p<0.001, Figure 4). On the other hand, nitrates in the drinking water showed no influence on serum creatinine of the CKDu patients in Thunukkai.

Geophysical distribution of total hardness level, TDS, nitrate, fluoride, phosphate and arsenic in the drinking water of the study area are illustrated in figure 5 & 6. Analysis of the spatial distribution data of the patients indicated that seven sampling points recorded creatinine levels over 2mg/dl in the order of, A12> A9> A4>A1>A13>B13>A2. When these locations are overlapped on the geophysical maps distinct interrelations were identified in TDS, As and phosphate with the creatinine levels (Figure 5), showing the same trend observed in the regression analysis. Higher serum creatinine levels, over 2.5 mg/dl were appeared to be linked with higher total dissolved solids contents 650.9-1516 ppm (Figure 5a) and higher arsenic contents 0.0016-0.0297 ppm (Figure 5b) and low phosphate contents, 0.789-0.904 ppm (Figure

5c). Conversely, the spatial distribution of nitrate, fluoride and total hardness contents showed no direct influence on serum creatinine levels in the study samples (Figure 6 a, b & c).

Discussion

The present study revealed that the male farmers, aged between 50-70 in Thunukkai Division of Mullaitivu developed higher risk of CKDu. Smoking and alcohol consumption may enhance the vulnerability towards the disease. The patients have developed hypertension and diabetes as secondary illnesses after developing the CKDu. Drinking water quality of the area was not at the desirable levels, particularly with higher ionic content leading to higher salinity, dissolved solids and hardness levels. Secondarily, turbidity, fluoride, chloride, and magnesium levels reported disturbingly higher values compared to SL standards. As predicted by the regression model, fluoride, phosphate, TDS, total hardness and arsenic levels together accounted for considerable variation in the serum creatinine of the studied patients. This trend was further validated by the association of geospatial distribution of TDS, arsenic and phosphates with the occurrence of higher serum creatinine levels of the study samples.

Demographic data of the study comply with many other studies conducted in CKDu issue in Sri Lanka. Sex distribution of the presents study, i.e 4:1, (Male: Female) was consistent with Wanigasuriya et.al., [19], Noble et.al., [20], Ranasinghe et al [2], and Balasooriya et al., [9] which reported male preponderance. However, the age distribution was not compatible with Wanigasuriya et.al., [19]) and Noble et.al., [20] who reported as the majority of patients were under 40-50 years, unlike 50-70 in the present study. On the other hand, many recent studies reported consistent findings where more than 60% of the CKDu patients were over 50 years of age [2,9] Spotty pigmentations on palms, observed by Paranagama [21] was consistent with similar type of patches observed in the patients of Thunukkai in the current study. Dermatological references of these patients suggested the development of early stage of arsenic related keratosis.

Drinking water quality parameters were compatible with the groundwater quality data available for North Central province through various CKDu based research conducted to date. However, the total hardness, calcium and magnesium ion concentration, electric conductivity levels of the groundwater of Thunukkai were substantially high compared to those of the groundwater of North Central province [5, 15, 22, 23]. On the other hand, the heavy metals such as As and Cd in the water of Thunukkai were significantly low compared to the levels detected in the North Central province [5, 15]. Nevertheless, in compliance with the present study, Wickramarathna et al., [10] reported insignificant levels of Cd and As in ground water of Girandurukotte, Wilgamuwa and Nikawewa areas. Chloride ion content in the groundwater of Thunukkai was exponentially high compared to the groundwater in CKDu high prevalent areas such as Padaviya, Kebithigollawa, Medawachchiya, & Kahatagasdigiliya, and moderate prevalence areas such as Mihintale, Talawa, and Nochchiyagama of the dry zone of Sri Lanka [24]. Similarly, fluoride levels were relatively high in Thunukkai compared to those of the dry zone of Sri Lanka, reported by Chandrajith et al., [25]. Water hardness and the conductivity levels in Thunukkai were compatible with those of the high and moderately prevalent areas [24].

