Research Square

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Association of serum markers of iron status with Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in chronic kidney disease patients undergoing Hemodialysis

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Abstract

Background & objectives:

Chronic kidney disease (CKD) is a major health issue worldwide which leads to end stage renal failure and cardiovascular events. Anemia in CKD patients is highly prevalent, the commonest one being iron deficiency anemia (IDA). Anemia leads to reduction in quality of life and cardiovascular performance and increased rate of hospitalization and mortality. Hematological indices such as Neutrophil-lymphocyte ratio (NLR) and Platelet lymphocyte ratio (PLR) are markers for systemic inflammation. We aimed to explore the association serum markers of iron status with NLR and PLR in CKD patients on Hemodialysis attending AMCH.

Method

This was a hospital-based cross-sectional study done on 74 adult CKD patients from the department of Nephrology ward and Dialysis ward. Iron profile was analyzed on Dimension® RxL Max clinical chemistry, CLIA (SIEMENS ADVIA Centaur® CP)and hematological parameters were analyzed on Coulter counter (HORIBA Yumizen H2500). Data were analyzed using SPSS version 20.

Result

The mean age of study population was 48.89 ± 15.92years.Seventy two (97.3%) had anemia. 56.8% had absolute iron deficiency, whereas the remaining 43.2% had functional iron deficiency. NLR had significant positive correlations with CRP and ferritin, but non-significant correlations with other parameters of iron profile. PLR was positively correlated with CRP, but had negative correlations with all other iron profile parameters.

Interpretations & Conclusions:

Anemia is highly prevalent among CKD patients, with almost similar proportions of absolute and functional iron deficiency based on iron profile parameters. NLR and PLR are cheaper and reliable markers that correlate well with CRP and ferritin.

Introduction

Chronic kidney disease (CKD) is defined as the presence of kidney damage or impaired kidney function for consecutive three or more months. CKD is highly prevalent in the world with a high burden of mortality and morbidity[1]. Diabetes, high blood pressure, glomerulonephritis, and polycystic kidney disease are the other primary causes of chronic kidney disease[2]. Reduced GFR≤60ml/min/1.73m^2 for > 3

months, albuminuria ≥ 30mg/day, urine sediment abnormalities (hematuria, red cell casts, etc.), electrolyte imbalance and other abnormalities due to tubular disorders, structural abnormalities detected by imaging are all diagnostic criteria for CKD[3]. CKD is divided into 5 stages based on the estimated glomerular filtration rate (eGFR) and evident kidney damage[4].

Due to severe oxidative stress and extracellular fluid overload, patients with CKD have high levels of inflammatory mediators[5]. Chronic systemic inflammation in CKD, also known as low-grade chronic inflammation, is defined by a 2–3 fold rise in plasma levels of acute-phase proteins and inflammatory mediators, which is slow-developing, persistent, and multifactorial in nature[6]. While the causes of chronic inflammation in CKD differ, the consequences of elevated inflammatory markers are consistent, including decreased renal function and an increased risk of mortality. Impaired renal function lead to a series of complications such as increased predisposition to urinary tract infections, generalized inflammation, anemia, low serum calcium levels, high serum potassium and phosphate levels in the blood[7].

In chronic kidney disease (CKD), the decline in endogenous erythropoietin production in kidneys and shortened erythrocyte survival are the main causes of anemia. Ferritin is the main iron storage protein whose production is regulated by the intracellular iron concentration. Each ferritin molecule can store more than 4000 iron atoms. Analysis of ferritin iron content has revealed that despite elevated ferritin concentration, hemodialysis patients might have a decreased ferritin iron content[8]. Serum ferritin is also an acute-phase reactant and can be increased in inflammation. Inflammation is quite common in dialysis patients, and its prevalence among hemodialysis (HD) patients may be as high as 40–60%. Hence, high levels of serum ferritin may be engendered by inflammation independently of iron stores[9].

Total body iron levels in CKD patients are likely to be depleted, resulting in absolute iron shortage, due to increased blood losses, especially in dialysis-dependent patients, as well as potentially reduced gastrointestinal iron absorption. In addition, many CKD patients have functional iron deficit, which is characterized as the inability to transfer enough iron to the site of erythroblast formation while having sufficient reserves, usually due to reticuloendothelial cell iron blockage[10].

