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Multivariate statistical techniques for the evaluation of hydrogeochemical data from areas with chronic kidney disease of unknown etiology (CKDu) in Sri Lanka

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Abstract

Chronic Kidney Disease of unknown etiology (CKDu) ranked as one of the most attended public health issues in Sri Lanka. The etiology of the disease is believed to be environmentally induced as it appears in geographically discreet regions of the dry zone of Sri Lanka. Girandurukotte and Wilgamuwa are two regions where CKDu is more prevalent. This study performs a comparative analysis to separately identify the quantitative differences of hydrogeochemical parameters of groundwater samples consumed by the patients and non-patients in two regions. Besides, groundwater quality data of 421 wells from Girandurukotte and Wilgamuwa were used for the analysis. Out of 421 samples, 310 samples were collected from wells where patients were reported. Descriptive statistics, Correlation analysis and Factor analysis were performed. Results of comparative analysis conclude that means of iron (Fe) content in Wilgamuwa CKDu (371 µg/L) and non-CKDu (731µg/L) exceeds the WHO permissible limits. The mean ion content of F^-, Cl^-, NO_3^-, SO_4^{2-} and Na^+ in the water wells consumed by the CKDu patients in the Wilgamuwa region is higher in concentration than non-CKDu wells. Out of major elements, Mg^{2+}, Ca^{2+}, K^+, Na^+, NO_3^- and hardness contents are higher in Wilgamuwa CKDu wells compared to the Girandurukotte CKDu wells. Trace elements such as As, Cd, and Pb do not exceed the WHO recommended limits in both regions. Generally, except for Fe ion concentration, almost all the other ion concentrations are within the WHO recommended limits. Correlation analysis reveals that Total Dissolved Solids (TDS) is highly positively correlated with alkaline earth metals Mg^{2+} (0.93), Ca^{2+}(0.86) and Sr^{2+}(0.85) in Wilgamuwa CKDu water sources. Also, Electrical Conductivity (EC) is highly positively correlated with Ca^{2+}(0.84), Ni (0.82) and Sr (0.81) in Wilgamuwa non-CKDu water sources. Factor analysis was applied to identify the chemical combinations in each cluster. Ni, Ca^{2+}, Sr^{2+} and Mg^{2+} appear as a factor in Wilgamuwa non-CKDu water sources. Hence, alkaline earth metals are chemically associated and appear as a factor combination in Wilgamuwa non-CKDu water sources. Also, Alkalinity, Hardness, Ca^{2+} and Sr^{2+} were found as a factor in Ginnoruwa CKDu samples.

Keywords: CKDu, comparative analysis, factor analysis, hydrogeochemical data

Introduction

Chronic Kidney Disease of undermined orgin (CKDu) is a significant public health issue throughout the world. Although Chronic Kidney Disease (CKD) is mainly due to chronic or severe hypertension, snake bites, diabetes milatues, urological diseases. However, a similar disease but with interstitial nephrities are apper in certain regions of the world, particularly in tropical terrains but without any known risk factors. Therefore the disease is yet denoted as CKDu. Sri Lanka, India, Thailand, Egipt, Central American nations of El Salvador, Mexico are among counties where CKDu is widely reported.[1] In Sri Lanka, dry zone regions in the North Central and the Uva province are the critical areas that are considered as endemic CKDu regions.[2] There has been a dramatic increase of patients with CKDu within these geographical locations over the past 20 years and over 15% of the population in some discreat areas are suffer from CKDu. North Central, North Western and Uva are the main CKDu affected provinces. Girandurukotte in Badulla district, Madawachiya, Padaviya and Siripura in Anuradhapura district, Medirigiriya and Hingurakgoda in Polonnaruwa district, Nikawewa in Kurunagala district are the areas where a significant number of patients were identified Figure 1). CKDu may involve many factors and therefore, it is considered as a multi-factorial pertaining to one or more ecological and lifestyle factors, and a possible genetic predisposition. Since groundwater is the main sources of drinking water in these regions more attention were made on investigating quality of groundwater in many aspects.[2–5]

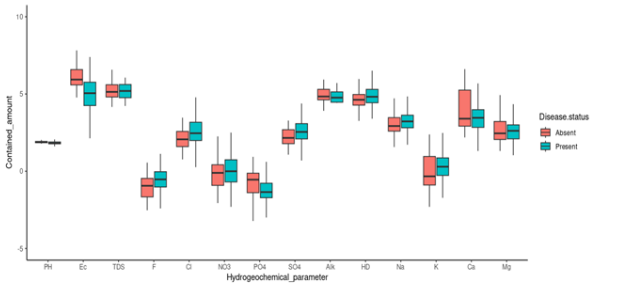

Figure 1 Comparison of Major element composition (log transformed) of groundwater in CKDu and non-CKDu wells from the Wilgamuwa region.

High concentration of water hardness, fluoride, and general alkaline nature of water with higher dissolved organic carbon (DOC) are proposed for the possible involving factors for the onset of the CKDu.[3–6] Although some studies highlight the involvement of heavy metals such as cadmium (Cd) and arsenic (As), aluminium (Al), etc.[7–10] but later studied did not confirmed high level of such elements in drinking water.