Correlation data of the water quality parameters and serum creatinine levels revealed a significant negative relationship with phosphate and positive relationships with total dissolved solid (TDS) and arsenic content of the drinking water. This observation was further confirmed by geospatial mapping of the water quality constituents and the occurrence of higher serum creatinine levels in study samples. Patients with extremely high levels of serum creatinine (over 4.5 mg/dL) appear to consume water from wells with higher TDS and As contents. In the same way several other constituents in drinking water and the diet were linked with the serum creatinine levels. For example, complying with the present study nitrate in the diet was only slightly linked with serum creatinine of CKDu patients [26, 27]. In contrast to our results, significant association between the fluoride content and the occurrence of CKDu were revealed by several other researchers, including Illeperuma et.al., [7], Balasooriya et al., [9], Wickramarathna [10], Jayasinghe [28] and Wijeratne et.al., [29].

As given in the present study, phosphate ions in water may negatively influence the serum creatinine of CKDu patients. Thus, the negative sign of the coefficient implies that when phosphate content in the water increases, possibility of occurring CKDu decreases. The coefficient of phosphate shows that by holding nitrate, fluoride, total dissolved solid, total hardness and arsenic content, possibility to occur CKDu decreases by 0.2113 times for every unit increase in phosphate content. This result found to be consistent with Eddington et.al., [30]. Furthermore, total dissolved solids positively influence the serum creatinine levels of the study participants. The positive sign of the coefficient implies that when TDS content in the water increases, possibility to occur CKDu increases. The coefficient of TDS shows that by holding nitrate, fluoride, phosphate, total hardness and arsenic constant, possibility to occur CKDu increases by 0.2113 times for every unit increase in TDS content in water. On the other hand, total hardness showed no association with the serum creatinine levels, i.e. not compatible with Jayasumana et.al., [31] and Paranagama [32] who concluded positive and significant relationship between total hardness in water and CKDu. However, low R^2 value obtained in the present study may affect the significance of the findings. Thus, further studies should be conducted with a higher sample number and a broader study area.

Conclusion

The chronic kidney disease of unknown aetiology in Thunukkai of the Mullaitivu District in the Northern Province of Sri Lanka showed male preponderance, M: F 4:1, at the age range of 50-70, revealing 80% occupational association with agricultural activities. Secondary development of hypertension and diabetes were observed in CKDu patients. Spotty pigmentation, similar to arsenic related keratosis was observed in the palms of the patients, lived in the areas where detectable arsenic levels in their drinking water were detected.

Evaluation of drinking water revealed substantially high ionic content leading to higher electric conductivity, salinity, total dissolved solids and total hardness levels compared to those of the Sri Lankan standards. Association of serum creatinine of the CKDu patients and water quality parameters showed significant positive correlation with dissolved solids and arsenic contents, contributing more than 50%

variation in serum creatinine. Geospatial distribution of higher TDS and As contents confirmed this trend by reporting higher serum creatinine levels in their respective locations. Thus, TDS and As may serve as major etiological factors for the occurrence of CKDu in Thunukkai of Mullaitivu District in Sri Lanka.

Abbreviations

COD-Chemical Oxygen Demand, CKDu-Chronic Kidney Disease of unknown etiology, EDTA-Ethylenediaminetetraacetic acid, GIS-Geographic information system, GN-"Grama Niladhari"-Public service officer, NTU-Nephelometric Turbidity Unit, SD- Standard Deviation, SLS-Sri Lanka Standards, TDS-Total Dissolved Solid, WHO-World Health Organization

Declarations

Ethics approval and consent to participate

Ethical approval (Ref: *J/ERC/17/82/NDR/0171*) was granted from Ethics Review Committee (ERC) of Faculty of Medicine, University of Jaffna, Sri Lanka. Authors declare that the experiments conducted, complied with the current laws of Sri Lanka. Patient's data was collected through an interviewer administered questionnaire, filled during the visit to their houses. Verbal consent was taken as per the guidelines given by the ERC.

Consent for publication

Not applicable

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

KG carried out the study under the guidance of PS, UAJ, GR, RS and PJ. KG drafted the manuscript and UAJ and PJ reviewed it before the initial submission. All authors read and approved the final manuscript.

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Tables

Table 1: Demographic data and baseline characteristics of the study population in Thunukkai.

