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ORIGINAL RESEARCH

Spectrum of Red Blood Cell Parameters and Morphology of Anemia in Chronic Kidney Disease

Dr. Alfa Babu[1], Dr. Cicy P.J.[2], Dr. Miriam Joseph[3]

[1]Junior Resident, Department of Pathology, Government Medical College, Kottayam, Kerala, India
[2]Associate Professor (CAP), Department of Pathology, Government Medical College, Kottayam, Kerala, India
[3]Assistant Professor, Department of Pathology, Government Medical College, Kottayam, Kerala, India

Corresponding Author
Dr. Miriam Joseph, Assistant Professor, Department of Pathology, Government Medical College, Kottayam, Kerala, India

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ABSTRACT

Background: Anemia in chronic kidney disease (CKD) is typically normocytic normochromic but can become microcytic hypochromic due to iron deficiency or hypoproliferative from reduced erythropoietin (EPO) activity. The aim is to investigate the spectrum of red blood cell parameters and morphology in anemia associated with chronic kidney disease.

Research questions

1. What are the types of anemia seen in patients with chronic kidney disease?
2. What is the severity of anemia in patients with chronic kidney disease?

Objectives

1. To describe the changes in various RBC parameters in Chronic Kidney Disease
2. To identify the morphologic spectrum of anemia in Chronic Kidney disease.
3. To assess the severity of anemia.

Methodology: 125 samples were chosen as per the KDIGO guidelines criteria regardless of its primary cause, during the study period. The selected patients were analysed for RBC Count, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin , Mean Corpuscular Hemoglobin Concentration and peripheral smear for type of anemia and features of hemolysis.All data were entered using Micosoft Excel and analysis was done using IBM SPSS software version 27.0.

Results: In the study, predominant age group was 60-80 years range, comprising 46.4% with a male preponderance (57.6%). The majority of patients, 54.4%, were in Stage 5 CKD. 53.6% cases showed moderate anemia. The vast majority of cases (74.4%) had normocytic normochromic anemia, indicating that red blood cells have normal size and hemoglobin concentration. There were statistically significant association between sex and CKD stage and hemoglobin levels and CKD stage.

Conclusion: The present study highlights the morphological spectrum and RBC parameters of anemia in chronic kidney disease (CKD) patients. These findings underscore the need for proactive management of anemia in CKD to improve patient outcomes and quality of life.

Keywords: Anemia, Chronic Kidney Disease, RBC indices.

INTRODUCTION

Chronic kidney disease (CKD) is a non-communicable condition commonly resulting from diabetes and hypertension.[1] The severity of CKD is assessed by a low serum creatinine-based estimated glomerular filtration rate and an elevated urinary albumin-to-creatinine ratio. CKD affects approximately 10% of the global population, making it a significant public health issue worldwide.[2] According to the KDIGO(Kidney Disease: Improving Global Outcomes) guidelines, anemia in chronic kidney disease is classified as hemoglobin levels below 13.0 g/dL in men and below 12.0 g/dL in women.[3] Anemia in chronic kidney disease is normocytic normochromic but can become microcytic hypochromic due to iron deficiency or hypoproliferative from reduced erythropoietin activity[4]. Macrocytic anemia can also occur due to Vitamin B12 or folate deficiency, dialysis-induced red cell volume changes, and bone marrow suppression[5]. The current study aims to investigate the spectrum of red blood cell parameters and morphology in anemia associated with chronic kidney disease.

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RESEARCH QUESTIONS

1. What are the types of anemia seen in patients with chronic kidney disease?
2. What is the severity of anemia in patients with chronic kidney disease?

OBJECTIVES

1. To describe the changes in various RBC parameters (Hb concentration and hematocrit, RBC count and RBC indices) in chronic kidney disease
2. To identify the morphologic spectrum of anemia in chronic kidney disease. (Microcytic/Normocytic/Macrocytic/Hypochromic/ Normochromic/Hemolytic)
3. To assess the severity of anemia.

