DOI: https://doi.org/10.4038/tsljh.v16i2.44

SLJH. 2024; **16**(2): 18-25

Research paper

Changes in haematological parameters in relation to the stage of chronic kidney disease in a cohort of patients receiving care at Teaching Hospital Karapitiya, Galle, Sri Lanka

HSSP Fernando[1], **MGM Mohotti**[2], **KAC Wickramaratne**[3]

Key words: chronic kidney disease, anaemia, thrombocytopenia, haemodialysis

Abstract

The incidence and prevalence of chronic kidney disease is rising in Sri Lanka. This study aimed to evaluate the degree and type of anaemia, erythrocyte sedimentation rate, thrombocytopenia, hypersegmented neutrophils, and burr cells in patients with chronic kidney disease across different stages. A descriptive cross-sectional study was conducted at Teaching Hospital Karapitiya, enrolling 114 CKD patients: 49 in stages 3 & 4, 26 in stage 5 non-dialysis, and 39 on haemodialysis. Data was collected via questionnaires and routine full blood counts. Anaemia was present in 82.3% of the cohort, with a mean haemoglobin of 10.61 g/dL. Prevalence rates were 75.5% in stages 3 & 4, 76.9% in stage 5 non-dialyzed and 94.7% in haemodialyzed patients. Haemoglobin levels in patients on haemodialysis were significantly lower by 2.4 g/dL compared to stages 3 & 4 and 1.86 g/dL compared to stage 5 non-dialyzed patients. (P<0.001). Normochromic normocytic anaemia was the most common (82.3%), while hypochromic microcytic anaemia was found in 16.8% overall and 42.1% of dialyzed patients. Thrombocytopenia affected 18% of patients, where prevalence increased from 10.2% in stages 3 & 4 to 25.6% in dialyzed patients. Moderate thrombocytopenia was seen only in dialyzed patients (10.3%), with their significantly lower mean platelet count, compared to stages 3 & 4 (P=0.04). Elevated ESR occurred in 75.2%, which increased with disease progression without statistical significance. Markedly elevated ESR (>100 mm/ hr) was observed in 3.53 % of patients. Hypersegmented neutrophils were present in all patients, being highest in stage 5 non-dialyzed (P=0.001) and next in dialyzed patients (P=0.004). Burr cells increased with CKD progression without statistical significance.

Introduction

Chronic kidney disease (CKD) is defined as abnormalities of structure or function of the kidneys which are present for more than three months with implication for health[1]. CKD leads to a gradual decline in renal function, often progressing to the point where intensive treatments such as dialysis or kidney transplantation become necessary. Based on the glomerular filtration rate (GFR), CKD is classified into six stages:

- **Stage 1:** Normal or high GFR (≥90 mL/min/ 1.73m^2)
- **Stage 2:** Mildly decreased GFR (60-89 mL/min/ 1.73m^2)
- **Stage 3a:** Mild to moderate decrease in GFR (45-59 mL/min/1.73m^2)
- **Stage 3b:** Moderate to severe decrease (30-44 mL/min/1.73m^2)

[1]*Department of Haematology, District General Hospital, Hambanthota, Sri Lanka.*

[2]*Department of Haematology, Teaching Hospital Karapitiya, Galle, Sri Lanka.*

[3]*Department of Pathology, Faculty of Medicine, University of Ruhuna, Sri Lanka.*

Correspondence: Dr. HSSP Fernando

E-mail: subodhapfernando@gmail.com

- **Stage 4:** Severe decrease (15-29 mL/min/ 1.73m^2)
- **Stage 5:** Kidney failure (GFR <15 mL/min/ 1.73m^2)

CKD affects approximately 9.1% of the global population, with an increasing prevalence in Sri Lanka[3], where it ranked as the fifth leading cause of death in 2019[4]. In recent decades, a new form, chronic kidney disease of uncertain aetiology (CKDu), has emerged in Sri Lanka's dry zone, placing a significant burden on the national healthcare system[5]. Anaemia is a common complication in CKD, often accompanied by thrombocytopenia, elevated erythrocyte sedimentation rate (ESR), hyper-segmented neutrophils and the presence of burr cells. This study aimed to evaluate these haematological parameters in a cohort of patients in a government hospital of Sri Lanka and contribute to the limited body of research in this area.