	Data	Number of patients (%)
Age distribution	30-40	4 (11.42%)
	41-50	5 (14.28%)
	51-60	12 (34.28%)
	61-70	12 (34.28%)
	71-80	2 (5.71%)
Sex	Male	28 (80%)
	Female	07 (20%)
Occupation	Agriculture related	31 (90%)
	Other	02 (5%)
	No occupation	02 (5%)
Impact of other habits	Smoking	09 (25.71%)
	Alcohol consumption	07 (20%)
	Both- smoking & alcohol	07 (20%)
Disease history	Hypertension	15 (42.82%)
	Diabetes	06 (17.14%)
	Both hypertension & diabetes	06 (17.14%)

Table 2. Physicochemical properties of the water samples collected from Thunukkai

Water quality parameter	Control (Mean±SD ¹)	Samples			SLS ² 614 (2013)	% of water sample exceeding SLS value
		Max	Min	Mean (±SD)		
Turbidity (NTU ³)	0.38±0.0916	26.5	0.3	2.3±4.4076	2	28.8
pH	8.02±0.01154	9.12	7.45	8.18±0.4061	6.5-8.5	17.1
Electric conductivity (µs/cm)	935.67±238.7	6900	330	1413.31±1275.4	750	17.1
Salinity (ppt)	0.416±0.1305	3.36	0.13	0.69±0.6984	0.5	50
TDS ⁴ (ppm)	417.66±0.130	3750	136.3	686.63±681.047	400	63.16
Nitrate (ppm)	51.66±31.459	295	0	28.13±53.3099	50	15.17
Fluoride (ppm)	0.37±0.2362	22.3	0.1	1.73±4.09903	1	39.5
Phosphate(ppm)	3.1±2.1232	4.84	0.06	0.84±0.6468	2	10.6
Total hardness(ppm)	340.2±76.103	683.2	39.84	295.76±147.522	250	65.4
Magnesium(ppm)	29.28±5.8925	68.13	0	30.19±1.87014	50	18.42
Calcium(ppm)	86.36±22.983	193.22	3.98	64.074±43.1998	100	21.05
Sodium(ppm)	7.2±5.4369	41.6	0.1	13.52±11.4608	200	0
Potassium(ppm)	4.8±4.1617	9.6	1.3	3.75±2.0033	10	0
Sulphate(ppm)	13.3±7.6376	160	0	37.10±31.3539	250	0
Alkalinity(ppm)	6.37±1.2663	31.87	2.46	7.2496±5.4155	200	0
Chloride(ppm)	184.96±139.9	2066.53	6.95	367.272±514.75	250	31.42
Arsenic (lowest detected value) (ppm)	Less than 0.0001±0	0.03	Less than 0.0001	0.0054±0.0051	0.01	23.7
Cadmium	Not detected	Not detected			0.003	0

¹Standard deviation, ²Sri Lanka Standards, ³NTU- Nephelometric Turbidity Units, ⁴TDS-Total dissolved solids

Table 3: Regression analysis between serum creatinine of CKDu patient and explanatory variables, phosphate, TDS and Arsenic

Explanatory variables	Coefficient	St. Error	T value	P value
(Intercept)	1.660e+00	1.767e-01	9.394	5.63e-11 ***
PO4 ³⁻	2.113e-01	1.009e-01	-2.095	0.0437 *
TDS	2.829e-04	1.592e-04	1.777	0.0845
As	1.065e+02	2.135e+01	4.986	1.79e-05 ***

