Comprehensive Groundwater Quality Analysis in Chronic Kidney Disease of Unknown Etiology (CKDu) Prevalence Areas of Sri Lanka to Investigate the Responsible Culprit

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Received 9 February 2022; Accepted 17 March 2022; Published 29 March 2022

Academic Editor: Rabia Rehman

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Chronic kidney disease of unknown etiology (CKDu) is a serious health problem in Sri Lanka especially among agricultural communities in dry zone since 19th century. In the present study, several water quality parameters were studied in well water samples and only four parameters, namely, hardness, cadmium level, fluoride level, and strontium level have shown a relationship with the CKDu prevalence. Higher percentage of well water samples collected from CKDu prevalent area in both pre and postmonsoon seasons have exceeded the WHO recommended levels of hardness. Further, water samples collected in postmonsoon seasons had significantly higher hardness than the well water samples collected in premonsoon season \( P < 0.05 \). This may be due to the dissolution of calcium carbonate by water recharge. Moreover, cadmium and fluoride contents have exceeded the recommended levels in high-risk area during the premonsoon period. Furthermore, according to principal component analysis (PCA), four clusters were identified depending on the different levels of fluoride, cadmium, hardness, and strontium contents. The control area (Am) fell in to separate cluster with low contents of fluoride, cadmium, hardness, and strontium than in CKDu prevalent area. Since it has been found that the above species are directly involved in renal damage, it can be concluded that a synergetic effect of cadmium, fluoride, hardness, and strontium in well water may be a main cause for CKDu in Sri Lanka.

1. Introduction

Chronic kidney disease is a heterogeneous disorder that affects the structure and function of the kidney. Due to this disorder, the kidneys do not function properly and this may lead to the inability of the kidneys to remove waste products from the blood, which will ultimately build up in the body causing many complications. The kidney disease is diagnosed as Chronic Kidney Disease (CKD) when the kidney damage and the improper functioning last for more than three months [1]. The main causes for the CKD are identified as diabetes, hypertension, and chronic glomerulonephritis. Other possible sources for the CKD are bacteria, allergies, kidney stones, tumors, and toxic chemicals while heart disease, smoking, obesity, and high cholesterol are considered to have an increased risk of CKD [2].

The Chronic Kidney Disease of unknown etiology (CKDu) is a burning health issue in Sri Lanka, especially in the agricultural areas in dry zone. This was initially reported in 1994. CKDu is not related to any of the known causes for kidney failures like, hypertension, diabetes mellitus, and infections [3]. CKDu in Sri Lanka is a chronic tubule interstitial disease which has a very low progression to the end stage [4]. It has been twenty years since this was reported and the problem is still remaining unsorted. Earlier, CKDu was mainly observed among agricultural communities in the
North Central Province (NCP) in Sri Lanka and now the disease has spread to new areas including Uva and North west provinces [5].

Increased use of fertilizers and pesticides for the enhancement of crop productivity eventually leads to soil and water contamination and several health issues [6, 7]. According to several studies, it has been found that the fertilizers and pesticides generally include several impurities including heavy metals. Zinc, copper, lead, arsenic, and cadmium were reported as major heavy metal impurities in fertilizers. These heavy metals were found in higher quantities in pesticides [8–10]. Furthermore, soil-bounded cadmium can be dissolved and mobilized when there is an enrichment in the soil acidity [11].

Although many local and foreign scientists are working on this problem, they have not yet found an exact cause for CKDu. However, some causative factors have been hypothesized and those factors have a large variation. Some of those are fluoride content, hardness, presence of heavy metals, cyanobacteria in water, and extraordinary dehydration conditions which prevail in people living in this area [12–15].

According to the epidemiological data, the distributions of CKDu patients vary between divisional secretariat (DS) in a district and also between Grama niladhari divisions (GND) within a divisional secretariat. Nevertheless, many studies were conducted without considering this fact and have taken mean values for a large area. Thus, there are many drawbacks in previous research studies.

Therefore, in the current study, CKDu prevalent areas were selected based on careful consideration of epidemiological data. Thus, the objective of this study was to assess the groundwater quality in Chronic Kidney Disease of unknown etiology (CKDu) prevalence.