MATERIALS &METHODS

The present Descriptive Study was conducted in the Department of Pathology, Government. Medical College, Kottayam on Patients with Chronic Kidney Disease with anemia who are coming for hematological evaluation in Central Laboratory-Hematology Division for a period of 18 months

Sample Size:

Calculated by the formula,

$N= (Z_{1-\alpha/2}\ \sigma)^2 / d^2$

N- SAMPLE SIZE

Z= 1.96 at 95% CI

α= 0.05

σ= Standard deviation

d= Estimation error= 1% of mean

Hb percentage ranged from 3.6 to 14.2 g/dl with a mean of 9.31 ± 0.52 g/dl[1]

σ= 0.52 d=0.093

N = **125**

Inclusion Criteria

1. Patients with chronic kidney disease of stage I-V with anemia
2. Patients with end stage renal failure on replacement therapy in the form of hemodialysis and/or peritoneal dialysis and/or medical line treatment.

Exclusion Criteria

1. Patients with end stage renal disease treated with renal replacement therapy in the form of renal transplantation.
2. History of erythropoietin therapy during the last 3 months.

Methods

Study procedure

125 Patients having CKD with anemia were chosen and analysed as per KDIGO guidelines criteria regardless of its primary cause.

STAGE OF CKD	GFR (ml/min/1.73m²)
Stage 1	≥90
Stage 2	60-89
Stage 3a	45-59
Stage 3b	30-44
Stage 4	15-29
Stage 5	<15

Table 1: Kidney Disease Improving Global Outcomes (KDIGO) Guidelines for Staging CKD6

KDIGO guidelines for diagnosis of anemia in CKD patients[7]:

- Adults and children > 15 years: Hb Concentration is < 13 g/dl in males and, 12 g/dl in females
- Children 0.5 – 5 years: <11 g/dl
- Children 5-12 years: < 11.5g/dl
- Children 12-15 years: <12 g/dl

Anemia is categorized with Hb% into mild (9-11 g%), moderate (7-9 g%) and severe (<7 g%) as per WHO anemia classification[8].The selected patients were analyzed for the following parameters: RBC Count, Hemoglobin (Hb), Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and peripheral smear for type of anemia and features of hemolysis.

Statistical Analysis

The collected data were entered in Microsoft Excel and analysed using IBM SPSS software, version 27.0.

RESULTS

- The majority of individuals fall within the 60-80 years age group, making up 46.4% of the sample indicating that most of the population is older, with the bulk of individuals over 40 years.
- Males make up the majority of the sample, constituting 57.6%.
- The majority 52.0%, had an MCH below 27 pg, indicating hypochromic red blood cells and 45.6% have an MCH within the normal range of 27-32 pg, reflecting normochromic red blood cells.

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- 52.0% cases have an MCHC within the normal range of 31.5 - 34.5 g/dL, indicating that most individuals have red blood cells with normal hemoglobin concentration. 41.6% have an MCHC below 31.5 g/dL suggesting hypochromic cells often associated with iron deficiency anemia.
- The distribution of RBC count among 125 patients shows that 48% have an RBC count in the range of >2-3 million. This is followed by 43.2% of individuals with an RBC count of >3-4 million. A smaller proportion of the population, 4.8% has an RBC count in the range of 1-2 million, while only 4% of patients exhibit an RBC count greater than 4 million.
- The distribution of hematocrit levels in this population shows that out of 125 individuals, 48% are females with hematocrit levels below 36%, while a smaller proportion, 4.8%, are males with hematocrit levels below 40%.

Stage of Chronic Kidney Disease

	Frequency	**Percent**
Stage 3	19	15.2
Stage 4	38	30.4
Stage 5	68	54.4
Total	**125**	**100.0**

Table 2: Chronic Kidney Disease staging

The majority of patients, 54.4% are in Stage 5 CKD indicating advanced kidney disease, who require dialysis or transplant.Overall, the data suggests a predominance of advanced CKD (Stage 4 and 5), highlighting the severity of kidney impairment in this population.