R²=0.5109

Figures

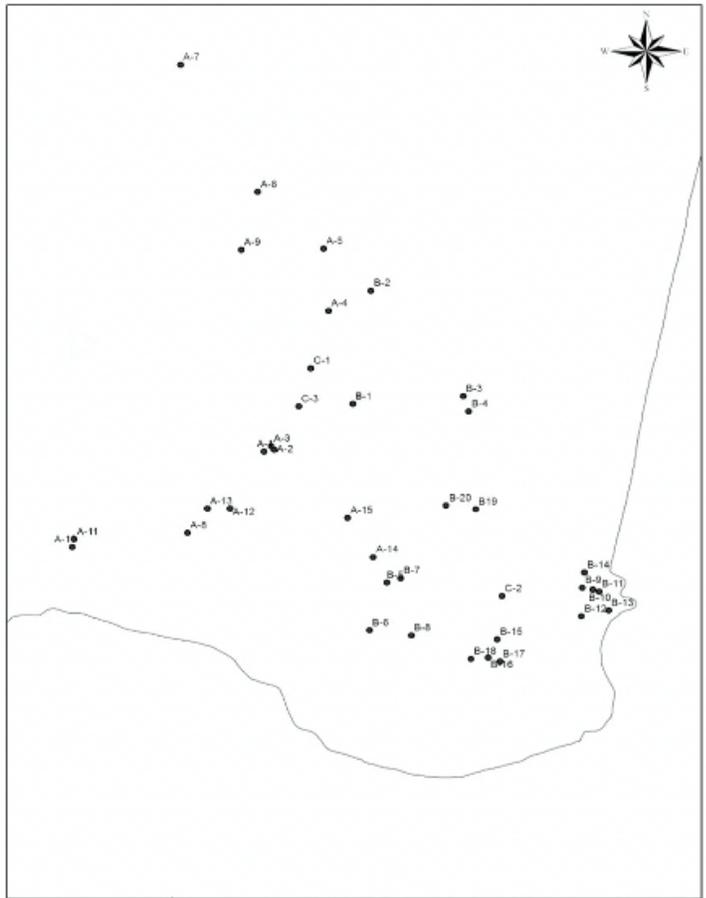
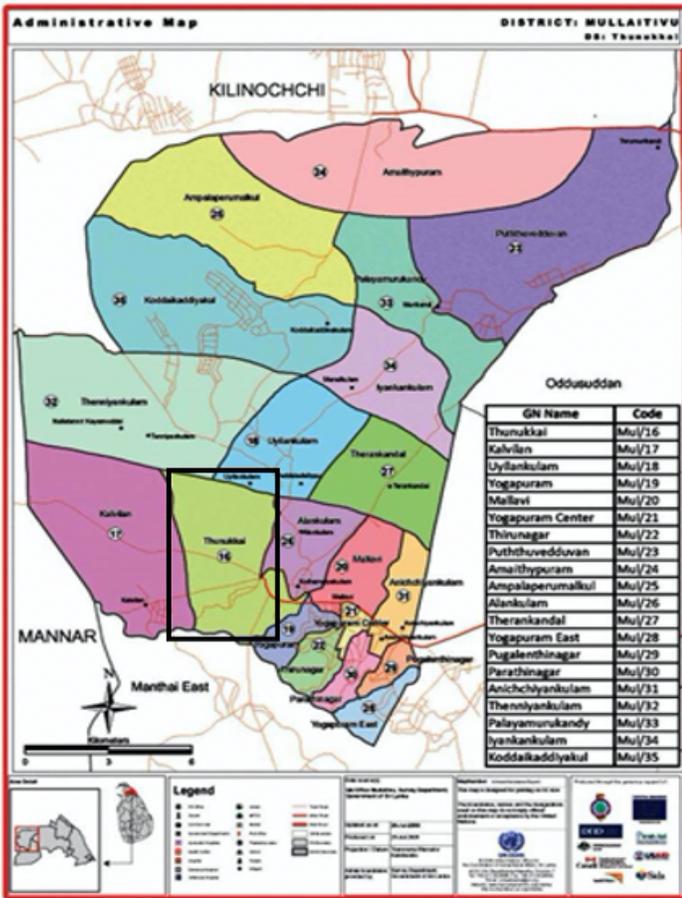


Figure 1

Thunukkai Divisional secretariat of the Mullaitivu District showing the distribution of drinking water sources (sampling points, A1-A15, B1-B20) of the patient households and control samples from C1-C3.

(Source: Original layer of the map was adopted from maps of Sri Lanka Surveys Department, Maps and Geoinformations, <https://www.survey.gov.lk/>, Locations drawn with ArcGIS 10.1, <https://www.arcgis.com/index.html>).