Methods

This descriptive cross-sectional study was conducted over one year at the Nephrology Clinic and Dialysis Unit of Teaching Hospital Karapitiya, Galle, Sri Lanka. A total of 114 adult CKD patients in stages 3 to 5 were enrolled, regardless of gender, underlying cause, clinical profile or treatment status. Patients were categorized into three groups, Stage 3 & 4, stage 5 not on dialysis and stage 5 on dialysis. Data were collected using interviewer-administered, pretested questionnaires covering detailed medical and treatment histories. Individuals with a recent history of blood transfusion within three months were excluded from the study. The samples with platelet clumps were excluded from the platelet related data and morphology suggestive of haemoglobinopathy were excluded from red cell related data.

Routine clinic Full blood count (FBC) samples, in ethylene diamine tetra acetic acid (EDTA) analyzed by Beckman coulter LH 780 analyzer, were used to assess haemoglobin, red cell indices, white cell count and platelet count. ESR was assessed by Westergren method. Blood films were stained manually in Leishman's stain and morphological assessment of red cells and enumeration of burr cells and hyper-segmented neutrophils were done by the principal investigator. Hypersegmented neutrophils were counted in ten x40 power fields and average was calculated. In an area of evenly distributed red cells, under the x100 power oil emersion field, total number of red cells (n) was counted in three consecutive fields. Mean red cell number was calculated dividing total number of red cells by three (n/ 3). The number of burr cells was counted in ten consecutives ×100 power fields (Y).

Burr cell percentage =Y/10 ÷ n/3 ×100.

Statistical analysis was performed using SPSS statistics for windows 2018 ANOVA. The statistical significance level was set to P<0.05.

Results

In the total of 114 patients, there were 49 in CKD stage 3 & 4, 26 in stage 5 non-dialyzed group and 39 in stage 5 dialyzed group. The total sample consisted of 72 males (63.15%) and 42 females (36.84%) with a male to female ratio of 1.71: 1. The mean age of the total sample was 59.5 years which ranged from 19 to 86 years.

Anaemia

Anaemia-related data were analysed for 113 patients (Figures 1 & 2). Overall, 82.3% of the cohort were anaemic, with prevalence rates of 75.5% in stage 3 & 4, 76.9% in stage 5 non-dialyzed (5 ND), and 94.73 % in dialysis (5 D) patients.

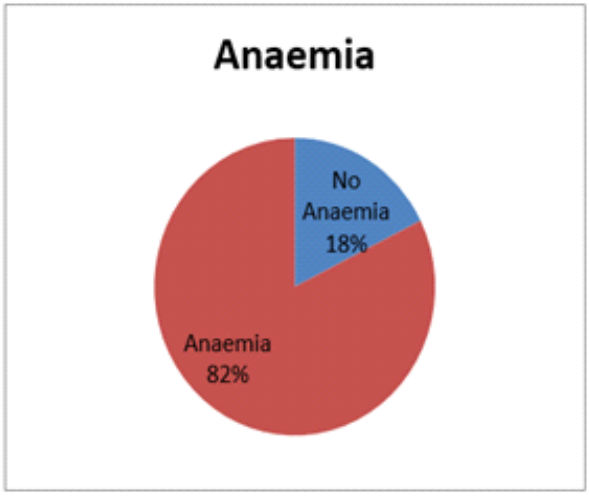

Figure 1. The prevalence of anaemia.

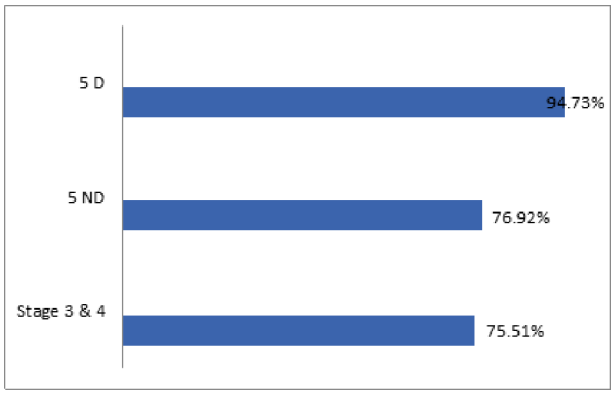

Figure 2. Prevalence of anaemia among the three study groups.

