Original Research Article

A Study of Red Cell Parameters in Treatment Naive Chronic Renal failure Patients: A hospital based cross-sectional study

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International Archives of Integrated Medicine, Vol. 13, Issue 5, May, 2026.
Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 10-5-2026 **Accepted on:** 22-5-2026
Source of support: Nil **Conflict of interest:** None declared.

DOI: **10.5281/zenodo.20541566**

How to cite this article: SJ Sanketh, Vishal K. A Study of Red Cell Parameters in Treatment Naive Chronic Renal failure Patients: A Hospital Based Cross-sectional Study. Int. Arch. Integr. Med., 2026; 13(5): 41-51.

Abstract

Background: Chronic renal failure (CRF) is associated with a wide spectrum of hematological abnormalities, of which anemia is the most predominant. Red cell parameters provide a simple, cost-effective, and readily available means to assess the nature and severity of anemia in CRF patients not receiving renal replacement therapy (RRT).

Objectives: To study red cell parameters in patients of chronic renal failure not treated with renal replacement therapy and to characterize the type and severity of anemia in such patients.

Methods: This cross-sectional observational study was conducted over a period of 18 months. A total of 300 patients diagnosed with chronic renal failure not on renal replacement therapy were enrolled. Complete blood count (CBC) with red cell indices including hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and peripheral blood smear examination were analyzed. Data were statistically evaluated using SPSS version 26.0.

Results: Anemia was present in 92.3% of patients. Normocytic normochromic anemia was the most common type (61.3%), followed by microcytic hypochromic anemia (22.7%) and dimorphic anemia (11.3%). Mean hemoglobin was 7.8 ± 1.9 g/dL. RDW was elevated in 68% of patients. A significant negative correlation was found between eGFR and severity of anemia ($p<0.001$).

Conclusion: Red cell parameters are valuable tools in the evaluation of anemia in CRF patients not on RRT. Normocytic normochromic anemia is most prevalent, correlating with declining renal function.

Key words

Chronic Renal Failure, Anemia**,** Erythrocyte Indices, Renal Replacement Therapy, Glomerular Filtration Rate.

Introduction

Chronic Renal Failure (CRF) is also known as Chronic Kidney Disease (CKD) at its end stage and it is the progressive and irreversible degeneration of renal function where the kidneys lose their ability to sustain the body's homeostasis of metabolism [1].

It has been identified as a large-scale global public health problem, with an approximate prevalence of 10-15% of all people in the world and significantly greater than this in developing countries [2].

In India, CKD afflicts about 17% of adults in the country, with hypertension and diabetes mellitus being the leading causes of CKD in adults [3].

One of the earliest and most frequent complications of CRF is anemia, which occurs in the majority of patients prior to initiating any form of renal replacement therapy [4].

Multiple factors contribute to the pathogenesis of anemia in CRF, but it is primarily caused by the relative deficiency of erythropoietin (EPO) produced by the peritubular cells of the kidney [5].

As the patient loses functional renal tissue, EPO production declines directly related to the reduction in renal tissue and consequently reduce erythropoiesis in the bone marrow. Other contributing mechanisms of anemia in CRF include decreased red cell survival, iron deficiency secondary to chronic blood loss and/or dietary restrictions, folate and vitamin B12 deficiencies, and bone marrow depression secondary to uremic toxins, and blunted response of the bone marrow to administered EPO [6, 7].

The effects of anemia in CRF patients can be profound. Anemia is correlated with poor quality of life, increased fatigue, decreased cognitive function, left ventricular hypertrophy, accelerated progression of renal disease, and increased cardiovascular morbidity and mortality [8].

Despite its potential clinical impact, anemia in CRF patients who are not on dialysis or any form of renal replacement therapy remain under-diagnosed and poorly managed in many settings [9].