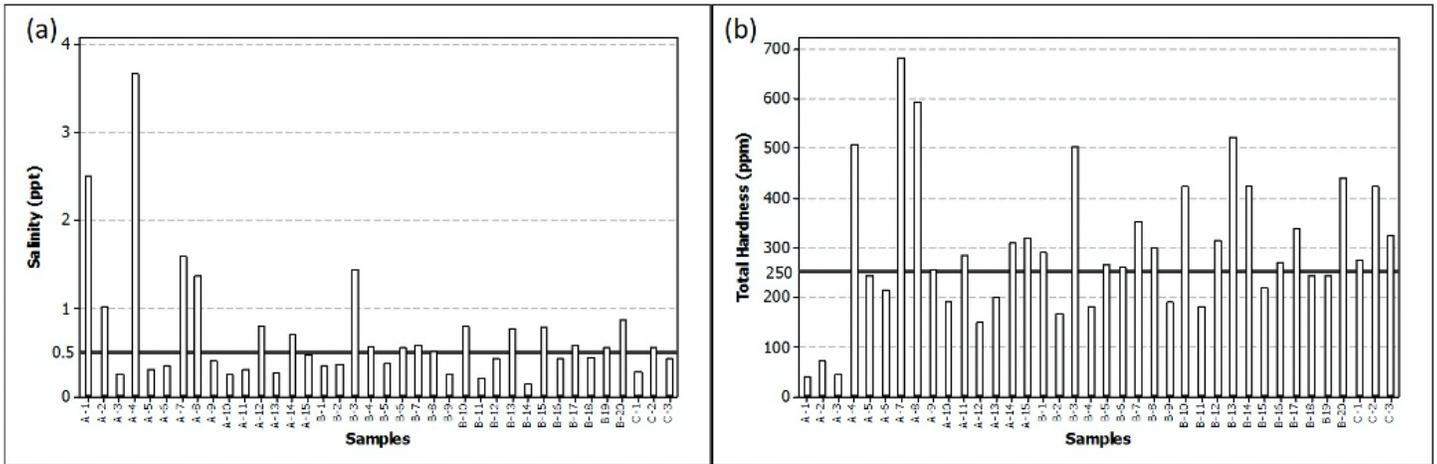


Figure 2

Salinity (a), and total hardness (b) of the water samples collected from various locations A1-C3 of Thunukkai (SLS standard value is marked in the Y axis). A1-B20 are CKDu endemic areas, C1-C3 non-CKDu areas