For more precise detection of inflammatory responses, a variety of diagnostic and monitoring markers are currently available which include: Ferritin, C-reactive protein (CRP), Interleukin assays, Tumor necrosis factor-α, and PCT[11]. Inflammation is common in the pre-dialysis CKD population and is an important predictive factor of patient health and outcome. Early and specific diagnosis of inflammation is expected to improve quality of life while lowering mortality and morbidity rates[12].

Hematological indices such as Neutrophil Lymphocyte ratio (NLR) and Platelet Lymphocyte ratio (PLR) are inexpensive, convenient tests, and can be measured easily. The neutrophil-to-lymphocyte ratio (NLR) can be calculated by determining the ratio of neutrophils to lymphocytes in peripheral blood, while the platelet-to-lymphocyte ratio (PLR) is obtained by dividing the absolute platelet count by the absolute

lymphocyte count. NLR and PLR have been regarded as novel markers of inflammation in ESRD patients[13].

Previous studies have demonstrated NLR to be a risk factor for arterial stiffness, CVD, and all-cause mortality in peritoneal dialysis (PD) and hemodialysis (HD) patients[14]. Platelets are important in the process of liver inflammation. Several platelet indices, such as platelet count, mean platelet volume, and platelet distribution width, have been shown to predict liver fibrosis[15]. However, the relationship between PLR and mortality in CKD patients is still limited and controversial[16].

Day by day, there has been an increase in the number of patients presenting with symptoms of chronic kidney disease. Anemia is common in CKD patients due to decline in endogenous erythropoietin production and serum iron profile will help in the detection of iron status in CKD patients. CKD patients are immune-compromised and have high possibility of acquiring infection. Since inflammation is common in hemodialysis patients, serum ferritin is increased inspite of the body iron content. In the present socioeconomic status, we must seek cost-effective biological markers. The platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) have been used in kidney patients, particularly as markers of inflammation, endothelial damage, and, more recently, as a predictor of death[17]. NLR predicts the progression rate of stage 4 CKD to dialysis while PLR was linked to inflammation and could predict mortality among haemodialysis patients[18]. NLR and PLR can be easily calculated from the routine blood cell count and are more stable and predictive than each parameter alone. Similar to serum CRP, NLR, PLR and ferritin served as novel markers of systemic inflammation, and these biomarkers are independently related to other inflammatory markers[19].

The purpose of this study was to explore the association of these hematological indices (NLR and PLR) with the serum markers of iron status and CRP in CKD patients under hemodialysis.

Material & Methods

Ethical considerations

This Cross-sectional study was conducted in the department of Pathology at tertiary care teaching hospital. Ethical approval was taken from AMCH-IEC (Institutional Ethical Committee) with Ref. no. of AMCH-IEC/21–104 and the Department of Laboratory Medicine of Arogyam Medical College & hospital, Roorkee, Uttarakhand. Verbal informed consent was taken from the patient before his/her inclusion in research. All required data for my research was taken from tests that were prescribed by clinicians, with no added costs to the patients. The samples were collected from November 2024 to April 2025. Total 74 samples were taken for the study.

Inclusion & exclusion criteria: All adult CKD-diagnosed patients under Hemodialysis were included in this study. CKD patients not under dialysis were excluded from the study. And other exclusion a) nephrotic-range proteinuria, (b) active infection, (c) autoimmune disease, and (d) secondary hyperparathyroidism

Data collection tools and techniques were used interview questionnaires and observation tools were used as data collection tools. Detail information of the patients from himself/herself, clinicians and nursing staffs, blood samples were collected. Samples were collected aseptically. Collection and processing of the sample about 5ml of the venous blood sample was collected from patients using the aseptic technique into a clot activator tube 3ml (Yellow capped vial) and EDTA tube 2ml (Purple capped vial). The serum sample was separated and utilized for analysis of different parameters.

Equipment and materials: Syringe, Tourniquet, Vacutainer (Purple capped & Yellow capped vial), 70% ethanol, Dry cotton, Rotator, Coulter counter (HORIBA Yumizen H2500), Biochemistry analyzer(Dimension® RxL Max clinical chemistry), CLIA(SIEMENS ADVIA Centaur® CP). NLR: It is calculated by dividing the absolute neutrophils by absolute lymphocyte count. PLR: It is calculated by dividing the absolute platelet count by the absolute lymphocyte count. Estimation of Iron-Serum Iron test is measured on Dimension ® clinical chemistry by Ferrozine method. Estimation of TIBC (total iron binding capacity) Normal Range:- 250–450µg/dl Estimation of Ferritin Normal Range:- 4.63-204ng/ml Transferrin Saturation Normal Range:- 20–50% Estimation of C-reactive protein: Normal Range :- <10mg/L

Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it application for the measurement of test and control samples

Data analysis

The data was entered in IBM SPSS 20.0 software. Then the descriptive analysis (frequency, percentage, mean, Median, standard deviation, cross tabulation) was done. Pearson's correlation analysis was performed to find out the correlation between continuous variables. $P < 0.05$ was considered to be statistically significant.