Last few years, large number of water quality studies were carried out in CKDu effected regions by the Department of Geology, University of Peradeniya, Sri Lanka and National Hospital, Kandy, Sri Lanka. Such studies gathered various water quality day and many of the findings were published as individual publications in various international journals. However no comprehensive statistical approach were carried out with these geochemical data. Therefore, this paper aims to analyses available water quality data gathereted from early studies using multivariate statistical methods.

Materials and methods

Water quality data was obtained from early studies from two well-known hotspots of CKDu in Sri Lanka, namely Ginnoruwa (in Girandurukotte) and Wilgamuwa. The data include water quality from drinking water wells used by CKDu patients and also healthy peoples (controls) in the same geographic region. A total of 65 wells from Ginnoruwa were selected and from which 45 wells were from non-CKDu households while 356 wells from which 66 were non-CKDu households were selected from the Wilgamuwa region were selected. After considering available data, 29 hydrogeochemical parameters were selected used for the statistical analysis. Temperature, pH, Electrical conductivity (EC), Total dissolved solids (TDS), Alkalinity, Hardness, F^-, Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+} are the major elements and paramters used for the analysis. Li, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Cd, Ba and Pb are the trace elements considered in this analysis. The analytical methods of these paramters are discussed in previous publications.[2,3]

In this study, several statistical techniques such as descriptive statistics, correlation analysis, non-parametric tests and factor analysis were employed. Under descriptive analysis, descriptive measurements such as mean, median, standard deviation, minimum, maximum and percentiles were measured. Correlation analysis was used to evaluate the relationship between hydrogeochemical variables. The objective of factor analysis is to group the variables based on their correlations. Variables within a factor are highly correlated, and the correlations among the factors are low.

Data preprocessing was carried out as the first step. Shapiro Wilk test was used to observe whether the sample data follows the normality. If the p-value is less than or equal to 0.05, the null hypothesis was rejected and concluded that the data do not follow the normality. Since most of the variables do not follow the normality, the non-parametric Mann–Whitney U test was used in order to compare the medians of two sets of groups. Descriptive measurements of hydrogeochemical data in CKDu and non-CKDu samples were measured under descriptive analysis. Mean contents of each parameter were calculated, to identify whether the parameter value follow the WHO recommended limits.

Under the comparative analysis, statistical tests such as ANOVA, Kruskal–Wallis, Mann–Whitney's post hoc test and Dunnett's T3 post hoc test were applied to distinguish possible influential elements in drinking water for CKDu.

Kaiser-Meyer-Olkin (KMO) test was also used to determine whether Factor analysis is applicable for the data set. Cronbach's Alpha value, a coefficient of reliability, was used to measure their liability of the factors. Higher Cronbach's Alpha value indicates that the reliability of the variables in a factor is high. 0.8 or above Cronbach's Alpha shows high reliability, and 0.6 - 0.7 indicates an acceptable level of reliability. When the original loadings may not be readily interpretable, rotational techniques had been applied until a simple structure was achieved.

Results and discussion

Out of the major elements, the mean content of F^-, Cl^-, NO_3^-, SO_4^{2-}, Na^+ and hardness were higher in Wilgamuwa CKDu wells compared to non-CKDu wells from the same area. Temperature, PH, Electrical condictivity, TDS, Alkalinity, Hardness, K^+, Ca^{2+} and Mg^{2+} are higher in the non-CKDu wells. Besides, it is observed that the mean Ca content in non-CKDu wells is twice larger than the mean Ca content of CKDu wells in the Wilgamuwa region. Mean calcium (Ca^{2+}) ion content 137.9 mg/L exceeds the WHO recommended limit (100 mg/L).[11] The average EC value of the Electrical conductivity is 606.6 µS/cm which is higher compared to the WHO recommended limits, 400 µS/cm.[12]

When considering the average chemical contents of trace elements, Al, Cr, Cu, Sr, and Pb are higher in Wilgamuwa CKDu ground water compared to the Wilgamuwa non-CKDu ground water. The rest of the elements are higher in the Wilgamuwa non-CKDu wells. Further, the mean content of iron (Fe) in the Wilgamuwa CKDu sample exceeds the WHO permissible limits in drinking water. It indicates 370.781µg/L amount of iron in the sample. Some elements such as Fe, Mn, Sr, Zn show high variability. In Wilgamuwa non-CKDu samples, the mean content of iron (Fe^{2+}) 731.229µg/L and nickel (Ni) 103.566µg/L lies far away beyond the permissible limits of WHO water quality standards. When considering the trace elements, Cadmium, Arsenic, and Lead has been identified as possible causative factors associated with CKDu.[10] The mean level of Cadmium is higher in non-CKDu wells(0.478µg/L) than the CKDu wells(0.14µg/L) in the Wilgamuwa region (Figure 2). WHO recommended the maximum level of Cadmium in drinking water is 3.00µg/L. However, the maximum cadmium level in Wilgamuwa non-CKDu wells was reported as 11.38µg/L, which is far beyond the permissible limit.