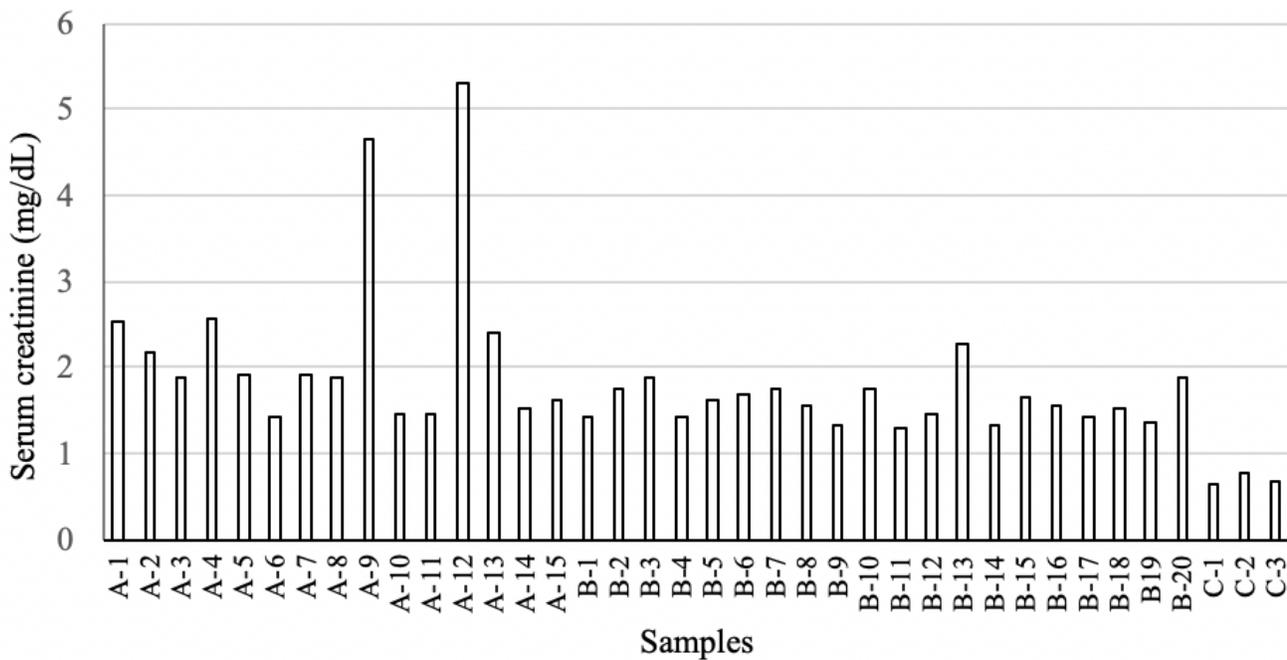


Figure 3

Serum creatinine data of CKDu patients, collected from different locations, A1-C2 in Thunukkai. A1-B20 are CKDu endemic areas, C1-C3 are non-CKDu areas