The mean haemoglobin level for the total sample was 10.61 g/dL (SD ±1.94 g/dL). In patients with stage 3 & 4 CKD, it was 11.55 g/dL (±1.54 g/dL), 11.00 g/dL (±1.34 g/dL) in stage 5 non-dialyzed (5 ND) patients and 9.15 g/dL (±1.91 g/dL) in those undergoing dialysis (5D). Based on red cell indices, 93 patients in the total sample had normochromic normocytic anaemia (NCNC), while 19 patients (16.81%) had hypochromic microcytic anaemia (HCMC) and one patient (0.8%) had macrocytic anaemia. Among stage 3 & 4 CKD patients (N=47), 95.91% had NCNC anaemia, while 2.04% (N=1) had HCMC anaemia and 2.04% (N=1) had macrocytic anaemia. In the stage 5 non-dialyzed group, 92.3% (N=24) had NCNC anaemia and 7.69% (N=2) had HCMC anaemia. Among dialysis patients, 57.89% (N=22) had NCNC anaemia, while 42.1% (N=16) had HCMC anaemia. No patients in the stage 5 groups had macrocytic anaemia (Figure 3).

Anaemia was defined and classified according to the WHO anaemia classification9. In stages 3 & 4, the prevalence of mild and moderate anaemia was 42.85% and 32.65%, respectively. Severe anaemia was not observed among these patients. Among stage 5 non-dialyzed patients, 42.3% had moderate anaemia, and 3.8% had severe anaemia. In the dialysis group, moderate and severe anaemia was prevalent in 71.05% (moderate anaemia in 50% and severe anaemia in 21.05%), indicating worsening anaemia with disease advancement (Table 1).

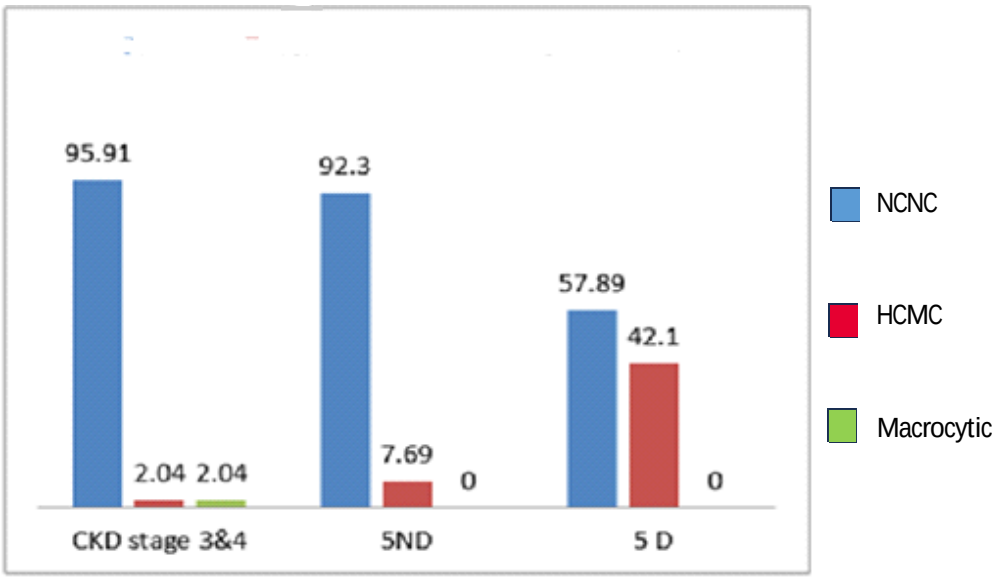

Figure 3. Morphological sub type of anaemia.

Table 1. Severity of anaemia among three study groups

	CKD stage 3 / 4	Stage 5ND	Stage 5D
Mild anaemia	42.85%	30.76%	23.68%
Moderate anaemia	32.65%	42.30%	50.0%
Severe anaemia	0	3.8%	21.05%
No Anaemia	24.49%	23.07%	5.26%

The mean haemoglobin of the dialyzed patients was 2.399 g/dL lower than that of CKD stage 3 & 4 and 1.858 g/dL lower than that of stage 5 non-dialyzed group. Both were statistically significant (P=<0.001). The mean haemoglobin of stage 5 non-dialyzed patients was 0.541 g/dL lower than that of stage 3 & 4 CKD which was statistically insignificant (P=0.177).