Using automated complete blood count (CBC) analyzers provides a quick, easy, and affordable means of assessing the type and severity of anemia through red cell parameters. Hemoglobin concentration, packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) provide important information regarding diagnosis. Peripheral blood smear examination provides additional diagnostic information to the previously mentioned parameters by demonstrating morphologic abnormalities such as burr cells (echinocytes), fragmented red cells (schistocytes), and hypochromia which are characteristic of uremic anemia [10].

Fewer studies have evaluated anemia in CKD patients who are not undergoing any form of renal replacement therapy than have evaluated anemia in CKD patients on dialysis.

This group of patients is especially significant because they represent a time when early interventions may occur.

Therefore, the objective of this study was to investigate red cell parameters in patients with chronic renal failure who were not receiving renal replacement therapy; to assess the type and severity of anemia in this group; and to correlate hematologic findings with the degree of renal impairment in this group.

Materials and methods

Study Design and Setting

This was a cross-sectional observational study conducted in the Department of Pathology in collaboration with the Department of Medicine and Nephrology at a tertiary care teaching hospital over a period of 18 months (January 2022 to June 2023). Ethical clearance was obtained from the Institutional Ethics Committee prior to commencement of the study (Ref No: IEC/2022/01/14). Written informed consent was obtained from all study participants.

Study Population

A total of 300 patients diagnosed with chronic renal failure not receiving renal replacement therapy (neither hemodialysis, peritoneal dialysis, nor renal transplantation) were enrolled consecutively.

Inclusion Criteria:

- Patients aged ≥18 years of either sex
- Diagnosed with chronic renal failure based on persistent reduction in eGFR<60 mL/min/1.73 m² for ≥3 months
- Patients not on any form of renal replacement therapy
- Willing to provide informed consent

Exclusion Criteria:

- Patients with hematological malignancies or primary bone marrow disorders
- Patients with active bleeding, recent blood transfusion within 3 months
- Patients on immunosuppressants, erythropoiesis-stimulating agents, or androgens
- Pregnant or lactating women
- Patients with hemolyticanemias, hemoglobinopathies, or thalassemia
- Patients with hepatic failure or malignancy

Sample Collection

Five milliliters of venous blood were collected from each patient under aseptic conditions in EDTA (ethylenediaminetetraacetic acid) vacutainer tubes. Samples were processed within 2 hours of collection.

Laboratory Investigations

Complete Blood Count (CBC): Performed using the automated 5-part differential hematologyanalyzer (Sysmex XN-1000). Parameters recorded included:

- Hemoglobin (Hb) in g/dL
- Red Blood Cell count (RBC) in millions/µL
- Packed Cell Volume / Hematocrit (PCV/HCT) in %
- Mean Corpuscular Volume (MCV) in fL
- Mean Corpuscular Hemoglobin (MCH) in pg
- Mean Corpuscular Hemoglobin Concentration (MCHC) in g/dL
- Red Cell Distribution Width (RDW-CV) in %
- Reticulocyte count (%)

Peripheral Blood Smear (PBS): Prepared using Leishman stain and examined under oil immersion for red cell morphology including:

- Normocytic normochromic pattern
- Microcytic hypochromic pattern
- Macrocytic pattern
- Dimorphic picture
- Specific red cell abnormalities (burr cells, schistocytes, target cells)

Renal Function Tests: Serum creatinine (enzymatic method), blood urea nitrogen (BUN),

and eGFR calculated using the CKD-EPI equation.

Serum Iron Studies: Serum iron, total iron-binding capacity (TIBC), transferrin saturation, and serum ferritin.

Serum B12 and Folate levels were measured where clinically indicated.