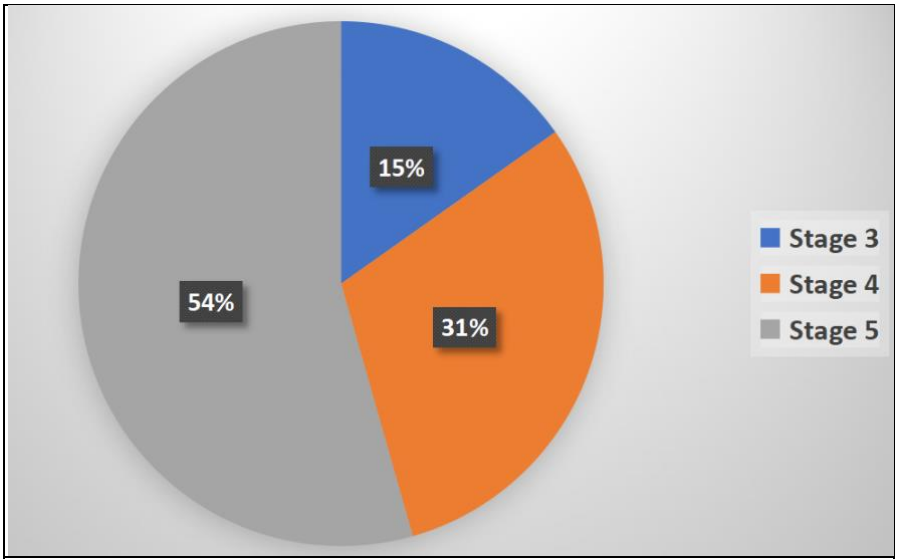

Chart 1: Piechart of Chronic Kidney Disease staging distribution

Severity of Anemia

	Frequency	Percent
Mild	29	23.2
Moderate	67	53.6
Severe	29	23.2
Total	125	100.0

Table 3 : Severity of Anemia

This distribution shows that while moderate anemia is most common, there are still substantial portions of the population with either mild or severe anemia, suggesting varying levels of anemia severity within the group.

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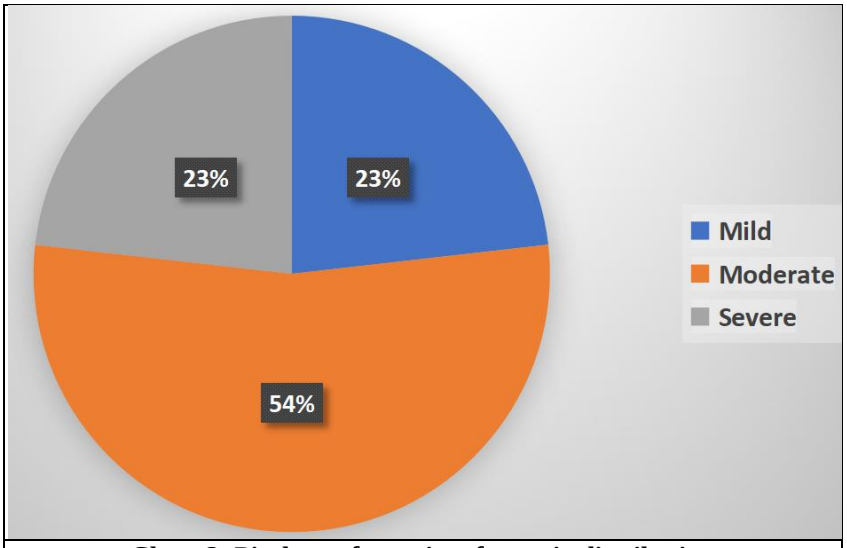

Chart 2: Piechart of severity of anemia distribution

Morphology of Anemia

	Frequency	Percent
Normocytic normochromic anemia	93	74.4
Microcytic hypochromic anemia	13	10.4
Macrocytic anemia	1	0.8
Dimorphic anemia with normocytes and microcytes	15	12.0
Dimorphic anemia with normocytes and macrocytes	2	1.6
Hemolytic anemia	1	0.8
Total	**125**	**100.0**

Table 4: Morphology of Anemia

74.4% cases have normocytic normochromic anemia, indicating red blood cells of normal size and hemoglobin concentration, which may suggest anemia of chronic disease.10.4% have microcytic hypochromic anemia and 12.0% exhibit dimorphic anemia with normocytes and microcytes, indicating a mixed population of RBCs.