Results

This chapter deals with analysis and interpretation of the data obtained from CKD patients. The data was collected from 74 patients visiting Nephrology ward and Dialysis ward AMCH.

Age distribution

The mean age of the patients was 48.9 ± 15.9years.

Table 1 shows the sex wise distribution of CKD patients. Out of 74 patients, 46 patients (62.8%) were males and 28 patients (37.2%) were females.

Table 1
Sex wise distribution of CKD patient (n = 74)

Sex	Numbers	Percentage%
Male	46	62.8
Female	28	37.2
Total	74	100

Personal History

Among total patients, 18 (24.3%) had Diabetes Mellitus. All of the patients (100%) hypertension. 8 (10.8%) were current consumers of alcohol, and 10 (13.5%) were current smokers.

Table 2 : Personal history of CKD patients (n=74).

Variable	Frequency	Percentage (%)
Diabetes Mellitus		
Yes	18	24.3
No	56	75.7
Hypertension		
Yes	74	100
Current Smoking		
Yes	10	13.5
No	64	86.5
Current Alcohol consumption		
Yes	8	10.8
No	66	89.2

Duration of CKD

The median duration of CKD among the patients was 2 years (IQR: 1 – 2.5 years).

Hematological parameters

The summary of the hematological parameters is shown in Table 3. The median Hb level was 7.8 g/dl (IQR: 6.8 – 9.3 g/dl).

Table 3 : Hematological parameters of CKD Patients (n=74)

Variables	Median	IQR
Hemoglobin (g/dl)	7.8	6.8 – 9.3
WBC count (/mm$^{3)}$	5680	4670 - 6967.5
Hematocrit or PCV (%)	25.9	22.0 – 30.7
RBC count (Million/mm^3)	2.8	2.3 – 3.1
MCV (fl)	92.6	87.6 – 100.1
MCH (pg)	28.6	27.9 – 29.7
MCHC(%)	30.5	29.0 – 31.7
Platelets (lakh/mm^3)	1.7	1.5 – 2.0
ESR (mm/hr)	40.0	15.3 – 70.5

Abbreviations: WBC, White blood cell; PCV, Packed cell volume; RBC, Red blood cell; MCV, Mean cell volume; MCH, Mean cell hemoglobin; MCHC, Mean cell hemoglobin concentration; ESR; Erythrocyte sedimentation rate

Iron profile parameters

Table 4 shows iron status of CKD patients in which median (IQR) of iron, TIBC, TSAT and Ferritin was found to be 9.4(6.6-11.4),215.0(196.0-244.5),23.0(17.0-29.8) and 420.7(173.2-1418.5) respectively.

Table 4 : Status of Iron profile Parameter in CKD patients (n=74)

Variables	Median	IQR
Iron (µmol/L)	9.4	6.6 – 11.4
TIBC (µg/dl)	215.0	196.0 – 244.5
TSAT(%)	23.0	17.0 – 29.8
Ferritin (ng/ml)	420.7	173.2 – 1418.5

Abbreviations: TIBC; Total iron binding capacity, TSAT; Transferrin saturation

Inflammatory markers

Table 5 shows inflammatory markers observed in the study population. The median CRP was 4.3, NLR was 3.67 and PLR was 160.97 respectively.

Table 5 : Status of Inflammatory Marker of CKD Patients (n=74)

Variables	Median	IQR
CRP (mg/L)	4.3	0.9 – 13.2
NLR	3.7	2.4 – 4.8
PLR	160.9	113.9 – 221.1

Abbreviations: CRP; C-reactive protein, NLR; Neutrophil-lymphocyte ratio, PLR; Platelet-lymphocyte ratio

Distribution of anemia among CKD patients

72 (97.3%) patients had anemia based on Hb criteria i.e. Hb<12 g/dL.