2. Material and Methods

2.1. Study Area and Sample Collection. Anuradhapura district, in North Central Province of Sri Lanka, has been selected as the study area. According to the CKDu epidemiological data in Sri Lanka, the highest number of CKDu patients was reported in Anuradhapura district. Nevertheless, the CKDu prevalence is not uniform in all over the Anuradhapura district. The distribution of CKDu patients varies between divisional secretariat (DS) and also between Grama niladhari divisions (GND) within a divisional secretariat. GNDs are the smallest administrative units in Sri Lanka.

Due to this reason, three study areas were selected for further studies depending on the CKDu prevalence in CKDu endemic area. Mahadivulwewa GND in Madawachchiya DS (M-Ma-PostM) (high risk), Bogahayaya GND in Mihinthale DS (B-Mi-PostM) (moderate risk), and Kalugala GND in Palagala DS (K-P-PostM) (low risk) were selected based on the CKDu prevalence in CKDu endemic area. Buddahangala GND in Ampara district (Am-PostM) was selected as the control area for this study due to the same climatic conditions and agricultural practices as Anuradhapura district with none of the reported CKDu patients. In these sampling sites, shallow drinking well water samples were collected in postmonsoon season.

Furthermore, shallow well water samples were collected from two GNDs, namely, Mahadivulwewa (M-Ma-PostM) and Puhudivula (P-Ma-PreM) in Madawachchiya DS (high risk) in premonsoon season (extremely dry season) as well. Highest numbers of CKDu patients were recorded from Madawachchiya DS (Figure 1).

In each sampling site, an average of thirty shallow drinking well water samples were randomly collected. Sampling locations were recorded using the global positioning system (GPS). Collected well water samples were stored at 4°C until analysis.

2.2. Analytical Methodology. Total dissolved solids (TDS), pH, salinity, and conductivity of each raw water sample were measured at the site without any prepreparation using an analyzer HACH: HQ40d Multi-parameter (HACH, Loveland, Colorado, USA). The calcium and magnesium contents were determined using flame atomic absorption spectrometry (GBC Avanta, Australia) and hardness of the collected water samples was obtained using Ca Mg calculation method (APHA -2340B) (APHA, 1998). Furthermore, fluoride content of the collected well water samples was determined using a fluoride ion-selective electrode (Thermo Orion Inc.) according to the APHA 4500-F-, using TISAB II buffer (APHA, 1998). The nitrate, sulphate, and total phosphate contents were analyzed using HACH DR/890 colorimeter (Hach, Colorado, USA). In addition, the metal analysis was carried out using an inductively coupled plasma-mass spectrometry (Agilent 7900, USA) to determine the selected heavy metal contents (APHA, 1998). All the samples were analyzed in triplicate and all the metal concentrations of samples were compared with the percentage recoveries of water (80–120%) through spike addition. The statistical analyses were carried out using IBM SPSS statistics 20 and Unscramble 11.0 software.

3. Results and Discussion

In the present study, physical and chemical water quality parameters were analyzed and tabulated in Table 1.

According to the results, total hardness, fluoride content, cadmium content, and strontium content have shown significant correlation with CKDu prevalence. All other water quality parameters were also compared with the permissible limits (WHO, SLS) [16, 17] and those parameters were not shown a significant deviation from the recommended levels (P < 0.05).

Hardness of water is mainly caused by the presence of polyvalent metal cations mainly calcium (Ca) and magnesium (Mg) and the degree of hardness can be varied depending on the Ca and Mg content. Depending on the level of hardness, water can be categorized as soft water (0–60 mg/L), moderately hard water (61–120 mg/L), hard water (121–180 mg/L), and very hard water (>181 mg/L) [18, 19]. Long-term intake of hard water can cause kidney dysfunction [20] and hardness contents in water can reform chemical configuration of fluoride and other trace metals.
According to the study, the highest percentage of well water sample that was collected in both post and premonsoons from CKDu endemic area has exceeded the maximum permissible level (MPL) of hardness (250 CaCO₃ mg/L [22]) except in the control area of Ampara (Am). Furthermore, various studies in the CKDu prevalence areas also reported high hardness contents in well water samples [21, 23-25]. There is no significant difference among K-P-PostM, M-Ma-PostM and B-Mi-PostM for the hardness content of well water samples (P > 0.05). However, hardness contents of well water samples in premonsoon season (M-Ma-PreM and P-Ma-PreM) were significantly lower than postmonsoon well water samples in CKDu prevalent Madawachchiya DS (M-Ma-PostM) (P < 0.05) (Figure 2). That may be due to the dissolution of calcium carbonate by water recharge [26].