Definitions

- **Anemia:**Hb<13 g/dL in males and <12 g/dL in females (WHO criteria) [11]
- **Severity of anemia:** Mild (Hb 10–12.9 g/dL in males, 10–11.9 g/dL in females), Moderate (Hb 7–9.9 g/dL), Severe (Hb<7 g/dL)
- **Normocytic normochromic:** MCV 80–100 fL, MCHC 32–36 g/dL
- **Microcytic hypochromic:** MCV <80 fL, MCHC <32 g/dL
- **Macrocytic:** MCV >100 fL
- **Elevated RDW:** RDW-CV >14.5%

CKD Staging (KDIGO 2012):

- Stage 3a: eGFR 45–59 mL/min/1.73 m²
- Stage 3b: eGFR 30–44 mL/min/1.73 m²
- Stage 4: eGFR 15–29 mL/min/1.73 m²
- Stage 5: eGFR<15 mL/min/1.73 m²

Statistical Analysis

Data were entered and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages. Chi-square test was used for comparison of categorical variables. Pearson's correlation coefficient was used to assess the relationship between eGFR and hemoglobin levels. One-way ANOVA was used for comparison across CKD stages. A p-value of <0.05 was considered statistically significant.

Results

Demographic Profile

A total of 300 patients were included in the study. The demographic characteristics are summarized in **Table - 1**.

The majority of patients were male (62.0%) with a mean age of 54.6 ± 13.8 years. The most common age group was 46–60 years (37.3%). Diabetic nephropathy was the most frequent underlying etiology (39.0%), followed by hypertensive nephrosclerosis (31.0%). The largest proportion of patients were in CKD Stage 5 (40.0%) [12].

Table - 1: Demographic and Clinical Characteristics of Study Patients (n=300).

Parameter	Category	Number (n)	Percentage (%)
Sex	Male	186	62.0
	Female	114	38.0
Age Group (years)	18–30	24	8.0
	31–45	58	19.3
	46–60	112	37.3
	61–75	82	27.3
	>75	24	8.0
Mean Age ± SD	54.6 ± 13.8 years		
Etiology of CRF	Diabetic Nephropathy	117	39.0
	Hypertensive Nephrosclerosis	93	31.0
	Chronic Glomerulonephritis	45	15.0
	Obstructive Uropathy	27	9.0
	Others/Unknown	18	6.0
CKD Stage	Stage 3a	30	10.0
	Stage 3b	54	18.0
	Stage 4	96	32.0
	Stage 5	120	40.0

Graph - 1: Prevalence and Severity of Anemia (n=300).