Hemoglobin and Stage of CKD

			CKD Stage			Total
			Stage 3	Stage 4	Stage 5	
Hemoglobin	Mild	N	14	11	4	29
		%	73.7%	28.9%	5.9%	23.2%
	Moderate	N	3	27	37	67
		%	15.8%	71.1%	54.4%	53.6%
	Severe	N	2	0	27	29
		%	10.5%	0.0%	39.7%	23.2%
Total		N	19	38	68	125
		%	100.0%	100.0%	100.0%	100.0%
χ^2=55.556, p<0.0001*						

Table 5: Crosstabulation of Hemoglobin and CKD

The crosstabulation of hemoglobin levels and CKD stage in 125 individuals highlights a significant relationship between the severity of anemia and CKD progression. Severe anemia is notably concentrated in Stage 5 CKD, where 39.7% of patients are affected, with no cases in Stage 4.

The χ^2 value of 55.556 and a p-value of less than 0.0001 indicate a highly statistically significant association between hemoglobin levels and CKD stage. As CKD progresses, anemia severity tends to increase, with severe anemia being predominantly associated with Stage 5 CKD.

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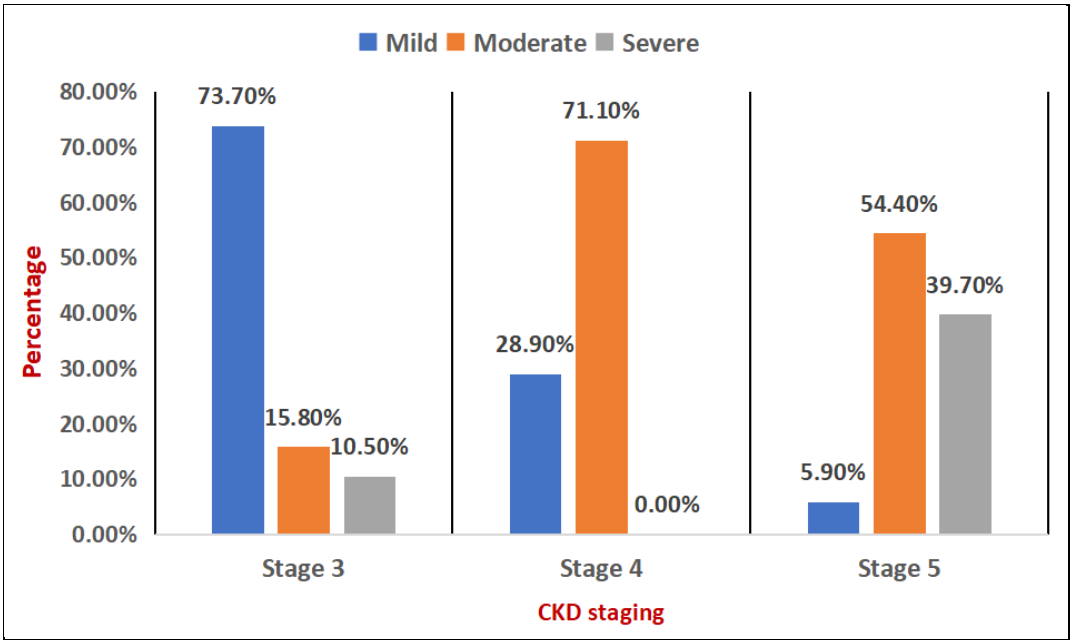