Thrombocytopenia

Platelet-related data were analysed for all 114 patients. Thrombocytopenia (platelet count <150 $\times10^3/\mu L$) was observed in 21 patients (18.42%) across the total cohort. The mean platelet count was $207.66\times10^3/\mu L$ (± 64.76). By subgroup, mean counts were $219.31\times10^3/\mu L$ (± 63.78) in stage 3 & 4 CKD, $211.08\times10^3/\mu L$ (± 62.18) in stage 5 non-dialyzed patients, and $190.74\times10^3/\mu L$ (±65.66) in the dialysis group.

Mild thrombocytopenia (platelet count 100-149 $\times10^3/\mu L$), is prevalent in 10.2% of stage 3 & 4 CKD patients, 23.07% of stage 5 non-dialyzed (stage 5 ND) patients and 15.38% of those on dialysis (5 D). Moderate thrombocytopenia (50-99$\times10^3/\mu L$) was found exclusively in dialysis patients (10.25%). No cases of severe thrombocytopenia (platelet count <50$\times10^3/\mu L$) were recorded in any group (Figure 4).

The mean platelet count in stage 5 non-dialyzed patients was $8.229\times10^3/\mu L$ lower than in stage 3 & 4 CKD patients, which was not statistically significant (P=0.598). Similarly, the mean platelet count in dialysis patients was $20.333\times10^3/\mu L$ lower than in stage 5 non-dialyzed patients, which was also not statistically significant (P=0.213). In contrast, the platelet count in dialysis patients was 28.563 $\times10^3/\mu L$ lower than in stage 3 & 4 patients, which is statistically significant (P=0.04).

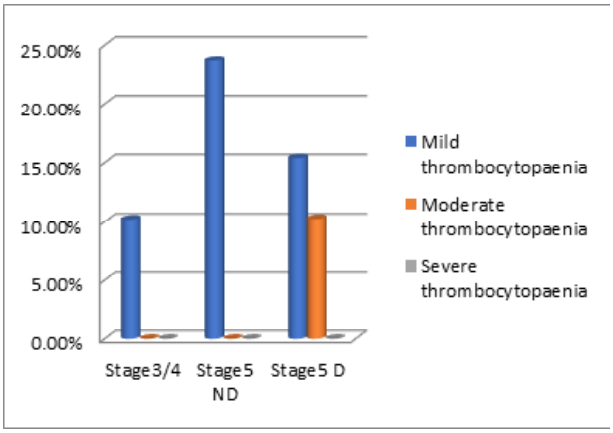

Figure 4. Severity of thrombocytopenia.

Erythrocyte sedimentation rate (ESR)

ESR higher than the age and sex-adjusted normal range was considered elevated[11], while values exceeding 100mm/hr were classified as very high. In this study, 85 patients (75.22%) had elevated ESR, while 28 patients (24.77%) had normal ESR levels. Elevated ESR was found in 75.51% of stage 3 & 4 CKD patients, 72% of stage 5 non-dialyzed (stage 5 ND) patients and 76.92% of dialysis (stage 5D) patients. Very high ESR values (>100mm/hr) were observed in only 3.53% of the total cohort (Figure 5).

The mean ESR for the entire group was 40.79mm/hr (±26.75). By category, the mean ESR was 38.29mm/hr (±26.25) in stage 3 & 4 CKD, 40.08mm/hr (±26.95) in stage 5 non-dialyzed group and 44.38mm/hr (±27.54) in dialyzed patients).

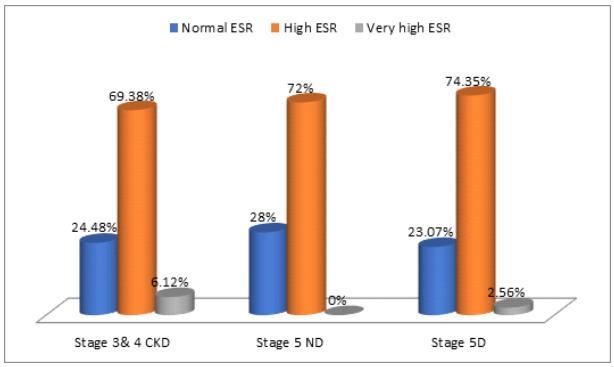

Figure 5. ESR among the three study groups.