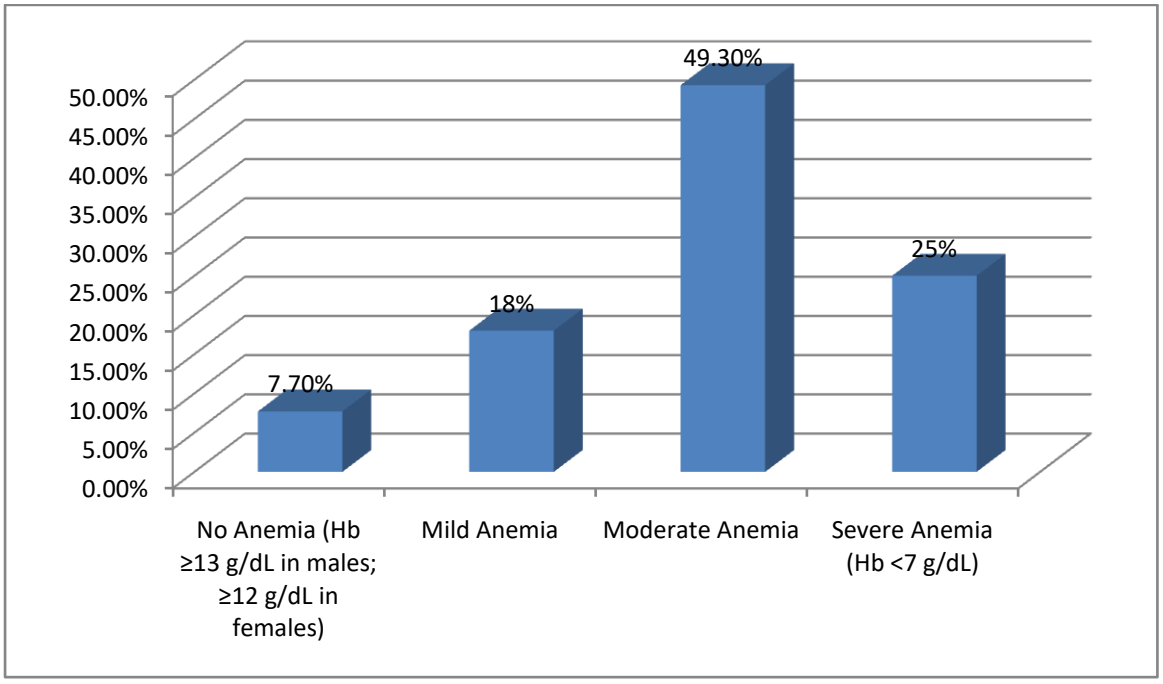

Table - 2: Mean Red Cell Parameters of Study Population (n=300).

Parameter	Mean ± SD	Reference Range
Hemoglobin (g/dL)	7.8 ± 1.9	M: 13–17; F: 12–15
RBC count (millions/µL)	2.9 ± 0.7	M: 4.5–5.5; F: 4.0–5.0
PCV/HCT (%)	24.6 ± 6.1	M: 40–54; F: 36–48
MCV (fL)	82.4 ± 10.2	80–100
MCH (pg)	27.6 ± 4.3	27–32
MCHC (g/dL)	31.8 ± 2.6	32–36
RDW-CV (%)	16.2 ± 2.8	11.5–14.5
Reticulocyte Count (%)	0.9 ± 0.4	0.5–2.0

Graph - 2: Morphological Classification of Anemia on Peripheral Blood Smear (n=277).

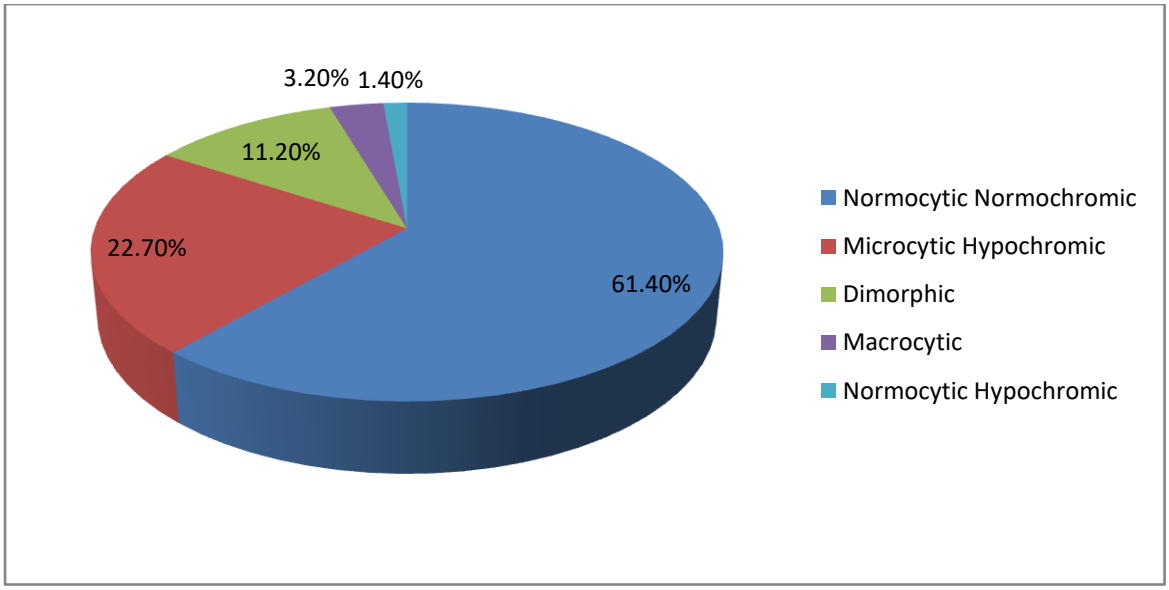

Table - 3: Red Cell Morphological Abnormalities on Peripheral Blood Smear (n=277).