Chart 3: Bargraph of showing severity of anemia in various stages of CKD

Morphology of Anemia and Stage of CKD

Morphology of anemia		CKD Stage			Total
		Stage 3	Stage 4	Stage 5	
Normocytic normochromic anemia	N	15	33	45	93
	%	78.9%	86.8%	66.2%	74.4%
Microcytic hypochromic anemia	N	2	0	11	13
	%	10.5%	0.0%	16.2%	10.4%
Macrocytic anemia	N	0	1	0	1
	%	0.0%	2.6%	0.0%	0.8%
Dimorphic anemia with normocytes and microcytes	N	2	2	11	15
	%	10.5%	5.3%	16.2%	12.0%
Dimorphic anemia with normocytes and macrocytes	N	0	2	0	2
	%	0.0%	5.3%	0.0%	1.6%
Hemolytic anemia	N	0	0	1	1
	%	0.0%	0.0%	1.5%	0.8%
Total	N	19	38	68	125
	%	100.0%	100.0%	100.0%	100.0%
χ^2=17.762, p=0.069					

Table 6: Crosstabulation of Morphology of anemia and CKD

The majority of patients (74.4%) have normocytic normochromic anemia, with its highest prevalence in Stage 4 CKD (86.8%) and slightly lower in Stage 5 (66.2%). Microcytic hypochromic anemia is present in 10.4% of the total population, mostly concentrated in Stage 5 CKD (16.2%). Dimorphic anemia, with normocytes and microcytes are predominantly in Stage 5 CKD (16.2%) while dimorphic anemia with normocytes and macrocytes(2 cases) are seen in Stage 4 CKD(5.3%). Macrocytic anemia and hemolytic anemia are rare, with only one case of each, and both are found in Stage 4 and Stage 5 CKD, respectively.

The χ^2 value of 17.762 and a p-value of 0.069 indicate that while there is a trend towards different anemia morphologies across CKD stages, the association is not statistically significant. This suggests that although the types of anemia may vary with CKD progression, the relationship is not strong enough to be conclusive in this population.

DISCUSSION

Anemia in chronic kidney disease (CKD) is primarily due to erythropoietin (EPO) deficiency. Other contributing factors include shortened red cell lifespan from the accumulation of toxic metabolic byproducts, uremia-related hemolysis, platelet dysfunction leading to blood loss, and deficiencies in iron, vitamin B12 and folate. Inflammatory conditions and malnutrition can worsen anemia and reduce treatment effectiveness

In our study most individuals in the sample are above 40 years, with the majority (46.4%) aged 60-80 years, followed by 43.2% within 40-60 years. Males are the predominant gender, making upto 57.6% of the sample. In a study by Mohammed MR, Mahmood B

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et al,[9] on 40 patients with chronic renal failure who developed anemia as the disease progressed, the demographic breakdown revealed that males were significantly affected (80%). The majority of patients were within the 60 to 70-year age, highlighting that older males were most frequently affected by anemia in the context of chronic renal failure.

CKD staging and severity of anemia

In our population, most CKD patients are in advanced stages, with 54.4% in Stage 5 and 30.4% in Stage 4, indicating severe kidney impairment, while 15.2% are in Stage 3. Severity of anemia show marked variation with 53.6% having moderate anemia, 23.2% each having mild and severe anemia. Overall, the data highlights significant kidney disease progression and a predominance of moderate to severe anemia within the population.

The analysis of hemoglobin levels across CKD stages reveals a clear association between severity of anemia and CKD progression. Mild anemia is most common in Stage 3 CKD (73.7%) with decreasing prevalence in Stage 4 (28.9%) and minimal prevalence in Stage 5 (5.9%). Moderate anemia is more prevalent in advanced stages, 71.1% in Stage 4 and present in over half of Stage 5 cases (54.4%), making up 53.6% of the total study population. Severe anemia is concentrated almost exclusively in Stage 5 CKD, affecting 39.7% of patients and absent in Stage 4. The χ^2 value of 55.556 and a p-value below 0.0001 confirm a highly significant relationship between anemia severity and CKD stage. This trend indicates that as CKD advances, anemia severity worsens, with severe anemia particularly linked to Stage 5 CKD.

Vikrant S et al[10] conducted a study among 512 CKD patients with 193 cases(33%) in Stage 4, and 319 (54.6%) in Stage 5 and a mean hemoglobin of 9.2 g/dL. Hemoglobin levels declined progressively with advancing CKD stages, averaging 10 g/dL in Stage 3, 9.4 g/dL in Stage 4 and 8.4 g/dL in Stage 5. Linear regression showed a significant positive correlation between hemoglobin and GFR.