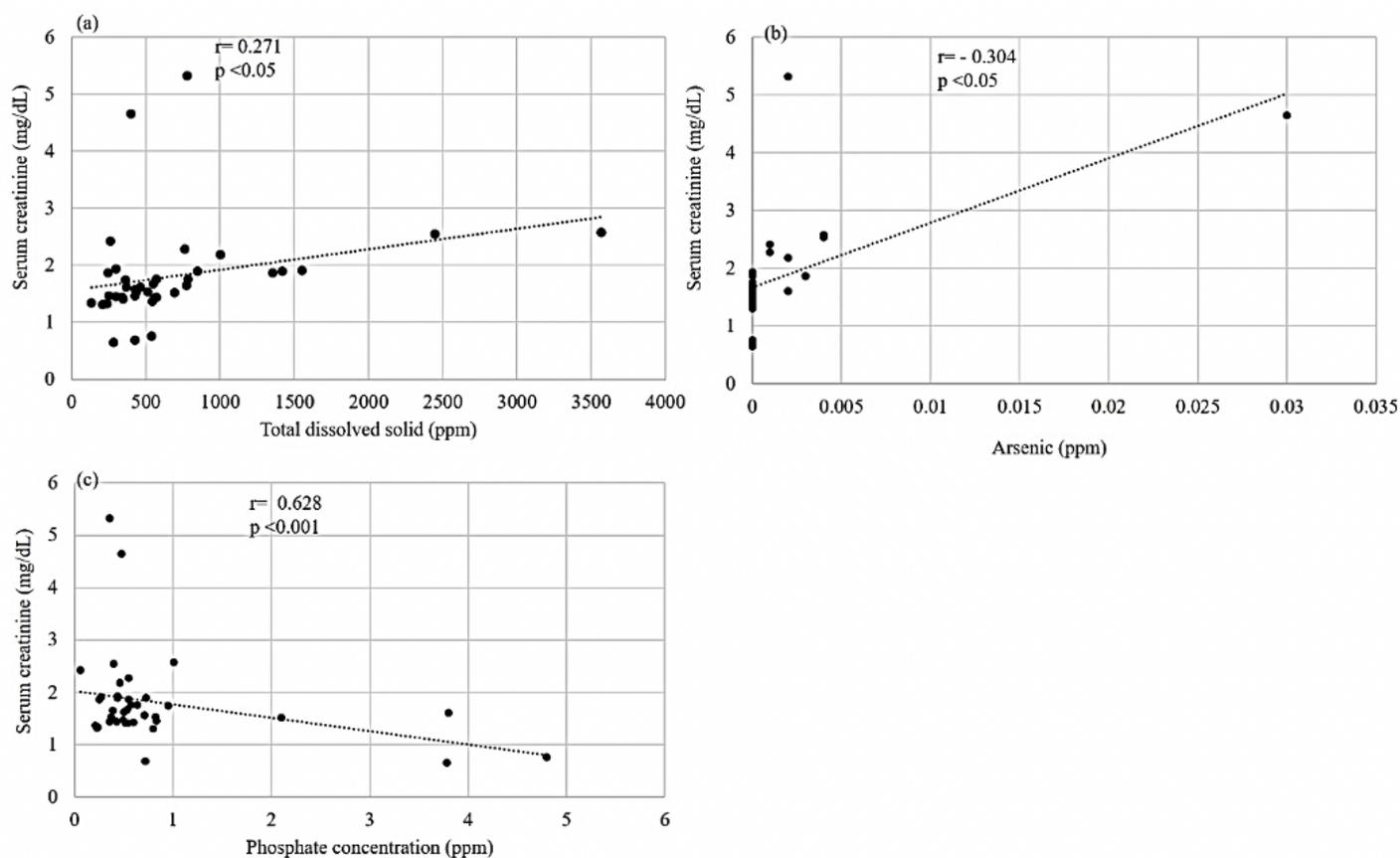


Figure 4

Scatter plots of total dissolved solids (TDS), arsenic and phosphate contents in drinking water showing significant correlations of with the serum creatinine levels of the CKDu patients in Thunukkai.

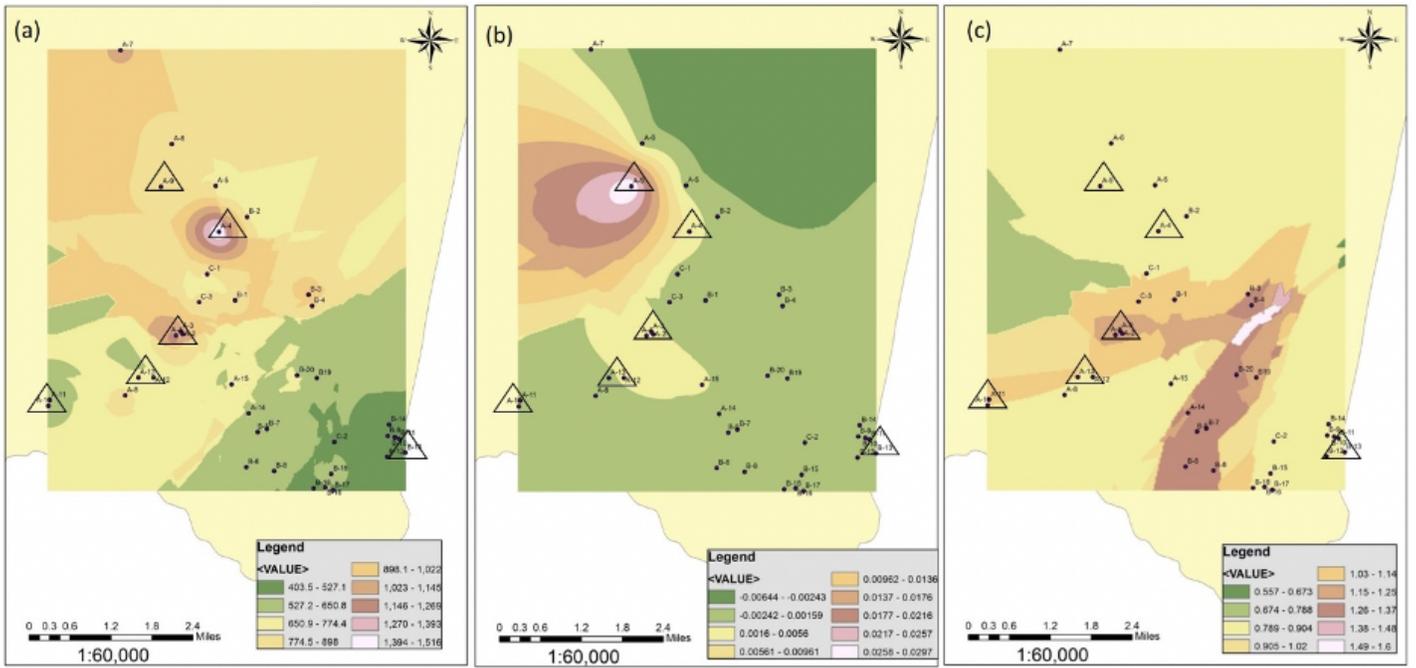


Figure 5

GIS mapping of (a) TDS-total dissolved solid, (b) arsenic and (c) phosphate distribution in the ground water in Thunukkai Division. Six triangles in each plot represent CKDu patients with higher serum creatinine content over 2 mg/dL. (Source: Created by the authors with ArcGIS 10.1, <https://www.arcgis.com/index.html>).

