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Research Article

ASSESSMENT OF IRON PROFILE AND ITS CORRELATION WITH MICRO R% AND OTHER RED CELL PARAMETERS IN PATIENTS WITH CHRONIC RENAL FAILURE ON DIALYSIS AND RECEIVING ERYTHROPOIETIN INJECTION

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ABSTRACT

Objectives: The objectives of the study are to correlate Micro R% with red cell indices and to assess their efficiency in detecting iron deficiency in chronic kidney disease (CKD) patients receiving erythropoietin (EPO).

Methods: Red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration, and red cell distribution width generated from automated analyzers frequently help in the diagnosis of iron deficiency. Micro R% is a research-only parameter in an automated analyzer while doing a complete blood count. This study is done to correlate and compare hemoglobin and biochemical parameters in healthy controls and CKD patients and to correlate the Micro R% with MCV and serum iron in detecting iron deficiency in CKD patients receiving EPO.

Results: Red cell indices showed a good correlation with serum iron studies. Micro R% is a reliable parameter for the identification of iron deficiency anemia and it correlates with other red cell indices and with serum iron studies.

Conclusion: Identification of iron deficiency in patients with chronic renal failure on dialysis helps us to start the treatment earlier. Anemia-related complications can be prevented and we can give a better quality of life in these patients.

Keywords: Micro R%, Red cell parameters, Chronic renal failure, Erythropoietin, Iron deficiency.

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INTRODUCTION

Chronic kidney disease (CKD) is regarded as a public health issue globally due to its high incidence and prevalence rates. Advanced CKD frequently leads to anemia and the prevalence of anemia in CKD ranges from 7% to 50% in the more severe phases of the disease, the prevalence of anemia rises with each stage of CKD [1]. Major factors associated with anemia in CKD are iron deficiency, iron-related dysfunction, and relative deficit of erythropoietin (EPO) [2]. Erythropoiesis-stimulating agents (ESAs) drugs are therefore widely regarded as a standard treatment for anemia in individuals with CKD. The use of ESAs to normalize hemoglobin in CKD patients has been shown to be effective in several well-done studies [3]. Coexisting iron deficiency reduces the benefit of EPO. Red blood cell (RBC) indices give us details on the hemoglobin content and size of RBCs. These abnormal results reveal anemia and provide information regarding the type of anemia and are useful in identifying the root of anemia [4]. Dialysis patients frequently experienced anemia as a result of endogenous EPO insufficiency, decreased RBC survival, and uremic inhibitors. Recombinant human EPO (rHuEPO) and its analogs are being widely used to treat anemia in CKD patients on dialysis. Transferrin saturation (TSAT), tissue iron binding capacity (TIBC), and serum ferritin are the three primary indicators of iron status. Both the serum ferritin concentration and TSAT, are used to guide anemia treatment in patients with CKD and are linked to clinical outcomes in dialysis patients [5-8]. Red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), and red cell distribution width (RDW) generated from automated analyzers frequently help in the diagnosis of iron deficiency. Micro R% is a research-only parameter in an automated analyzer while doing a complete blood count. It gives the percentage of microcytic red cells excluding the macrocytic red cells. It is claimed to be the real indicator of microcytosis as it is not influenced by the factors affecting the red cell size. It helps in redefining the diagnosis of anemia [9].

Plasma transferrin is a glycoprotein with two iron-binding domains and is synthesized by the liver [10]. It is the most important vehicle for transporting iron into cells and preventing iron-mediated free radical toxicity. Approximately 3 mg of iron (0.1% of total body iron stores) circulates in the plasma and is bound to transferrin. The sum of all iron binding sites on plasma transferrin is known as TIBC. TSAT is the ratio of the total number of occupied iron binding sites to TIBC. The number of available iron-binding sites is known as unsaturated iron binding capacity. In healthy individuals, approximately one-third of the iron binding sites are occupied (TSAT approximately equal to 33%) [10]. In CKD patients, low TSAT (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 >30% [11-13]. Anemia is a common complication of CKD. It is associated with left ventricular dysfunction and heart failure, in addition to a reduction in exercise capacity and quality of life. The use of iron therapies and ESAs has allowed improvement in patients with anemia of CKD. Treatment with rHuEPO in dialysis patients has been shown to be highly effective in terms of correcting anemia and improving quality of life [14]. This study is done to evaluate the effectiveness of Micro R% in detecting iron deficiency in CKD patients receiving EPO, so that

treatment can be started earlier. This study also assesses the red cell parameters and iron status in EPO injection-receiving hemodialysis patients.