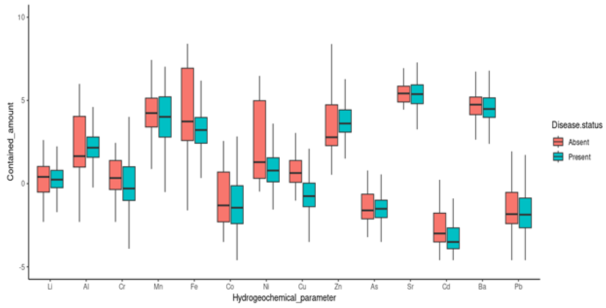

Figure 2 Comparison of Trace element composition (log transformed) of groundwater in CKDu and non-CKDu wells from the Wilgamuwa region

There is a significant relationship between Cadmium and arsenic concentration in urine among CKDu patients.[9] In this study, investigators have taken arsenic, Cadmium, lead, selenium and pesticides as the risk factors for the analysis. The Lead concentration of Wilgamuwa CKDu and non-CKDu wells were respectively 0.91µg/L and 0.70µg/L, which is below the WHO recommended value of 10µg/L.[11] It was suggested that phosphate fertilizers are a major source of inorganic arsenic in CKDu endemic areas in Sri Lanka.[7] In the Wilgamuwa region, the mean arsenic content in CKDu wells (0.40µg/L) is almost the same as that in non-CKDu wells(0.40µg/L). However, arsenic levels in both types of wells are below the WHO recommended limit10µg/L (Figure 2).[11] Maximum Arsenic content was recorded as 4.8µg/L, which is also less than the recommended limit.

Citation: Rathnayake HMAD, Nawarathna LS, Chandrajith R. Multivariate statistical techniques for the evaluation of hydrogeochemical data from areas with chronic kidney disease of unknown etiology (CKDu) in Sri Lanka. *Biom Biostat Int J.* 2023;12(6):196–202. DOI: 10.15406/bbij.2023.12.00403

When considering the major element composition Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}, NO_3^{-} and hardness are higher in Wilgamuwa CKDu wells compared to the Ginnoruwa CKDu wells (Figure 3). The rest of the parameters are higher in Ginnoruwa CKDu water samples. All the chemical (trace elements) contents except Cadmium are higher in Wilgamuwa CKDu wells compared to the Ginnoruwa CKDu wells. Also, note that the mean content of iron (370.78 µg/L) and nickel (47.45 µg/L) are comparatively higher in Wilgamuwa CKDu wells (Figure 3). None of the Hydrogeochemical parameters of Ginnoruwa CKDu does not exceed the permissible drinking water quality standards recommended by WHO.

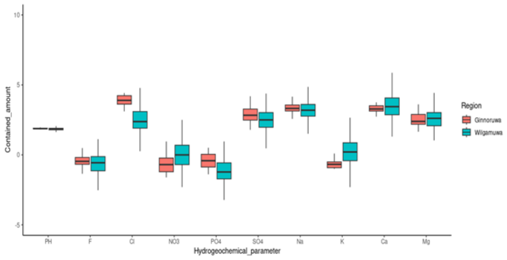

Figure 3 Comparison of Major element composition (log transformed) of groundwater in Wilgamuwa and Ginnoruwa CKDu wells.

A comparative analysis was performed to identify the significant difference parameters in groups. The comparison was performed under two sections. One section explains the comparison between CKDu and non-CKDu in the Wilgamuwa region. The other section contains the comparison between Wilgamuwa CKDu and Ginnoruwa CKDu. The null hypothesis is median values of geochemical parameters are equal in both regions. According to the Mann-Whitney U test, it shows that pH, EC, Hardness, F, Cl, $PO4^{3-}$, Na^{+}, K^{+}, Al, Cr, Fe, Ni, Cu, Zn and Cd had significant difference ($p < 0.05$) between water sources used by patients and non-patients in Wilgamuwa region (Table 1).

Also, statistically significant differences between CKDu wells in Wilgamuwa and Ginnoruwa were observed in variables pH, EC, Cl^{-}, NO_3^{-}, PO_4^{3-}, K^{+}, Li, Cr, Ni, As, Cd and Pb. A small p-value indicates strong evidence against the null hypothesis. Since the p-values of Cl^{-}, PO_4^{3-}, K^{+} and Cr are extremely small, there is a very high significant difference between these variables in the two samples. Out of those twelve significant values, the mean concentration of NO_3^{-}, K^{+}, Li, Cr, Ni, As, and Pb are higher in the Wilgamuwa CKDu wells compared to the Ginnoruwa CKDu wells (Table 1).

Table 1 Results of Mann-Whitney U (MWU) tests for Wilgamuwa CKDu Vs non-CKDu and Wilgamuwa CKDu Vs Ginnoruwa CKDu.