Morphological Finding	Number (n)	Percentage (%)
Burr Cells (Echinocytes)	156	56.3
Hypochromia	112	40.4
Anisocytosis	201	72.6
Poikilocytosis	134	48.4
Schistocytes	38	13.7
Target Cells	29	10.5
Pencil Cells	45	16.2

Note: Multiple findings could be present in the same patient

Table - 4: RDW Distribution Across CKD Stages (n=300).

CKD Stage	n	Mean RDW-CV (%) ± SD	Elevated RDW (>14.5%) n (%)
Stage 3a	30	13.8 ± 1.4	8 (26.7)
Stage 3b	54	14.9 ± 1.7	28 (51.9)
Stage 4	96	16.1 ± 2.3	64 (66.7)
Stage 5	120	17.8 ± 3.1	104 (86.7)
Total	**300**	**16.2 ± 2.8**	**204 (68.0)**

p< 0.001 (ANOVA)

Table - 5: Mean Hemoglobin Levels Across CKD Stages (n=300).

CKD Stage	eGFR Range (mL/min/1.73 m²)	n	Mean Hb (g/dL) ± SD
Stage 3a	45–59	30	10.9 ± 1.4
Stage 3b	30–44	54	9.6 ± 1.6
Stage 4	15–29	96	8.1 ± 1.7
Stage 5	<15	120	6.4 ± 1.5

p< 0.001 (One-way ANOVA)

Table - 6: Serum Iron Study Findings (n=300).

Parameter	Mean ± SD	Normal Range
Serum Iron (µg/dL)	54.2 ± 28.4	60–170
TIBC (µg/dL)	242.6 ± 48.3	250–370
Transferrin Saturation (%)	18.4 ± 9.2	20–50
Serum Ferritin (ng/mL)	286.4 ± 198.6	12–300
Iron Status	**Number (n)**	**Percentage (%)**
Absolute Iron Deficiency	78	26.0
Functional Iron Deficiency	132	44.0
Normal Iron Stores	60	20.0
Iron Overload	30	10.0

Prevalence and Severity of Anemia

Anemia was detected in 277 of 300 patients (92.3%). The severity distribution is presented in **Graph - 1**.

Moderate anemia was the most prevalent form (49.3%), followed by severe anemia (25.0%). Only 7.7% of patients had normal hemoglobin levels. The overall mean hemoglobin in the study population was 7.8 ± 1.9 g/dL.

Red Cell Parameters

The mean values of red cell parameters in the study population are summarized in **Table - 2**.

The mean MCV of 82.4 ± 10.2 fL indicated a predominantly normocytic picture. However, MCHC was mildly reduced (31.8 ± 2.6 g/dL), and RDW-CV was elevated above the normal range (16.2 ± 2.8%). Reticulocyte count was inappropriately normal/low, suggesting hypoproliferative marrow activity [13].

Morphological Classification of Anemia

The type of anemia based on peripheral blood smear and red cell indices is summarized in **Graph - 2**.

Normocytic normochromic anemia was the predominant morphological type (61.4%), consistent with the anemia of chronic kidney disease. Microcytic hypochromic pattern, indicative of concomitant iron deficiency, was observed in 22.7% of cases. Dimorphic anemia, suggesting mixed nutritional deficiency, was noted in 11.2% [14].

Red Cell Morphological Abnormalities on PBS

Special red cell morphological changes identified on peripheral smear are detailed in **Table - 3**.

Anisocytosis was the most common finding (72.6%), followed by burr cells (56.3%) and hypochromia (40.4%). Schistocytes were identified in 13.7% of patients, primarily those with severe uremia [15].