Distribution of anemia based on TSAT and Ferritin

Table 7 shows TSAT ≤ 30% was 75.7% and >30% was 24.3% and also Ferritin ≤ 500ng/ml was 56.8% and Ferritin > 500ng/ml was 43.2% respectively.

Table 6 : Distributions of CKD Patients based on TSAT and Ferritin (n=74)

Variables	Frequency	Percentage (%)
TSAT		
TSAT ≤ 30%	56	75.7
TSAT > 30%	18	24.3
Ferritin		
Ferritin ≤ 500ng/ml	42	56.8
Ferritin > 500ng/ml	32	43.2

Distribution of type of anemia based on Ferritin and TSAT

Table 8 shows 56.8% had Absolute iron deficiency and 43.2% had Functional iron deficiency in patients of CKD.

Table 7 : Iron deficiency status of CKD Patients based on TSAT and Ferritin (n=74)

Variables	Frequency	Percentage (%)
Absolute iron deficiency (TSAT ≤ 30% and Ferritin ≤ 500ng/ml)	42	56.8
Functional iron deficiency	32	43.2

Correlation between iron profile marker and inflammatory marker (NLR, PLR and CRP)

NLR: There was a significant positive correlation between NLR and CRP (r = 0.74, P<0.001) and NLR and ferritin (r = 0.26, P<0.05). Other parameters of iron profile had negative, but non-significant correlation with NLR. [Table 9]

PLR: There was significant association between PLR and CRP.

Table 8 : Correlation between iron profile marker and inflammatory marker (NLR, PLR and CRP) of CKD patients (n=74)

	Iron	TIBC	TSAT	Ferritin	CRP
NLR	- 0.165 (P = 0.160)	- 0.216 (P = 0.065)	- 0.137 (P = 0.244)	0.265* (P = 0.02)	0.745** (P = 0.000)
PLR	- 0.348** (P = 0.002)	- 0.133 (P = 0.259)	- 0.313** (P = 0.007)	- 0.026 (P = 0.827)	0.498** (P = 0.000)
CRP	- 0.223 (P = 0.056)	- 0.270* (P = 0.020)	- 0.175 (P = 0.136)	0.176 (P = 0.134)	

**Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

Chronic kidney disease is a medical condition characterized by irreversible renal dysfunction caused by a variety of diseases. The most common underlying disorders resulting in CKD include Diabetes Mellitus, Hypertension and Chronic glomerulonephritis. Progressive damage to kidneys ultimately results in involvement of every organ system of the body. The most frequent hematological manifestation in patients of CKD is anemia[20].Our study evaluated the Association of serum markers of iron status with neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in chronic kidney disease patients undergoing hemodialysis at AMCH.

In our study, it was observed that out of 74 CKD patients, males were 46 (62.8%) and females were 28 (37.2%).The proportion is consistent with a study done in Nepal by S Bhatta, et.al 2011, where there were 25 (62.5%) males and 15 (37.5%) females[21]. Our study had a higher proportion of males compared to females. This might be due to the reason that the male patients had more years of education, higher income, a significantly higher percentage of hypertension, cigarette smoking and alcohol consumption

than the female patients. Other reason is that men with diabetes have higher risk of nephropathy than women with diabetes[22].

In our study, regarding the co-existing conditions, it was found that hypertension was found in 74 (100%) and diabetes mellitus was found in 18 (24.3%) patients. The findings are consistent with the study done by G Brito et.al, 2021, where Hypertension was found in 92.94% and Diabetes mellitus in 47.06%[23]. A similar research conducted at a Rural Tertiary care Centre in North India showed that 67% of the CKD patients had hypertension and diabetes mellitus was found in 34% of CKD patients[20].

In our study, the patient with absolute and functional iron deficiency was found 56.8% and 43.2% respectively. In CKD patients, low TSAT. (<20%) combined with low serum ferritin is diagnostic of absolute iron deficiency. Low TSAT combined with normal or elevated serum ferritin is diagnostic of functional iron deficiency. Retrospective analyses of erythropoietic responsiveness showed that maximum erythropoietic response is achieved at TSAT levels greater than 30%[24]. Iron repletion/supplementation protocols used by most dialysis organizations in the United States specify TSAT target range of 30% to 50%.