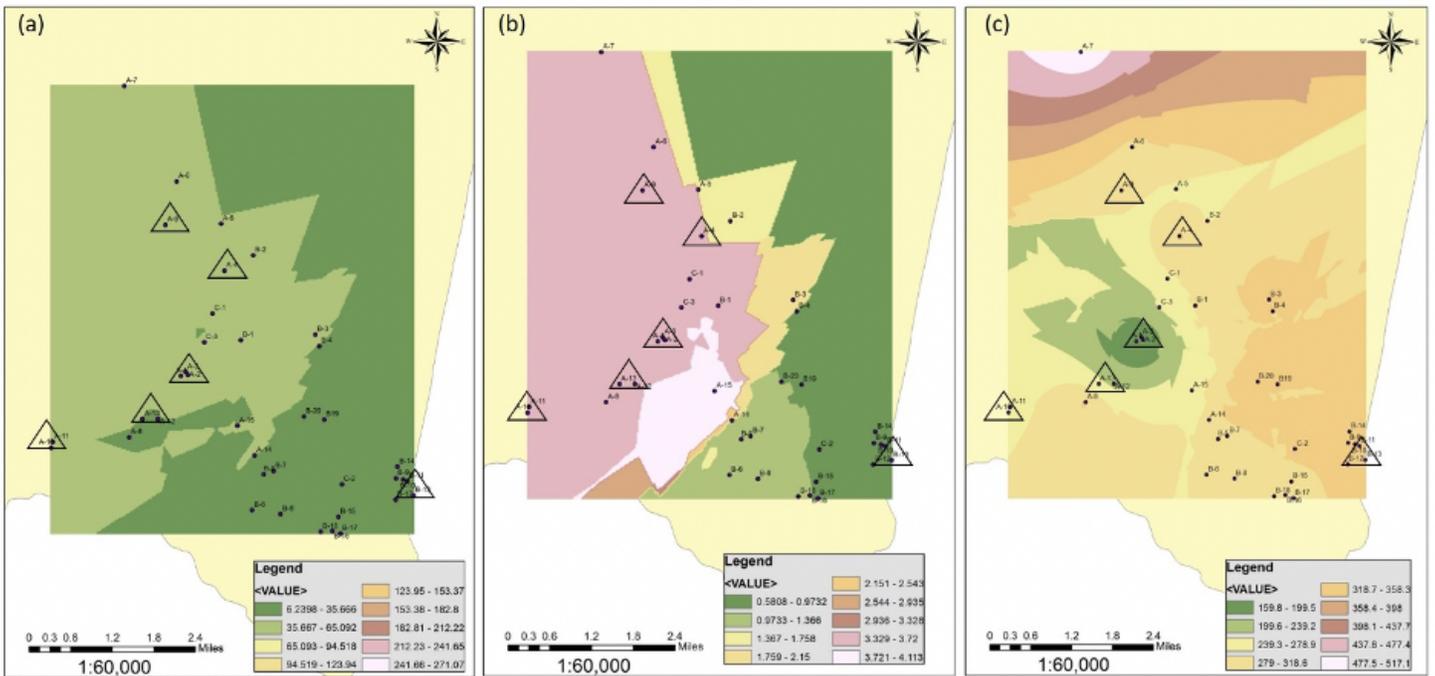


Figure 6

GIS mapping of (a) Nitrate, (b) fluoride and (c) total hardness content in the ground water in Thunukkai Division. Six triangles in each plot represent CKDu patients with higher serum creatinine content over 2 mg/dL. (Source: Created by the authors with ArcGIS 10.1, <https://www.arcgis.com/index.html>).

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