RDW Distribution

Elevated RDW (>14.5%) was observed in 204 (68.0%) of all 300 patients. Among anemic patients, 188 (67.9%) had elevated RDW. RDW elevation was progressively greater with advancing CKD stage (**Table - 4**).

Correlation Between eGFR and Hemoglobin

A statistically significant positive correlation was found between eGFR and hemoglobin levels ($r = +0.612$, $p < 0.001$), indicating that hemoglobin levels declined progressively as eGFR decreased. Mean hemoglobin values across CKD stages are shown in **Table - 5**.

Iron Studies

Serum iron studies revealed iron deficiency anemia (serum ferritin <30 ng/mL, transferrin saturation <20%) in 78 patients (26.0%). Elevated serum ferritin (>300 ng/mL), suggestive of functional iron deficiency or anemia of chronic inflammation, was observed in 132 patients (44.0%). Results are summarized in **Table - 6**.

Discussion

The current study evaluated all of the red blood cell tests in 300 patients who had chronic renal failure that were not on dialysis to give a complete picture of their blood tests. We found that there were significantly more men (62.0%) than women in the study, and we also confirmed the previously reported demographic data on chronic kidney disease (CKD) as noted in [2, 3], with a majority of participants (63.6%) falling into the 46-60 year old age range. Our data indicated that diabetic nephropathy (39.0%), and hypertensive nephrosclerosis (31.0%) combined accounted for 70% of the reasons why these patients developed chronic kidney disease, and this reflects the shift from infectious and parasitic causes of CKD toward non-communicable diseases as has been shown in both India and other developing countries [16].

We found that 92.3% of the patients in our study were anemic, which was very close to the results reported by Shristi S, et al. [17] (89.17%), and Suresh, et al. [18] (94.1%) when evaluating a similar population of patients. This data clearly indicates that anemia is nearly universal in all patients with chronic renal failure who are not undergoing dialysis. Our data also demonstrated that moderate anemia (49.3%), and a mean hemoglobin of 7.8 ± 1.9 g/dl in the cohort of patients in our study demonstrate a clinically

significant burden of anemia that needs treatment.

In the study by Habbab, et al., there were two primary patterns of anemia; a normocytic, normochromic type (61.4%) that is consistent with the expected classical type for chronic renal failure due to erythropoietin (EPO) deficiency and resulting in a hypoproliferative anemia [5, 6], and a microcytic hypochromic type (22.7%), indicating a concurrent iron deficiency that was also biochemically confirmed in 26.0% of the patients studied as having an absolute iron deficiency. As noted in other studies, such as those by Bhat WH, et al. [19] (75%) and Mohammed MR et al. [20] (62.5%), the normocytic pattern is indicative of reduced red cell production, although it is typically morphologically normal, and the bone marrow is unable to adequately respond to the decreased oxygen carrying capacity due to lack of sufficient EPO stimulation.

Iron deficiency in CRF patients can result from several different factors including reduced dietary intake, malabsorption due to gut dysfunction caused by uremia, occult gastrointestinal bleeding, and impaired release of iron from reticuloendothelial stores [7]. Furthermore, functional iron deficiency was identified in 44.0% of the patients studied (elevated ferritin levels with low transferrin saturation), indicative of the inflammatory status of CRF and the iron restricted erythropoiesis characteristic of this disease [21].

Dimorphic anemia, where both iron and folate/B12 deficiencies are present, was found in 11.2% of the patients studied. Dimorphic anemia is often found in poorly nourished CRF patients [14]. Macrocytic anemia, which would be suggestive of either folate or B12 deficiency, was found in 3.2% of the patients studied. Macrocytic anemia may be related to inadequate diet and/or loss of these nutrients during dialysis although the latter was not a factor in this particular non-renal replacement therapy cohort.