The mean ESR in stage 5 non-dialyzed patients was 1.79mm/hr higher than that of stage 3 & 4 CKD patients; however, this difference was not statistically significant (P=0.78). Similarly, the mean ESR in dialysis patients was 4.3mm/hr higher than in stage 5 non-dialyzed patients, which was also not statistically significant (P=0.53).

Hypersegmented neutrophils

Hypersegmented neutrophils are defined as neutrophils with six or more lobes, or when ≥3% of neutrophils have at least five lobes in a count of 100 neutrophils on a peripheral blood smear[25]. In this study, all blood smears showed the presence of hyper segmented neutrophils. The mean percentage in the total cohort was 70.09% (± 26.69). By CKD category, the mean percentage of hypersegmented neutrophils was, 58.16% (± 26.11) in stage 3 & 4 CKD patients, 89.62% (± 16.36) in stage 5 non-dialyzed patients and 72.05% (± 25.15) in dialysis patients.

The mean percentage in stage 5 non-dialyzed patients was 31.45% which was higher than in stage 3 & 4 CKD patients with a statistically significant difference (P=0.001). Dialysis patients had a mean hypersegmented neutrophil percentage that was 13.8% higher than stage 3 & 4 patients (P = 0.008) and 17.56% lower than stage 5 non-dialyzed patients: both differences were statistically significant (P=0.004). (Table 2).

Burr cells

A total of 114 peripheral blood smears were examined for the presence of burr cells. The mean burr cell percentage across the entire sample was 0.59% (±1.47%). According to disease stage, the mean percentages were 0.38% (±0.92%) in stage 3 & 4 CKD, 0.43% (±0.86%) in stage 5 non-dialyzed patients and 0.96% (±2.15%) in stage 5 patients on dialysis respectively. Although the mean percentage of burr cells showed a gradual increase with advancing stages of CKD, this trend was not statistically significant.

Table 2. Comparison of hypersegmented neutrophils among study groups

(I) Group	(J) Group	Mean Difference (I-J)	Standard error	Significance
Stage 3 & 4 CKD	Stage 5ND	-31.45	5.80	0.001
	Stage 5 D	-13.88	5.13	0.008
Stage 5 ND	Stage 3 & 4 CKD	31.45	5.80	0.001
	Stage 5D	-17.56	6.05	0.004
Stage 5D	Stage 3 & 4 CKD	13.88	5.13	0.008
	Stage 5 ND	17.56	6.05	0.004

(Stage 5 ND – Stage 5 CKD patients not on dialysis, Stage 5 D – Stage 5 CKD patients on dialysis) (clinical significance < 0.05)

Discussion

Chronic kidney disease is becoming a significant health problem around the globe including Sri Lanka[3]. Most of the Sri Lankan studies on chronic kidney disease were carried out on the patients with chronic kidney disease of uncertain aetiology (CKDu)[5]. The age-standardized global prevalence of CKD was higher in women and girls than in men and boys[2]. However, this study found a male predominance in the total cohort (male: female ratio of 1.71:1) and in each subcategory.

CKD is generally a disease of elderly[1]. In this study, 80.7% of patients were over 50 years old. The mean age of the study sample was 59.5 years, comparable to findings from a previous Sri Lankan study[6]. Most patients in advanced stages of CKD (stages 3, 4 and stage 5 non-dialyzed) were between 61 and 70 years of age. However, patients undergoing dialysis were generally younger, with the majority falling within the 51-60 age group.

Anaemia associated with chronic kidney disease (CKD) is typically normochromic and normocytic, primarily resulting from erythropoietin deficiency[24]. In Sri Lanka, most research in this area has focused on chronic kidney disease of uncertain aetiology (CKDu), while studies specifically examining the haematological profiles of CKD patients remain limited. A descriptive cross-sectional study conducted at a tertiary care centre in Sri Lanka[14] reported an overall anaemia prevalence of 93%. The study also found that more severe anaemia was significantly associated with female gender, advanced stages of renal dysfunction with the need for haemodialysis. Among the anaemic patients, 60.4% had iron deficiency anaemia, 27.5% had anaemia of chronic disease and 5.5% had mixed deficiency anaemia. Consistent with that study, the majority of patients in our sample cohort also exhibited normochromic normocytic anaemia, with worsening of the severity of anaemia with the disease progression. A statistically significant reduction in haemoglobin level was observed in patients undergoing dialysis compared to those in both stage 3/4 and stage 5 non-dialyzed groups ($P<0.001$).