According to the point of view of scientists, fluoride can be accumulated in the kidney than all other soft tissues and high concentration of fluoride in water is suspected to be responsible for the CKDu in Sri Lanka [21]. But according to the results, none of the samples collected in postmonsoon season from the CKDu prevalence and control areas has exceeded the recommended level of 1.5 mg/L set by WHO for fluoride content. However, the majority of well water samples which were collected in premonsoon season from...
### Table 1: Physical and chemical water quality parameters.

<table>
<thead>
<tr>
<th>Study area parameter</th>
<th>M-Ma_PostM</th>
<th>MahadivulwaPreM</th>
<th>PuhudivulvaPreM</th>
<th>B-Mi-PostM</th>
<th>K-P-PostM</th>
<th>Ampara</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>pH</td>
<td>7.63 ± .36</td>
<td>6.84 ± .52</td>
<td>6.88 ± .42</td>
<td>7.75 ± .38</td>
<td>6.36 ± 1.20</td>
<td>7.18 ± .35</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>6.30 ± 1.61</td>
<td>6.27 ± .98</td>
<td>6.40 ± 1.36</td>
<td>6.11 ± 2.29</td>
<td>4.73 ± 1.45</td>
<td>6.42 ± .69</td>
</tr>
<tr>
<td>Conductivity (μS/m)</td>
<td>765.54 ± 337.92</td>
<td>821.30 ± 199.51</td>
<td>952.51 ± 369.69</td>
<td>854.31 ± 419.70</td>
<td>717.17 ± 234.49</td>
<td>515.22 ± 126.17</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>415.05 ± 273.46</td>
<td>373.35 ± 111.13</td>
<td>463.35 ± 186.62</td>
<td>420.78 ± 212.04</td>
<td>358.92 ± 116.71</td>
<td>261.22 ± 80.61</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>.39 ± .27</td>
<td>.37 ± .10</td>
<td>.46 ± .19</td>
<td>.42 ± .22</td>
<td>.35 ± .11</td>
<td>.25 ± .05</td>
</tr>
<tr>
<td>Total hardness (as CaCO₃) (mg/L)</td>
<td>683.45 ± 312.26</td>
<td>304.50 ± 82.99</td>
<td>293.71 ± 93.10</td>
<td>742.89 ± 399.07</td>
<td>800.00 ± 452.26</td>
<td>121.49 ± 44.26</td>
</tr>
<tr>
<td>Fluoride (mg/L)</td>
<td>.50 ± .29</td>
<td>.99 ± .69</td>
<td>1.40 ± 1.25</td>
<td>.15 ± .08</td>
<td>.30 ± .15</td>
<td>.65 ± .29</td>
</tr>
<tr>
<td>Sulphate (mg/L)</td>
<td>28.17 ± 34.15</td>
<td>35.03 ± 38.24</td>
<td>24.07 ± 29.33</td>
<td>17.38 ± 13.15</td>
<td>10.54 ± 9.02</td>
<td>36.00 ± 34.79</td>
</tr>
<tr>
<td>Phosphate (mg/L)</td>
<td>1.14 ± .46</td>
<td>1.17 ± .41</td>
<td>1.13 ± .47</td>
<td>1.51 ± .53</td>
<td>1.42 ± .45</td>
<td>1.02 ± .46</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>.67 ± .33</td>
<td>.73 ± .35</td>
<td>.62 ± .29</td>
<td>.58 ± .30</td>
<td>.98 ± .93</td>
<td>1.39 ± .99</td>
</tr>
<tr>