	Wilgamuwa CKDu Vs Wilgamuwa non CKDu			**Wilgamuwa CKDu Vs Ginnoruwa CKDu**		
Variable	**MWU value**	**p-value**	**Significance**	**MWU value**	**p-value**	**Significance**
pH	5120	<0.00001	***	3935.5	0.00137	***
EC	3757	<0.00001	***	4015	0.00049	***
TDS	357	0.9410		153	0.73213	
F^{-}	12478	0.00003	***	3039	0.43582	
Cl^{-}	12234	0.00009	***	4951	<0.00001	***
NO^{3-}	5694	0.12934		903	0.00250	***
$PO4^{3-}$	6448	0.00008	***	4421	<0.00001	***
$SO4^{2-}$	11430	0.00084	***	3336.5	0.09633	
Alk	308	0.46946		172	0.31896	
HD	10953	0.02165	***	2981.5	0.46089	
Na^{+}	11256	0.00677	***	2966	0.48677	
K^{+}	11191	0.01188	***	879.5	0.00000	***
Ca^{2+}	8083	0.20017		2277.5	0.32736	
Mg^{2+}	8991	0.74515		2547	0.68396	
Li	9019	0.67756		1630.5	0.00335	***
Al	10904.5	0.03332	***	2447.0	0.45507	
Cr	7224.5	0.00505	***	992.5	<0.00001	***
Mn	8191	0.22505		2436.5	0.55689	
Fe	7501	0.01370	***	2359.0	0.32580	
Co	8243	0.14327		2192.5	0.15303	
Ni	7154	0.00418	***	1266.5	0.00010	***
Cu	3636.5	<0.00001	***	3420.5	0.06332	
Zn	11537.5	0.00285	***	2013.0	0.05625	
As	8994	0.65296		1979.0	0.04542	***
Sr	9114.5	0.77422		2442.5	0.44783	
Cd	6695	0.00034	***	3482.5	0.04152	***
Ba	8531	0.28258		2127.0	0.10877	
Pb	8525.5	0.27906		1867.5	0.02146	***

Correlation analysis

Under this section, attention was paid to extracting the relationship between Total hardness (HD) and Electrical conductivity (EC) and Total dissolved solids (TDS) with the metallic ions. Hardness shows a low positive correlation with Mg^{2+}(+0.13), Sr^{2+}(+0.17) and Ca^{2+}(0.07). Although hardness relies on the amount of calcium and magnesium in the water, the correlation of hardness with calcium and magnesium is not very significant in Wilgamuwa CKDu water samples. Correlation between CKDu and high groundwater hardness has been reported frequently.[14] A study conducted based on kidney patients in the central highlands of Sri Lanka reveals an association between high hardness and calcium content with kidney stone formation.[13] The hardness of the water used by the kidney patients in Wilgamuwa is found to be 159.07 mg/L (Table 1). It was reported that hardness levels above 500 mg/L are generally considered aesthetically unacceptable.[8] Although previous studies have been reported hardness is highly associated with CKDu, the hardness level in water consumed by Wilgamuwa patients lies below the WHO standards. Further, EC is low positively correlated with HD (+0.16), Mg^{2+}(0.19) and Na^{2+}(0.17). Also, TDS is highly positively correlated with alkaline earth metals Mg^{2+} (0.93), Ca^{2+}(0.86) and Sr^{2+}(0.85).

It was mentioned that total dissolved solids and Arsenic in drinking water might positively correlate with the occurrence of CKDu in the Thunukkai region in the Mullaitivu District of Sri Lanka.[15] Water consumed by the Wilgamuwa CKDu patients contained 204.85mg/L (Figure 1) of average TDS level, which does not exceed the maximum WHO recommended limit 600mg/L.[11] Figure 4

When considering the Wilgamuwa non-CKDu samples, the correlation between hardness and magnesium gives a moderately positive value of +0.26. Also, the correlation coefficient between hardness and calcium (+0.13) is slightly lower than the correlation between hardness and magnesium. The average hardness level of water consumed by Wilgamuwa non-patients is measured as 134.35 mg/L (Figure 1), which is less than the WHO recommended limit (500mg/L) and the Wilgamuwa CKDu wells 159.07 mg/L. Alkalinity gives a moderate positive correlation with strontium, magnesium and TDS, respectively, +0.62, +0.6 and +0.47 in Wilgamuwa non-CKDu samples. Electrical conductivity is positively correlated with almost all the cations and the anions. Electrical conductivity is highly positively correlated with calcium, nickel and strontium with correlation coefficients +0.84, +0.82 and +0.81, respectively. The rest of the geochemical parameters shows a moderate positive correlation with the electrical conductivity. Figure 5 demonstrates the correlation among the variables in Wilgamuwa non-CKDu wells.