In a study by Khadayate R et al,[11]62.1% of Stage 5 CKD patients and 57.1% of Stage 4 patients had severe anemia, while only 3.16% of Stage 3 patients had severe anemia. The average hemoglobin (Hb) level in CKD patients was 7.49 g/dL, with the highest mean Hb in Stage 3, demonstrating a positive correlation between CKD stage and anemia severity. The p-value for the presence of anemia in CKD patients was <0.5%, confirming a significant relationship, and Stage 5 patients, in particular, showed statistically significant anemia with Hb levels under 9 g/dL ($p<0.05$).

Morphology of anemia

In our study, morphology of anemia varies slightly across CKD stages. Normocytic normochromic anemia is the most common, affecting 74.4% of patients, with its highest occurrence in Stage 4 (86.8%) and followed by Stage 5 (66.2%). Microcytic hypochromic anemia comprised 10.4% of the population, mostly in Stage 5 (16.2%). Dimorphic anemia, comprising 13.6% of cases are primarily found in Stage 5 (16.2%). Macrocytic and hemolytic anemias are rare, with one case each in Stages 4 and stage 5, respectively. The χ^2 value of 17.762 and a p-value of 0.069 suggest that anemia types show some variation across CKD stages and the association is not statistically significant, indicating no strong relationship in this cohort.

Bhatta S et al,[12] compared morphology of anemia between predialysis and postdialysis patients, with a sample of 40 individuals in each group. In predialysis patients, the predominant anemia type is normocytic normochromic, accounting for 90% while microcytic hypochromic and macrocytic normochromic anemia were relatively rare with 7.5% and 2.5% respectively. In the postdialysis group, normocytic normochromic anemia remains the most common(77.5%), while microcytic hypochromic anemia and macrocytic normochromic anemia rised to 15% and 7.5% respectively. This shift indicates that while normocytic normochromic anemia is predominant in both groups, there is a trend toward more microcytic and macrocytic anemia types in postdialysis patients.

In a study by Afshar R et al,[13]morphology of anemia showed normochromic-normocytic (80%),followed by microcytic hypochromic (15%) and macrocytic (5%) anemia. Macrocytosis is slightly more common in hemodialysed patients (5.9%) compared to predialysed patients (4.8%). In a study by Khadayate R et al,[11] normocytic normochromic anemia is the most common, affecting 91.1% of patients, with 76% of these cases in Stage 5 CKD. Microcytic anemia is seen in 5.6% of patients, while macrocytic and dimorphic anemia are rare, each present in only 1.56% of cases.

CONCLUSION

- Most participants were aged 60-80(46.4%), indicating a predominance of older population with male preponderance(57.6%).
- The majority of patients were in Stage 5(55.4%) highlighting advanced kidney disease.
- Moderate anemia (53.6%) was the most prevalent form of anemia.
- Majority had normocytic (52.8%) or microcytic (44.8%) red blood cells.
- MCH showed predominance of hypochromic anemia (52%) with normal MCHC levels (52%).
- Normocytic normochromic anemia was the most frequent morphology of anemia accounting for 74.4%.

Hemoglobin Levels and CKD Stage - Highly significant association ($\chi^2 = 55.556$, $p < 0.0001$), with increased severity of anemia correlating with advanced stage of CKD.

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Morphology of anemia and CKD Stage - No significant assosciation. (χ^2 = 17.762, p = 0.069)- Morphology of anemia across CKD stages reveals predominance of normocytic normochromic anemia in all stages.

The present study highlights the morphology and RBC parameters of anemia in chronic kidney disease (CKD) patients, thereby understanding the spectrum of anemia in various stages of CKD. The study showed a significant association between the increased severity of anemia and advancing stage of CKD. Our observations regarding the prevalence, RBC indices and morphological patterns of anemia, align closely with existing western literature, thereby addressing a significant gap in the Indian literature on CKD. These findings emphasize the importance of conducting thorough investigations to initiate timely treatment and enhance the quality of life for these individuals.

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