<td>Li (μg/L)</td>
<td>1.69 ± 1.66</td>
<td>2.00 ± 1.49</td>
<td>3.17 ± 3.78</td>
<td>1.59 ± .94</td>
<td>1.49 ± 1.18</td>
<td>5.63 ± 8.33</td>
</tr>
<tr>
<td>Be (μg/L)</td>
<td>.03 ± .05</td>
<td>.01 ± .01</td>
<td>.01 ± .02</td>
<td>ND ± —</td>
<td>ND ± —</td>
<td>.08 ± .09</td>
</tr>
<tr>
<td>V (μg/L)</td>
<td>8.23 ± 5.30</td>
<td>10.16 ± 6.48</td>
<td>7.82 ± 5.62</td>
<td>7.25 ± 6.21</td>
<td>8.21 ± 6.84</td>
<td>1.00 ± 1.08</td>
</tr>
<tr>
<td>Cr (μg/L)</td>
<td>3.61 ± 2.72</td>
<td>ND ± —</td>
<td>.46 ± 1.10</td>
<td>2.39 ± 1.31</td>
<td>2.30 ± 2.36</td>
<td>3.31 ± 1.86</td>
</tr>
<tr>
<td>Mn (μg/L)</td>
<td>38.55 ± 166.62</td>
<td>5.42 ± 4.44</td>
<td>15.49 ± 35.00</td>
<td>7.84 ± 6.15</td>
<td>27.03 ± 76.52</td>
<td>107.97 ± 258.91</td>
</tr>
<tr>
<td>Fe (μg/L)</td>
<td>503.41 ± 616.91</td>
<td>.12 ± .06</td>
<td>.24 ± .37</td>
<td>135.64 ± 298.89</td>
<td>157.85 ± 92.59</td>
<td>.81 ± .72</td>
</tr>
<tr>
<td>Co (μg/L)</td>
<td>.85 ± .31</td>
<td>.01 ± .07</td>
<td>.05 ± .11</td>
<td>.89 ± .42</td>
<td>.62 ± .29</td>
<td>.05 ± .11</td>
</tr>
<tr>
<td>Ni (μg/L)</td>
<td>5.58 ± 4.27</td>
<td>2.14 ± 4.63</td>
<td>5.06 ± 10.22</td>
<td>8.59 ± 3.49</td>
<td>4.64 ± 3.81</td>
<td>2.26 ± 1.84</td>
</tr>
<tr>
<td>Cu (μg/L)</td>
<td>7.02 ± 17.00</td>
<td>6.37 ± 6.98</td>
<td>73.32 ± 409.75</td>
<td>3.96 ± 2.51</td>
<td>12.10 ± 28.67</td>
<td>10.54 ± 15.15</td>
</tr>
<tr>
<td>Zn (μg/L)</td>
<td>57.16 ± 48.80</td>
<td>52.70 ± 25.97</td>
<td>78.51 ± 169.51</td>
<td>57.32 ± 39.74</td>
<td>32.12 ± 17.17</td>
<td>88.92 ± 39.54</td>
</tr>
<tr>
<td>Ga (μg/L)</td>
<td>.22 ± .17</td>
<td>.27 ± .48</td>
<td>.17 ± .42</td>
<td>.09 ± .04</td>
<td>.05 ± .03</td>
<td>.30 ± .65</td>
</tr>
<tr>
<td>As (μg/L)</td>
<td>.01 ± .04</td>
<td>ND ± —</td>
<td>.03 ± .16</td>
<td>ND ± —</td>
<td>ND ± —</td>
<td>ND ± —</td>
</tr>
<tr>
<td>Sr (μg/L)</td>
<td>395.72 ± 195.22</td>
<td>367.73 ± 163.99</td>
<td>384.02 ± 153.96</td>
<td>581.75 ± 255.86</td>
<td>236.83 ± 104.56</td>
<td>39.91 ± 30.63</td>
</tr>
<tr>
<td>Cd (μg/L)</td>
<td>.47 ± .85</td>
<td>5.39 ± 3.65</td>
<td>3.40 ± 3.00</td>
<td>2.41 ± 1.47</td>
<td>1.44 ± 4.16</td>
<td>1.41 ± .40</td>
</tr>
<tr>
<td>Ba (μg/L)</td>
<td>173.40 ± 75.77</td>
<td>132.51 ± 82.97</td>
<td>103.00 ± 57.16</td>
<td>201.69 ± 52.13</td>
<td>121.77 ± 51.88</td>
<td>35.24 ± 16.37</td>
</tr>
<tr>
<td>Pb (μg/L)</td>
<td>1.19 ± 1.00</td>
<td>3.64 ± 1.44</td>
<td>4.80 ± 4.31</td>
<td>5.70 ± 10.93</td>
<td>4.39 ± 6.82</td>
<td>4.72 ± 2.19</td>
</tr>
</tbody>
</table>