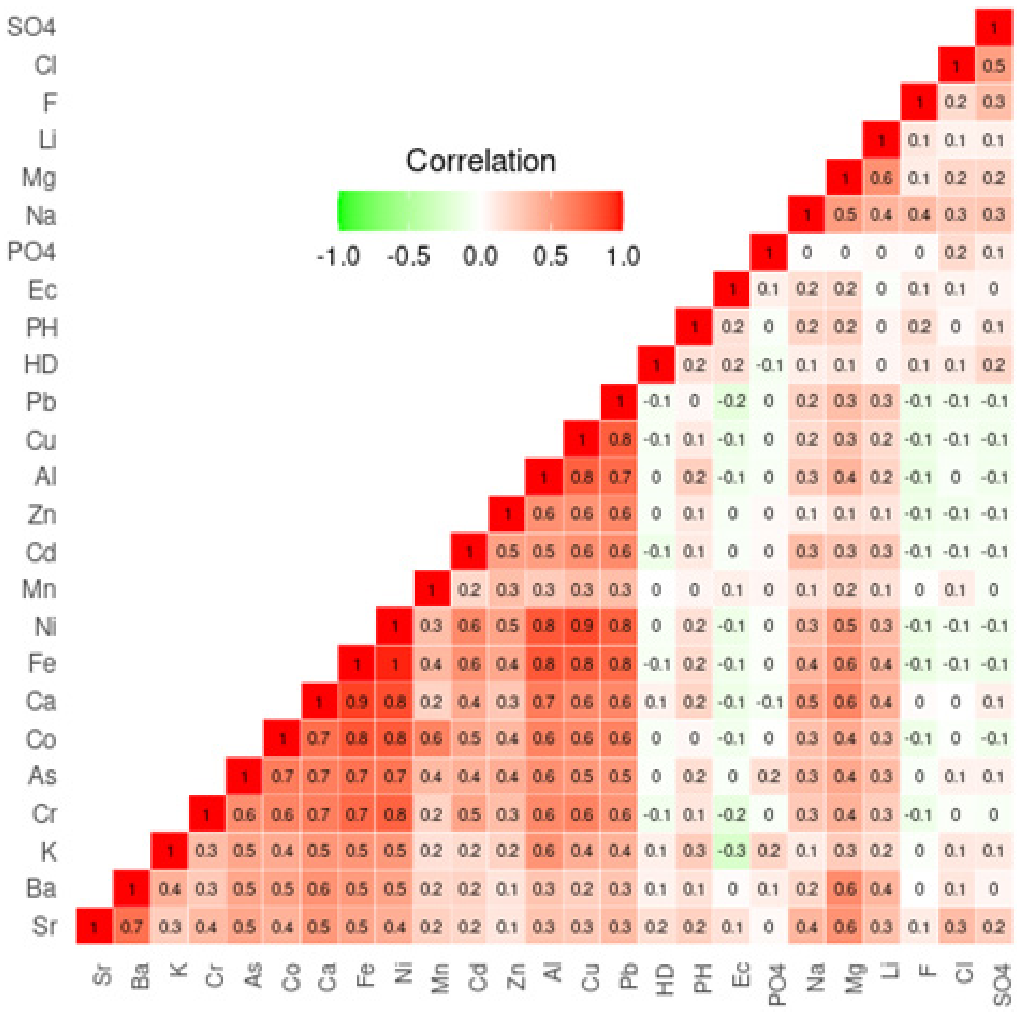

Figure 4 Correlation between geochemical parameters in Wilgamuwa CKDu wells (HD hardness, EC electrical conductivity, Temp temperature).