METHODS

This is the retrospective, analytical, case–control, and hospital-based study that includes 25 patients, who underwent dialysis and received EPO injections and another 25 individuals without any abnormalities as a control group. The hematological parameters such as RBC, hemoglobin (HB), packed cell volume, MCV, MCH, MCHC, Micro R%, and Macro R% were performed using a Sysmex XN10 analyzer. TIBC, iron TSAT was analyzed using automated chemistry analyzer cobas. This study was approved by the institutional ethical committee.

Inclusion criteria

Dialysis patients over the age of 80 who received rHuEPO treatment and patients with CKD received EPO. People of the same age without CKD and no abnormalities in complete blood count were chosen as the control group.

Exclusion criteria

Individuals with a history of malaria within the past month, pregnant women, thalassemia, acute leukemia, hemoglobinopathies, coagulation problems, gastric carcinoma or other malignancies, chronic diseases such as chronic liver disease, liver cirrhosis, chronic renal failure (CRF), tuberculosis and cancers.

Statistics

Using the Statistical Packages for the Social Sciences software, we carried out the statistical analysis. Version 16 and a 0.05 significance threshold were considered. Using the Shapiro–Wilk test, we first determined the data and normality before analyzing it. Then, for normal data, we used the Shapiro–Wilk test, and for non-normal data, we used the Mann–Whitney test.

RESULTS

In this study, the ratio of Male: Female was 1:1 in both control and cases. The age group varies from 18 years to 79 years in study cases and 20 years to 75 years in the control group. The lowest and highest range of red cell and iron parameters are given in Table 1. Both dialyzed patients and the control group showed statistically significant decreases in red cell and iron parameters in varying ranges (Table 2). The box and whisker plots of analysis of various parameters are given in Figs. 1 and 2. Micro R% is the estimation of % of microcytic RBC in the peripheral blood. A research parameter from the Sysmex XN 10 analyzer, micro r% is exclusively used for study. It has the advantage over MCV and MCH in that it is not influenced by normal cells mixed with microcytic red cells. Correlation of Micro R% with MCV and MCH was done (Fig. 3). Micro R% correlated with MCV in patients and controls. There was a correlation between Micro R% and MCH in patients and no correlation in controls.

The results of this study showed that the dialysis patients receiving EPO therapy showed a significant reduction in HB in comparison to control groups. Their relationship with serum iron, serum ferritin, TIBC, and TSAT was not statistically significant. Micro R% was a reliable indicator of iron deficiency in controls. Micro R% was not helpful in identifying iron deficiency in CRF patients receiving EPO.

DISCUSSION

Insufficient EPO production by damaged kidneys is the primary cause of anemia in CKD. The anemia that these people experience may also be caused by iron deficiency, chronic inflammation, hyperparathyroidism, and blood loss. Since 1986, rHuEPO has been utilized to treat the anemia associated with CKD. The majority of CKD patients, especially those with stage-5 non-hemodialysis CKD (NDCKD) patients, are iron deficient because of an imbalance in iron availability. Before starting EPO therapy, it is necessary to evaluate the patient's iron status and ensure that there are sufficient iron reserves on hand. Iron is a crucial component in the production of HB. In patients with CKD, iron supplementation is necessary for an effective response to EPO because the demands for iron by the erythroid marrow frequently exceed the amount of iron that is immediately accessible for erythropoiesis (as determined by % TSAT) as well as iron reserves (as measured by serum ferritin). To effectively treat anemia in CKD patients, iron indices must be estimated and regularly monitored. The assessment of tissue ferritin and transferrin is uncommon, despite the fact that patients' serum iron, TIBC, and ferritin levels are frequently assessed. The normal range (66–139 g/dL) is where serum iron levels on average fall for all CKD groups [6]. When compared to controls, the transferrin levels of group-carbamylated EPO hyporesponsive patients (31 mg/dL) and CKD patients on hemodialysis (HDCKD) patients on intravenous (IV) iron (184 mg/dL) dramatically decreased (p=0.0001). Transferrin levels among patients with HDCKD, EPO-resistant HDCKD, and NDCKD were also significantly different (p=0.0001) [6]. In our study, there is a significant difference in TSAT, TIBC, and iron between cases and control. Renal failure is a disorder brought on by the kidneys' insufficient ability to remove waste products and poisons from the blood. It was divided into two categories: acute and chronic. CRF is characterized by anemia, which is typically normochromic and normocytic. In patients with CRF, EPO deficiency is the most frequent cause of anemia [5]. There is a significant decrease in HB