ND: not detected; DO: dissolved oxygen; TDS: total dissolved solid.
CKDu high prevalence Madawachchiya DS have exceeded this recommended level (Figure 3). As a result of rain, the aquifers were recharged and fluoride concentration were diluted [27, 28]. This may be the main reason for reporting lower fluoride contents in rainy seasons comparing with dry season.

Although there are several metals that were analyzed, only Cd and Sr have shown a relationship with the CKDu prevalence. According to the results, only a few numbers of well water samples that were collected in postmonsoon season have exceeded the WHO recommended level of Cd (3 μg/L) [16, 17] in the high risk (M-Ma-PostM) and moderate risk (P-Ma-PostM) CKDu prevalence areas. None of the samples in the control area (Am) and low CKDu prevalence K-P-PostM has exceeded the recommended level of Cd (3 μg/L). However, in the premonsoon season, a higher percentage of well water samples in highly CKDu prevalence Madawachchiya DS have exceeded the recommended levels of Cd in 78.1% and 62.3%, respectively, for M-Ma-PreM and P-Ma-PreM (Figure 4). According to the results, a low concentration of Cd in well water samples was found during postmonsoon season. That may be due to the dilution of well water by rain water. However, some water samples that were collected during postmonsoon also have exceeded the MPL of Cd.

According to the previous studies, it has been shown that long-term intake of Cd can cause kidney failure in different pathways [21, 29, 30]. Furthermore, high concentrations of Cd (>3 μg/L) of well water were reported in several studies [31, 32] and low contents of Cd (<3 μg/L) were also reported [4, 23] in CKDu endemic areas.

Cd is occurring naturally in soils. Phosphate fertilizers are considered a major source of Cd. Furthermore, the solubility of Cd in water is affected by the acidity. Soil-bounded Cd can be dissolved when there is an enrichment in acidity [11]. On the other hand, glyphosate was the most consumed herbicide in agricultural areas in Sri Lanka [33] and a study has revealed that glyphosate reacts with Cd to form water-soluble complexes which has lower affinity to soil surface[34]. Therefore, the Cd content of ground water in those areas can be increased.

In this study, the Sr content of well water samples taken from the control area (Am) was significantly different from all other samples taken from the CKDu prevalence area (P < 0.05) (Figure 5) in both pre and postmonsoons except low risk K-P-PostM. Nevertheless, none of the samples has exceeded the recommended level of 1.5 mg/L [35]. Previous studies have shown that the elevated levels of Sr in drinking water can cause nephrotoxic actions [36, 37]. However, according to SLS guidelines, there is no recommended permissible level that has been established for Strontium in drinking water.

Furthermore, principal component analysis (PCA) was carried out to water quality parameters of hardness, fluoride,
Cd, and Sr in well water samples by examining the differences among the study areas. The results of PCA analysis are presented as a score plot (Figure 6), which displays clusters or groupings of samples based on compositional similarities of water quality parameters. A loading plot (Figure 7) which shows the variable correlation is also presented. In the PCA model, the first two principal component axes of PCA1 and PCA2 resolve 98% of the total data variance (Figure 6). According to the PCA loading plot, positive correlations were examined between Cd and fluoride contents, as well as between hardness and Sr contents. And also, hardness content and Sr contents have a negative correlation with fluoride and Cd contents.

The main trend on the PCA1 axis in well water was expressed as high positive PCA1 loadings for hardness and Sr, as well as negative loadings for Cd and fluoride. Moreover, the PC2 axis presented negative loadings for Cd, fluoride, and Sr, as well as positive loadings for hardness. From the PCA score plot, four groups were identified based on natural groupings in the PCA2 versus PCA1 plot (Figure 6). Those clusters were renamed as C1, C2, C3, and C4.