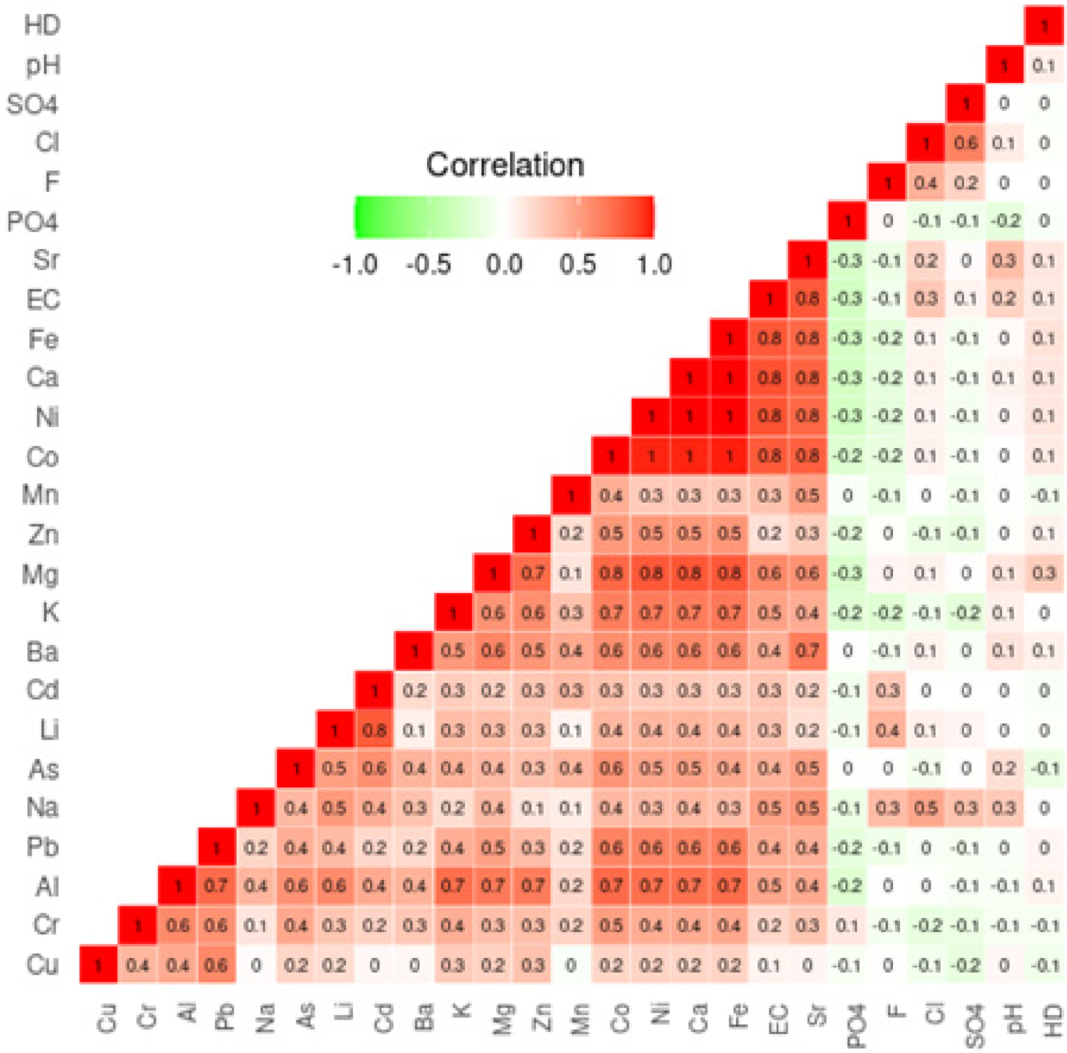

Figure 5 Correlation between geochemical parameters in Wilgamuwa non-CKDu wells (HD hardness, EC electrical conductivity, Temp temperature).

Due to the considerable number of missing values in TDS and Alkalinity variables, those two variables are not included in Figures 4 & 5. Also, note that the correlation of TDS and Alkalinity with other geochemical parameters have been measured separately.

Factor analysis

Table 2 summarize the results obtained by the factor analysis, and factor analysis is applicable because all KMO values are adequate.

Table 2 Summarization of factor analysis results

Group	KMO value	Factor	Initial eigen value	Factor variables	% variance	Cronbach's alpha value
Wil_CKD	0.85	1	9.88	Ca, Co, As, Ni	0.4	0.65
		2	2.7	SO_4^{2-}, Cl, F, Na	0.11	0.5
		3	1.86		0.07	
Wil_nCKD	0.78	1	9.74	Ni, Ca, Sr, Mg	0.37	0.86
		2	2.68	Cl, SO_4, Na	0.1	0.55
		3	2.66		0.1	
Gin_CKD	0.5	1	5.6	Alk, HD, Sr, Ca	0.19	0.83
		2	4.7	SO_4^{2-}, Cd, Li	0.16	0.25
Gin_nCKD	0.54	1	5.79	F, Na, Mg, Cl, SO_4^{2-}	0.21	0.81
		2	3.91	PH, EC, TDS	0.14	0.65
		3	2.92		0.1	

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Factor analysis results after factor rotations showed that three factors could be extracted from Wilgamuwa patient samples. The first factor had an initial eigenvalue of 9.88 and a percentage variance of 40%. Figure 6 (a) visualize the scree plot of Wilgamuwa CKDu samples. The horizontal line passes across the cutoff value for the eigenvalues. Since two factors lie above the cutoff value, two factors can be retained for the analysis. Further, BIC values for the two-factor model and three-factor model have been measured and observed that the three-factor model shows a lower BIC value than the two-factor model. For the three-factor and two-factor models, BIC values were 242.248 and 672.533, respectively.

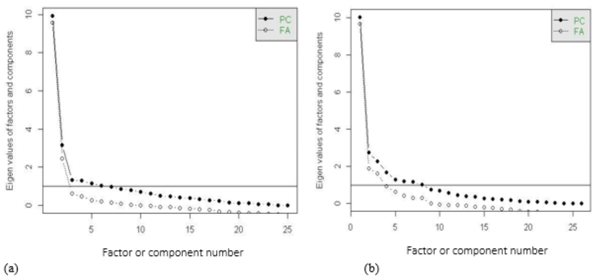

Figure 6 Scree plot of Wilgamuwa (a) CKDu group (b) non-CKDu group.

Three factors were selected for factor analysis of Wilgamuwa CKDu samples by considering the scree plot and BIC values. Ca, Co, As and Ni are the factor variables that belong to the first factor. According to the summarized results in Table 2, the second factor gives an eigenvalue of 2.7 and explains 11% of the total variance. Factor variables obtained for the second factor were SO_4^{2-}, F^-, Cl- and Na^{+}, containing three anions and one cation.

Scree plot suggests retaining only three factors for the factor analysis of Wilgamuwa non-CKDu samples (Figure 6(b)). As Table 2 shows, the initial eigenvalue of the first factor was 9.74, which explains 37% of the total variance. Three-factor variables belonged to the second factor. The factor variables of the second factor were Na^+, SO_4^{2-} and Cl^-. Figure 6

Factor variables of the first factor of Wilgamuwa non-CKDu samples were Ni, Ca^{2+}, $Sr^{2+}$and Mg^{2+}. It's worth highlighting that Ca, Sr and Mg are group 2 elements in the periodic table. These elements belong to a particular chemical combination named alkaline earth metals. Therefore, these alkaline earth metals are chemically associated and appear as a factor combination in Wilgamuwa non-CKDu water sources. Alkaline earth metals are grey or silver in colour. The density and the softness of these metals are relatively low. Although not as soft and lightweight as alkali metals.[16] Specific attention was paid to combinations of Alkali and Alkaline earth metals as possible causative influences for CKDu in Sri Lanka.[3]