In theory, dialysis should improve anaemia by alleviating uraemia-related bone marrow suppression. However, the data suggest that dialysis itself may contribute to anaemia. Potential contributing factors include blood loss through retention in the dialysis circuit, frequent phlebotomy, uraemia-induced platelet dysfunction and intermittent heparin use during dialysis sessions[7]. Supporting this hypothesis is the notably high prevalence of hypochromic microcytic anaemia (42.1%) among dialyzed patients.

A descriptive cross-sectional study using data from the U.S. National Health and Nutrition Examination Survey (NHANES)[16] reported an anaemia prevalence of 15.4% in individuals with chronic kidney disease (CKD), compared to 6.3% in those without renal disease. These rates are significantly lower than those observed in our study population.

Thrombocytopenia was observed in the cohort, affecting one-fifth of patients. The mean platelet count decreased with the advancement of renal impairment, particularly in dialyzed patients. This finding is compatible with the study done by Akbar Dorgalaleh et al[22]. Haemodialysis is known to cause a transient drop in platelet count during the procedure, primarily due to platelet adhesion, dialyzer materials, and complement activation[23]. However, the platelet reduction observed in our dialysis patients cannot be attributed to these mechanisms, as samples were collected prior to dialysis.

The study noted elevated ESR values in patients, indicating potential underlying inflammation associated with renal disease although statistical significance was not observed with disease progression. Additionally, hypersegmented neutrophils were consistently present, notably in non-dialyzed patients, suggesting a link to uraemia, which improved with dialysis.

The study observed elevated ESR levels in patients, though no significant correlation was found with disease progression. While Bathon et al[19]. found that 20% of end-stage renal disease patients had ESR levels over 100 mm/ hr, and a Saudi study[18] reported similar findings, only 3.53% of our total sample and 2.56% of dialyzed patients had ESR over 100 mm/ hr.

The presence of burr cells was very minimal among the study sample. The increment of burr cells with advancing renal impairment and presence in very low numbers in the peripheral blood smear were compatible with the findings of Indian study by Rathod et.al[20].

Conclusion

Normochromic normocytic anaemia was the most common type of anaemia observed, consistent with both local and international data. There was an increase in the severity of anaemia with the progression of CKD. Hypochromic microcytic anaemia was notably more common among dialysis patients, who also had significantly lower haemoglobin levels compared to those in stages 3-4 and non-dialyzed stage 5. The platelet counts declined with advancing disease, with the lowest levels seen in the dialysis group. ESR values generally increased with disease severity, though differences were not statistically significant, and only a small number of patients had ESR above 100 mm/ hr.

A significant rise in hypersegmented neutrophils was seen from stages 3-4 to stage 5 non-dialyzed, followed by a reduction in dialysis patients, likely due to toxin clearance. Burr cell percentages also rose with disease progression, though without statistical significance.

Limitations

The main limitation of the study was the small sample size, especially in the stage 5 non-dialyzed and dialyzed groups. A larger sample may have revealed more statistically significant results. Additionally, the gender imbalance and wide variation in patient characteristics and lack of representation of Sri Lanka's full ethnic diversity, may have affected the results highlighting the need for multi-centre studies to better assess haematological effects of CKD.

Recommendations

A large multi-centre study is needed to better understand the haematological profile of chronic kidney disease (CKD) patients in Sri Lanka. CKD patients with anaemia, high ESR and thrombocytopaenia require detailed investigations to identify the cause.

Acknowledgements

We gratefully acknowledge the patients who participated in the study; Dr. Bilesha Perera and Dr. C.J. Wijesinghe for their assistance with study design and statistical analysis; Dr. Nalaka Herath, Dr. Iresha Hettiarachchi, and Dr. Nadeeka Rathnamalala, Consultant Nephrologists at Teaching Hospital, Karapitiya, for their guidance; and the Haematology laboratory staff for their support with sample analysis. We also extend our sincere thanks to the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, for their approval and guidance.

Authorship contributions and Disclosure of conflicts of interests

The work presented in this research study is the own work of the authors and there were no conflicts of interests.