CRP, a widely accepted biomarker of inflammation was positively related to an elevated value of NLR & PLR, which is consistent with Pineault et al ,2011 [25]. However, we did not find any significant relationship between these hematological indices (NLR and PLR) with serum iron, TIBC and TSAT. NLR & PLR had significant correlation with ferritin. Serum ferritin is both a marker of iron status and an indicator of inflammation or malnutrition in dialysis patients. The level of ferritin in plasma represents the balance between its secretion, which is directly related to intracellular iron synthesis, and its clearance, mainly in liver and other organs [26]. However, liver dysfunction and inflammatory factors may interfere with the synthesis and clearance of ferritin, thereby increasing serum ferritin levels due to circumstances not related to iron metabolism [27], since serum ferritin is an inflammatory marker, it may be increased in the setting of infection. Infection may be the primary cause of death and also associated with hyper ferritinaemia as a secondary phenomenon. Thus, considering high ferritin levels as the primary cause of increased mortality in the setting of inflammation or infection may be flawed as long as longitudinal studies do not show any temporal relationship between iron administration and poor outcome in dialysis patients.

Conclusion

The study showed that anemia is highly prevalent among CKD patients under hemodialysis that constitutes of both absolute and functional iron deficiency. NLR & PLR was positively correlated with CRP and ferritin these patients, but not with other iron status parameters. NLR and PLR are cheaper and reliable markers that correlate well with CRP and ferritin.