Initial eigenvalues of Ginnoruwa CKDu and non-CKDu groups were respectively 5.6 and 5.79. Factor variables extracted from the first factor of Ginnoruwa CKDu were Alkalinity, Hardness, Sr^{2+} and Ca^{2+} (Table 2). While Sr and Ca are known as alkaline earth metals, Hardness depends on Ca ion concentration. Also, Alkalinity totally depends on the alkali metals. Therefore, the first factor of Ginnoruwa CKDu can be identified as a chemical combination that includes alkaline aspects. Factor variables of the first factor of the non-CKDu group of Ginnoruwa non-CKDu region were observed as F^-, Na^+, Mg^{2+}, Cl^- and SO_4^{2-} (Table 2). Factor variables of the second factor of the Ginnoruwa non-CKDu group were pH, Electrical Conductivity (EC) and Total Dissolved Solids (TDS), which can be identified as a non-anionic and non-cationic group.

Table 3 shows the factor's reliability variation when each factor variable was eliminated from the factor. Wilgamuwa non-CKDu samples show the highest Cronbach Alpha value, which is 0.86. Except Wilgamuwa CKDu rest of the regions gives higher alpha values. The alpha value of Wilgamuwa CKDu samples was 0.65, and all the other alpha values lie above 0.80, which shows higher reliability on their factors. Also, note that Table 3 includes only the first factor of each group. If Cronbach's alpha values decreased after a factor variable was dropped from a factor that intended better reliability of the factor with the variable. In the Wilgamuwa CKDu group, Ca, Co and as are the factor variables contained in the first factor. After eliminating Ca from the factor, the alpha value drastically dropped to 0.043. Ca is a reliable variable in this factor. Since the other two variables Co and as, increased the alpha value, those two variables are less reliable on this factor.

Table 3 Reliability of factors (Group 1,2, 3, 4 respectively refers to Wilgamuwa CKDu, Wilgamuwa non- CKDu, Ginnoruwa CKDu and Ginnoruwa non-CKDu)

Group	Factor variables	Cronbach's alpha if item dropped										
		Alpha	Ca	Co	As	Ni	Sr	Mg	F	Cl	Na	SO_4^{2-}
1	Ca, Co, As, Ni	0.65	0.043	0.72	0.73	-	-	-	-	-	-	-
2	Ni, Ca, Sr, Mg	0.86	0.71	-	-	0.72	0.81	0.94	-	-	-	-
3	Alk, HD, Sr, Ca	0.83	0.87	-	-	-	0.8	-	-	-	-	-
4	F, Na, Mg, Cl, SO_4^{2-}	0.81	-	-	-	-	-	0.77	0.85	0.7	0.71	0.72

Wilgamuwa non-CKDu samples consist of variables Ni, Ca, Sr and Mg with a Cronbach alpha of 0.86. When Ca, Ni, and Sr respectively were eliminated from the factor, Cronbach's alpha value dropped to 0.71, 0.72 and 0.81. Mg was not a reliable variable in this factor because it increased the alpha value to 0.94 after getting eliminated. Also, Ca is more reliable than Ni and Sr because Ca was the variable that decreased the alpha value than Ni and Sr. F^-, Na, Mg, Cl^- and SO_4^{2-} are the variables that the first factor was made within Ginnoruwa non-patients samples. The elimination of Mg, Cl, Na and SO_4^{2-} from the factor dropped the Cronbach alpha value to 0.77, 0.7, 0.71 and 0.72, respectively. Since the decrement of Cronbach alpha value was higher in Na^+ than Mg^{2+}, Na^+ was more reliable to combine with Cl^- and SO_4^{2-} than Mg^{2+}. Also, Cl^- was more reliable to combine with Mg^{2+} and Na^+ than SO_4^{2-} and F^-. As such, it was concluded that NaCl, Na_2SO_4, $MgCl_2$ were reliable chemical combinations in water consumed by Ginnoruwa non-patients.

Conclusion

CKDu depends on several factors relating to more ecological factors and potential hereditary inclination. Results of descriptive analysis conclude that mean iron (Fe^{2+}) content in Wilgamuwa CKDu (370.781μg/L) and non-CKDu (731.229 μg/L) exceeds the WHO permissible limits. Also, Wilgamuwa non-CKDu samples show the mean ion contents of Ca^{2+} (137.874 mg/L), Ni (103.566 μg/L), and Electrical conductivity (606.58 μS/cm) exceeds the WHO recommended limits of drinking water. Correlation analysis reveals high correlations between Hardness, Electrical conductivity and total dissolved solids with the Alkali and Alkaline earth metals in Wilgamuwa CKDu samples.