References

1. Kidney International Supplements, KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. 2013; **3**(1).
2. Carney E. The impact of chronic kidney disease on global health. *Nature Reviews Nephrology.* 2020; **16**(5):251
3. Annual Health Bulletin 2018. Ministry of Health Sri Lanka. Page 70.
4. Sri Lanka Institute for Health Metrics and Evaluation. 2021.
5. Rajapakse S, Shivanthan MC, Selvarajah M. Chronic kidney disease of unknown etiology in Sri Lanka. *International Journal of Occupational and Environmental Health* 2016; **22**(3): 259-64.
6. Kafle K, Balasubramanya S, Horbulyk T. Prevalence of chronic kidney disease in Sri Lanka: A profile of affected districts reliant on groundwater. The Science of the Total Environment. 2019; 694: 133767.
7. Tsagalis G. Renal anemia: a nephrologist's view. *Hippokratia.* 2011; **15** (Suppl1): 39-43.

8. Kidney International Supplements, KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, p 19-32.
9. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011, page 3.
10. Osei-Bimpong A, Burthem J. Supplementary techniques including blood parasite diagnosis. Dacie and Lewis practical haematology. 11th ed. 2011. p103-104.
11. Bain B. Blood cell morphology in health and disease, Dacie and Lewis practical haematology. 11th ed; 2011. p93.
12. Babitt JL, Lin HY. Mechanisms of anaemia in CKD. *Journal of American Society of Nephrology.* 2012; **23**(10): 1631-34.
13. Belurkar S, Shastry I. The spectrum of red blood cell parameters in chronic kidney disease: A study of 300 cases. *Journal of Applied Hematology.* 2019; **10**(2): 61-66
14. Luke N, De Silva S, Lakmini S, Dilhani N, Chronic kidney disease and anaemia: prevalence, association and treatment outcome in patients attending a tertiary care hospital Sri Lanka. *Kidney International Reports* 2019: **4**(7): 1.
15. Fernando WBNT, Hettiarachchi TW, Sudeshika T, Badurdeen Z, Abeysundara H, Ranasinghe S, Rathnayake MP, Nanayakkara N. Snapshot view on anaemia in chronic kidney disease of uncertain aetiology. *Nephrology (Carlton).* 2019; **24**(10): p1033. doi: 10.1111/nep.13545. Epub 2019 May 2.
16. Stauffer ME, Fan T. Prevalence of anemia in chronic kidney disease in the United States. *PLoS One.* 2014; **9**(1).
17. Stuart J and Lewis S M. Recommendations for standardization, safety and quality control of Erythrocyte sedimentation rate for World Health Organization.
18. Iwamoto Y, Ando M, Tsuchiya K, Nihei H. [Clinical analysis of thrombocytopenia in chronic dialysis patients]. *Nihon Jinzo Gakkai Shi.* 1999; **41**(7): 712-8.
19. Al-Homrany M. The significance of extreme elevation of the erythrocyte sedimentation rate in hemodialysis patients. *Saudi Journal of Kidney Disease and Transplantation.* 2002; **13**(2): 141-5.
20. Bathon J, Graves J, Jens P, Hamrick R, Mayes M. The erythrocyte sedimentation rate in end-stage renal failure. *American Journal of Kidney Disease.* 1987; **10**(1): 34-40.
21. Rathod SG, Arvind KA. A, Pravin P. Shekokar. A study of Haematological changes in Chronic Renal Failure. *Scholars Journal of Applied Medical Sciences* 2014; **2** (4A): 1232-34.
22. Dorgalaleh A, Mahmudi M, Tabibian S, Khatib ZK, Tamaddon GH, Moghaddam ES, Bamedi T, Alizadeh S, Moradi E. Anemia and thrombocytopenia in acute and chronic renal failure. *International Journal of Hematology Oncolology and Stem Cell Research.* 2013; **7**(4): 34-9.
23. Daugirdas JT, Bernardo AA. Hemodialysis effect on platelet count and function and hemodialysis-associated thrombocytopenia. *Kidney International.* 2012; **82**(2): 147-57. doi: 10.1038/ki.2012.130. Epub 2012 May 16.
24. Tsagalis G. Renal anemia: a nephrologist's view. *Hippokratia.* 2011; 15(Suppl1): 39-43.
25. Palmer L, Briggs C, McFadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin SJ. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *International Journal of Laboratory Hematology.* 2015; **37**(3): 290,292,297.