Table 1: Mean, standard deviation, and interquartile range and p-value of red cell parameters and iron parameters in cases and control

S. No	Parameters	Cases			Control		
		Mean	SD	p-value	Mean	SD	p-value
1	Hb	8.5	1.2	0.0	13.5	1.4	0.0
2	MCV	90.3	7.4	0.135	87.6	4.7	0.135
3	MCH	28.4	2.2	0.511	28	1.8	0.511
4	MCHC	32	1	0.159	32	1.1	0.159
5	RDW-CV	14.7	1.5	0.0	13	0.9	0.0
6	RBC	3.2	0.5	0.0	4.8	0.4	0.0
		Median	IQR	p-value	Median	IQR	p-value
7	Micro R%	2.2	(1.1–1.4)	0.3	1.7	(1.1–2.4)	0.3
8	Macro R%	3.5	(2.9–4.4)	0.71	3.9	(3.6–4.4)	0.71
9	Iron	50.3	(26.8–76.4)	0.17	34.7	(15.6–66.8)	0.17
10	TIBC	233.7	(163–259.7)	0.12	237.4	(202–393)	0.12
11	TSAT	22.7	(14.1–30.8)	0.15	15.5	26.67052	0.15

Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation, RBC: Red blood cell, TIBC: Tissue Iron binding capacity, TSAT: Transferrin saturation, SD: Standard deviation, IQR: Interquartile range

Table 2: Lowest and highest values of red cell parameters and iron parameters in cases and control

S. No	Parameters	Cases		Control	
		Lowest	Highest	Lowest	Highest
1	Hb	7 g/dL	11.6 g/dL	11.1 g/dL	16.6 g/dL
2	MCV	79.4 fL	103.5 fL	77.5 fL	96.9 fL
3	MCH	26.1 pg	32.8 pg	25.1 pg	31.3 pg
4	MCHC	29.8 g/dL	32.3 g/dL	30.1 g/dL	33.3 g/dL
5	RDW-CV (%)	12.6	18.3	11.8	14.7
6	RBC	2.4×10^6 µL	4.33×10^6 µL	4.06×10^6 µL	5.96×10^6 µL
7	Micro R% (%)	0.5	4.9	0.5	11.7
8	Macro R% (%)	2.4	9.3	3.3	4.5
9	Iron	20.88 µg/dL	138.88 µg/dL	10.68 µg/dL	288.37 µg/dL
10	TIBC	118	357.1	100	277.4

Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation, RBC: Red blood cell, TIBC: Tissue iron binding capacity