Declarations

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Conflicts of interest: None

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Figures

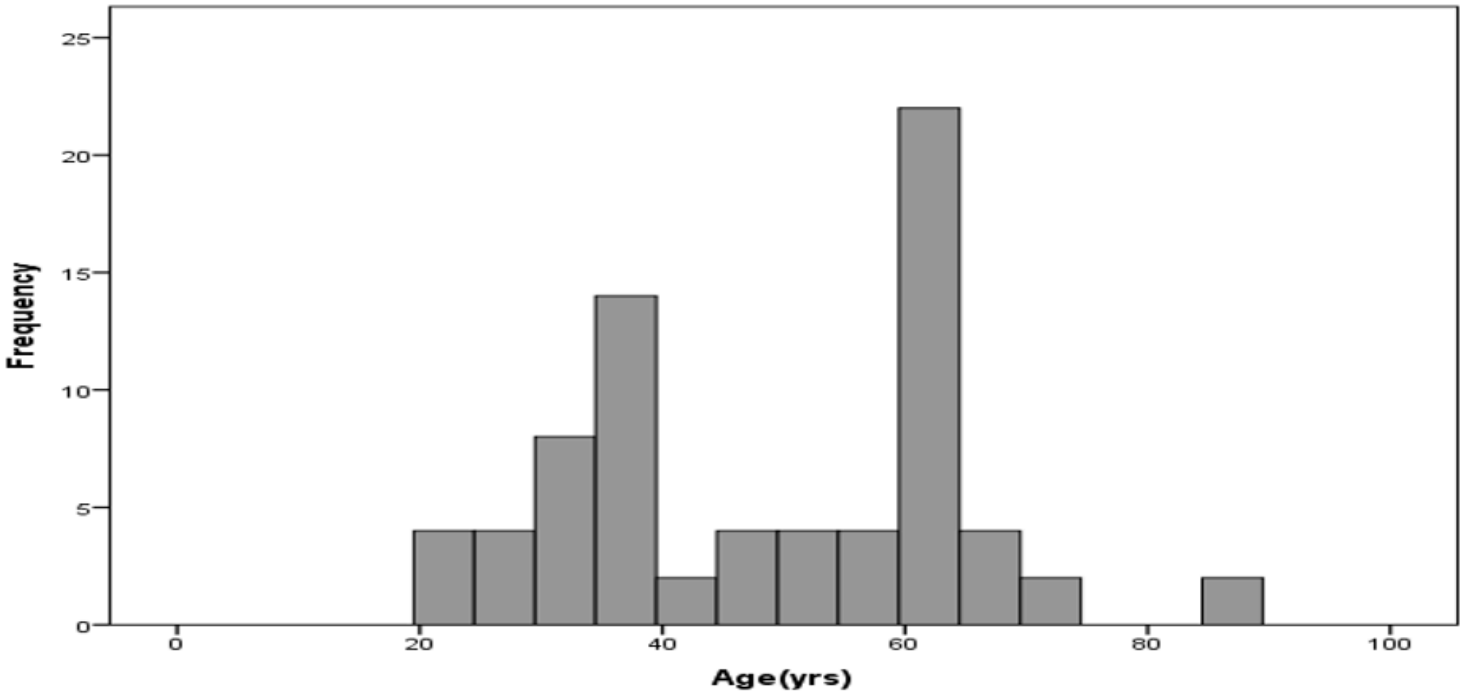


Figure 1

Age histogram of the CKD patients (n = 74)4.2 Sex distributions

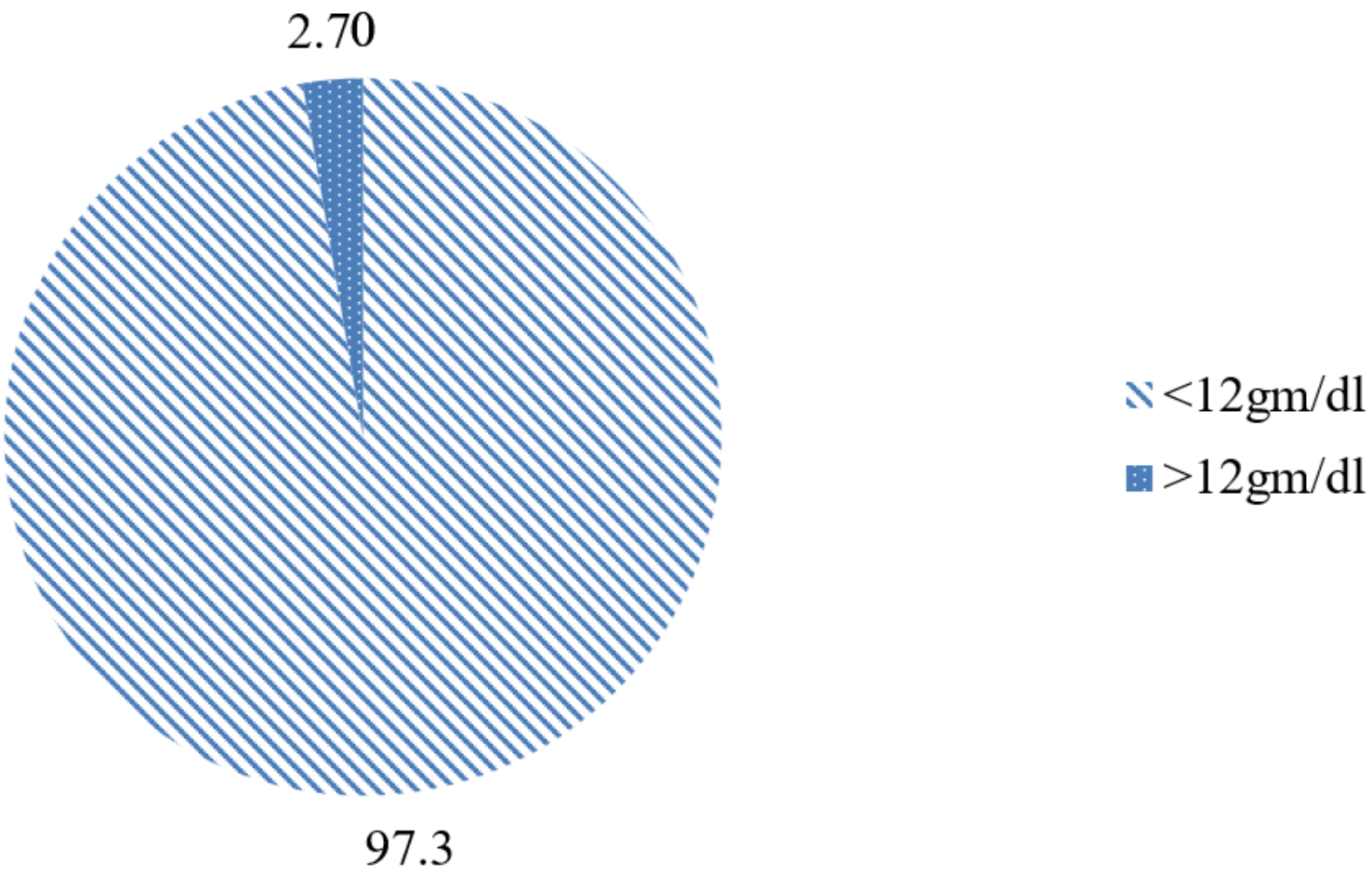


Figure 2

Distribution of anemia in CKD patients (n=74)