Out of the major elements, mean contents of F^-, Cl^-, NO_3^-, SO_4^{2-}, Na^+ and hardness in CKDu affected wells were higher than non-CKDu wells in Wilgamuwa. pH, EC, Hardness, F^-, Cl^-, $PO4^{3-}$, Na^+, K^+, Al, Cr, Fe, Ni, Cu, Zn, and Cd contents are significantly different ($p < 0.05$) in Wilgamuwa CKDu and non-CKDu wells. Also, out of major elements, Mg^{2+}, Ca^{2+}, K^+, Na^+, NO_3^- and hardness contents are higher in Wilgamuwa CKDu wells compared to the Ginnoruwa CKDu wells. Cl^-, PO_4^{3-}, K^+ and Cr concentrations are highly significantly different ($p<0.0001$) among Wilgamuwa and Ginnoruwa CKDu samples.

Results obtained by Factor analysis tends to identify chemical combinations, which are shown as factor combinations—observed that Ni, Ca^{2+}, Sr^{2+} and Mg^{2+} appears as a factor in Wilgamuwa non-CKDu water sources. Since all these elements are alkaline earth metals, we conclude that alkaline earth metals are chemically associated and appear as a factor combination in Wilgamuwa non-CKDu water sources. Also, Alkalinity, Hardness, Ca^{2+} and Sr^{2+} were found as a factor in Ginnoruwa CKDu samples, denoted as a chemical combination that includes alkaline aspects. By considering the reliability of the factors, it was concluded that NaCl, Na_2SO_4, $MgCl_2$ were reliable chemical combinations in water consumed by Ginnoruwa non-patients.

The study highlights an ecological and hereditary inclination contributing to Chronic Kidney Disease of unknown etiology (CKDu) in Wilgamuwa and Ginnoruwa. Notably, elevated iron levels in water pose a significant health risk, exceeding WHO limits. Recommendations include implementing water quality monitoring, public awareness campaigns, policy interventions to regulate alkaline earth metals, and interdisciplinary research to unravel genetic and ecological factors influencing CKDu. These measures aim to minimize the health problem with drastic consequences, emphasizing the importance of sustainable solutions.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

1. Navas–Acien A, Tellez–Plaza M, Guallar E, et al. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol*. 2009;170(9):1156–1164.
2. Chandrajith R, Dissanayake CB, Ariyarathna T, et al. Dose–dependent Na and Ca in fluoride–rich drinking water- another major cause of chronic renal failure in tropical arid regions. *Sci Total Environ*. 2011;409(4):671–675.
3. Balasooriya S, Munasinghe H, Herath AT, et al. Possible links between groundwater geochemistry and chronic kidney disease of unknown etiology (CKDu): an investigation from the Ginnoruwa region in Sri Lanka. *Exposure Health*. 2019;12:823–834.
4. Bandara JMRS, Wijewardena HVP, Liyanege J, et al. Chronic renal failure in Sri Lanka caused by elevated dietary cadmium: Trojan horse of the green revolution. *Toxicol Lett*. 2010;198(1):33–39.
5. Senevirathna L, Abeysekera T, Nanayakkara S, et al. Risk factors associated with disease progression and mortality in chronic kidney disease of uncertain etiology: a cohort study in Medawachchiya, Sri Lanka. *Environ Health Prev Med*. 2012;17(3):191–198.
6. Cooray T, Wei Y, Zhong H, et al. Assessment of groundwater quality in CKDu affected areas of Sri Lanka: implications for drinking water treatment. *Int J Environ Res Public Health*. 2019;16(10):1698.
7. Jayasumana C, Fonseka S, Fernando A, et al. Phosphate fertilizer is a main source of in areas affected with chronic kidney disease of unknown etiology in Sri Lanka. *SpringerPlus*. 2015;4(1):1–8.
8. Wasana HM, Perera GD, Gunawardena PDS, et al. WHO water quality standards Vs synergic effect (s) of fluoride, heavy metals and hardness in drinking water on kidney tissues. *Sci Rep*. 2017;7(1):1–6.
9. Jayatilake N, Mendis S, Maheepala P, et al. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrol*. 2013;14(1):180.
10. Bandara JMRS, Senevirathna DMAN, Dasanayake DMRSB, et al. Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (Tilapia). *Environ Geochem Health*. 2008;30(5):465–478.
11. Edition F. Guidelines for drinking- water quality. WHO chronicle. 2011;38(4):104–108.
12. World Health Organization (WHO). Cholera and other epidemic diarrhoeal disease control: fact sheets on environmental sanitation (No. WHO/EOS/96.4). World Health Organization. 1996.
13. Abeywickarama B, Ralapanawa U, Chandrajith R. Geo environmental factors related to high incidence of human urinary calculi (kidney stones) in central highlands of Sri Lanka. *Environ Geochem Health*. 2016;38(5):1203–1214.
14. Nanayakkara S, Stmld S, Abeysekera T, et al. An integrative study of the genetic, social and environmental determinants of chronic kidney disease characterized by tubulointerstitial damages in the north central region of Sri Lanka. *J Occup Health*. 2013;56(1):28–38.
15. Gobalarajah K, Subramaniam P, Jayawardena UA, et al. Impact of water quality on chronic kidney disease of unknown etiology (CKDu) in Thunukkai division in Mullaitivu district, Sri Lanka. *BMC Nephrol*. 2020;21(1):1–11.
16. Speight JG. *Natural water remediation: chemistry and technology*. Butterworth–Heinemann. 2019.