As previously stated, one of the hallmarks of hypoproliferative anemia secondary to EPO deficiency in CRF is an inappropriately low reticulocyte count (mean 0.9 ± 0.4%) in conjunction with significant anemia [13]. This finding has major clinical implications regarding treatment because it indicates that erythropoiesis stimulating agents would be appropriate in treating this patient population.

An elevated RBC distribution width (RDW) was observed in 68.0% of the patients; it also significantly increased with increasing CKD stage ($p < 0.001$). Elevated RDW is due to anisocytosis that is produced by two types of red cell populations that respond differently to erythropoietin (EPO) stimulation -- EPO responsive and EPO resistant red cells along with iron deficient microcytic red cells and normal or macroyctic red cells when there is a mixed deficiency of iron [22]. Elevated RDW has recently been shown to be an independent predictor for mortality and cardiovascular events in patients with CKD [23].

Anisocytosis (72.6%), and burr cells/echinocytes (56.3%) were the two most commonly observed morphologic abnormalities on peripheral blood smears. The presence of burr cells is a well-recognized morphologic indicator of uremia, resulting from the interaction of the red cell membrane with uremic plasma components that alter the lipid bilayer structure of the red cell membrane [15]. As such, their high frequency within this cohort supports the conclusion that the degree of uremia present in the CKD Stages 4 and 5 patients comprised 72% of our study population. Schistocytes were found in 13.7% of the patients studied and were primarily found in those CKD Stages 5 and with evidence of severe uremia, suggesting they were indicative of the microangiopathic changes that occur with severe uremic endothelial damage.

There is a strong correlation between eGFR and hemoglobin ($r=+0.612$, $p < .01$), indicating that the degree of anemia increases as kidney function decreases. Hemoglobin averaged 10.9

g/dl at stage 3a and decreased to 6.4 g/dl by stage 5, indicating that both hemoglobin and EPO levels decrease as renal mass decreases; this relationship has been observed in numerous studies and provides the rationale for utilizing hemoglobin as a marker of disease progression in CKD [8, 24].

Our results will have a significant impact on patient care. Using simple red blood cell indices, clinicians can identify anemia types in patients with chronic renal failure and target treatment accordingly. For example, if a patient has a normocytic anemia secondary to EPO deficiency, then they should receive an EPO-stimulating agent. If a patient has anemia due to iron deficiency, then he/she should be supplemented with iron. If the patient has anemia secondary to poor nutrition (dimorphic/macrocytic anemia), then their diet should be improved. These tests are relatively inexpensive, widely available, and provide information regarding what action should be taken next without the need for further expensive testing.

There are limitations to this study. It is a cross-sectional study therefore we cannot draw conclusions about cause and effect. The sample size is also limited to one center therefore our results may not be representative of all patients with CRF. Due to budgetary restrictions, we were unable to measure serum EPO levels. These measurements would have provided additional support for our mechanistic explanation.

Conclusion

Anemia occurs as a virtually universal complication of chronic kidney disease (CKD) in patients who have not received RRT with a rate of occurrence of 92.3 % in our investigation. The most common form of anemia is the normocytic normochromic type that is caused by low levels of erythropoietin (EPO), leading to hypoproliferative anemia. There is a highly significant inverse correlation of the degree of anemia with the eGFR values that progresses from stage III CKD through stages IV-5 CKD. In addition to elevated RDW and anisocytosis, the presence of "burr cells" can also be an important finding in red blood cells obtained from a peripheral smear. Using red blood cell parameters that are easily obtained from a routine complete blood count, along with a peripheral blood smear, provides a cost-effective and clinically useful method for assessing and characterizing anemia in patients with chronic kidney disease who do not require renal replacement therapy. Performing a comprehensive hematologic assessment early after diagnosis allows clinicians to initiate appropriate treatment promptly, potentially improving the quality of life of affected individuals and altering the adverse cardiovascular consequences resulting from renal anemia.

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