The cluster of C1 represents the well water samples collected from CKDu prevalent area of M-Ma-PostM (high risk) and B-Mi-PostM (moderate risk) in postmonsoon period. Considering water quality parameters, these areas could not be separated into clusters by PCA analysis due to their similarities. C1 cluster is with positive PC1 and with slightly negative PC1 or positive PC2 and slightly negative PC2. This is characterized by high hardness and Sr contents and low Cd and fluoride contents. And also, the cluster of C2 stands for the well water samples collected from K-P-PostM (low risk) in postmonsoon. This C2 cluster is with positive PC1 and PC2 loadings and that is characterized by high hardness content and low contents of Sr, Cd, and fluoride.

Furthermore, the cluster of C3 illustrates the well water samples collected from CKDu prevalent M-Ma-PostM and P-Ma-PostM GNDs (high risk) in premonsoon period. This cluster is with negative PC1 and positive PC2 loadings. Thus, it is distinguished by high fluoride and Cd contents and low levels of hardness and Sr. Moreover, the cluster of C4 denotes the control area of Ampara. This is with negative PC1 and positive PC2 loadings. Therefore, this cluster is specified by low contents of Cd, fluoride, hardness, and Sr.

According to these results, four clusters have been obtained and well water samples in the control area of Ampara clearly represent a separate cluster (C4) without any overlapping. That denotes low levels of Cd, Fluoride, hardness, and Sr contents in the control area. And also, according to PCA analysis, two overlapping clusters (C1 and C2) have been obtained for the well water samples collected from three GNDs in CKDu prevalent area in the postmonsoon period (M-Ma-PostM, P-Ma-PostM, and K-P-PostM). The main reason behind separating into two clusters was the low Sr content in K-P-PostM. However, well water samples that were collected from all of these three GNDs in postmonsoon were high in hardness content and low in Cd and fluoride content. Furthermore, the samples collected from CKDu prevalent high-risk area in premonsoon (M-Ma-PreM) and postmonsoon (M-Ma-PostM) were separated by high Cd and fluoride contents and low hardness and Sr contents in premonsoon well water samples. Therefore,
Figure 4: Cadmium content of well water samples in the study area.

Figure 5: Strontium content of well water samples in the study area.
Figure 6: PCA score plot.

Figure 7: PCA loading plot.
according to the obtained results, well water samples in CKDu area are at a high risk in both seasons due to comparatively high hardness and Sr contents in postmonsoon and high cadmium and fluoride contents in premonsoon seasons. However, the hardness content of well water sample in high-risk area at premonsoon also exceeded MPL of hardness (250 CaCO₃ mg/L).

Thus, according to this study synergic effect of hardness, Fluoride, Cd and Sr contents in water samples may be the cause for this CKDu problem in Sri Lanka. Further clinical studies are essential to prove this finding.

4. Conclusion

In this study, well water samples were analyzed in the CKDu prevalence area to identify the responsible culprit behind CKDu. Accordingly, several water quality parameters were studied and only four parameters have shown a relationship with CKDu prevalence. Those identified water quality parameters are hardness, Cd, fluoride, and Sr contents which are responsible for renal damage. However, the damaging mechanism may be different in two seasons due to variation of these elements with the seasonal change. The hardness content of well water in both seasons has shown higher values compared with the recommended level of hardness in drinking water. However, postmonsoon well water samples have shown significantly higher values with higher variation when comparing with premonsoon well water. That may be due to the dissolution of calcium carbonate by water recharge. The Cd and fluoride contents of well water in high CKDu prevalent area were significantly higher and have exceeded the recommended levels in premonsoon season. However, in postmonsoon, there was no issue associated with fluoride and Cd contents. Furthermore, Sr in well water has shown relationship with CKDu prevalence. Finally, it can be deduced that synergic effect of Cd, fluoride, hardness, and Sr on the kidney by well water may be the main cause for CKDu in Sri Lanka. Further clinical studies are essential to prove this finding.

Data Availability

The data are available on request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the University Grant commission, Sri Lanka under the research grant RP/03/SR/02/06/01/2016. We want to greatly thank Mr. Amila Kannangara and Mr. S. A. A. K. Sooriyaarachchi for providing technical support for this research. Furthermore, we would like to express our sincere gratitude to Prof. Shashikala Rajapakse and Mrs. D.H Perera for giving us support throughout this research.

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