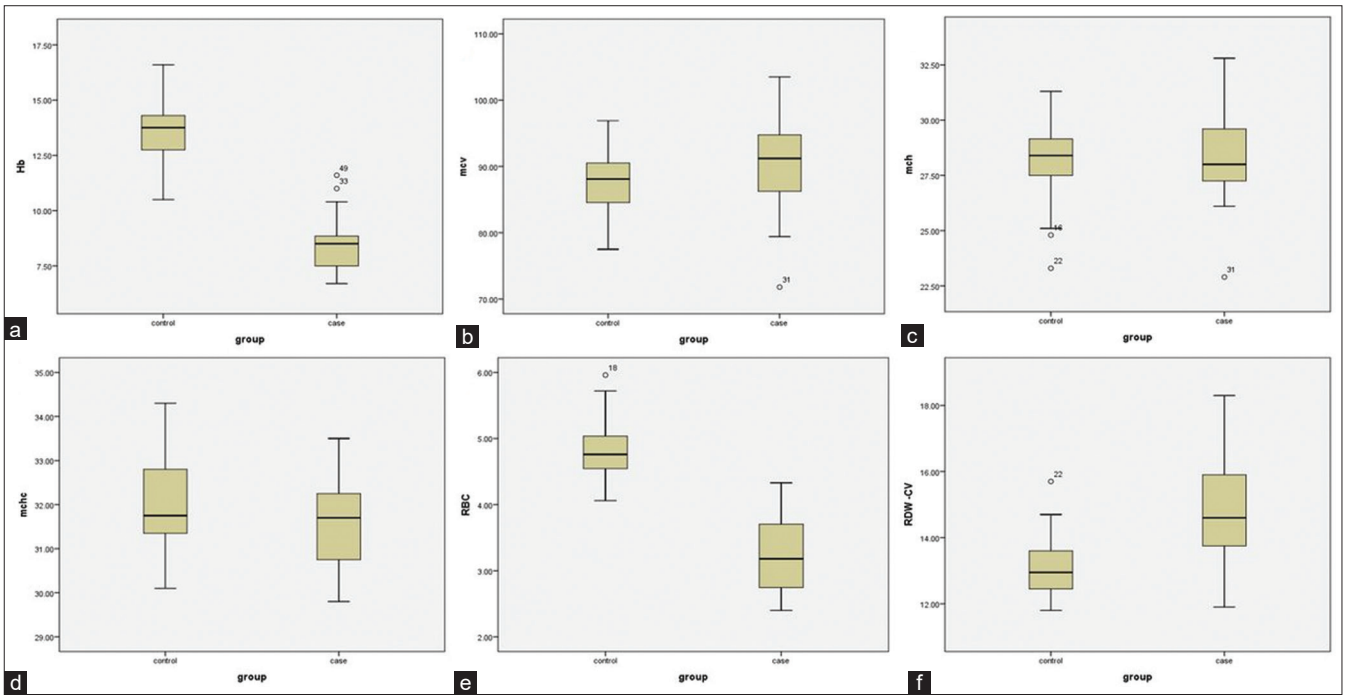

Fig. 1: Box and whisker plots of analysis of (a) hemoglobin; (b) mean corpuscular volume; (c) mean corpuscular hemoglobin; (d) mean corpuscular hemoglobin concentration; (e) red cell count; (f) red cell distribution width-coefficient of variation among the cases and control groups

concentration, which has been linked to chronic kidney failure [4]. It has been observed in our study that there was a decrease in HB concentration in CKD in comparison with a control group. Due to the massive blood losses involved with this procedure, iron insufficiency is a prevalent issue in chronic hemodialysis patients. After 1986, when rHuEPO was made accessible, most patients with end-stage renal disease anemia could be successfully treated with the medication. According to a study of dialysis patients receiving rHuEPO, patients had significantly lower serum levels of total iron and TSAT than healthy individuals [1]. A higher level of RDW was linked to a higher probability of death from any cause in stage 3–4 CKD. Along with the rise in death risk and kidney function loss, RDW also increased. RDW and HB showed a statistically significant correlation [8]. According to Gafter-Gvili *et al*., the proactive administration of high-dose iron was non-inferior to the reactive administration of a low-dose IV iron regimen and was not linked to an increased risk of death, serious adverse cardiovascular events, or infection. High-dose iron also made it possible to reduce the amount of ESA required, the frequency of blood transfusions, and the number of heart failure hospitalizations [3].

RBC volume-dependent measures such as %Hypo-He, %Hyper-He, %Micro-R, and Macro-R% are new reticulocyte and erythrocyte characteristics available on the Sysmex hematology analyzer which provide a quick and affordable substitute for conventional laboratory testing, especially in regions with limited resources. These were stable for <12 h when held at room temperature 4–8°C [7] and are used to develop discriminant indices for the differential diagnosis of microcytic anemia in conjunction with other RBC parameters (mostly hypochromic erythrocytes) [2]. The goal of this study was to examine if these red cell parameters were useful as a substitute for the iron indices which are expensive; however, it was found that the research parameter of Micro R% only corresponds with hematological parameters and not biological factors (Iron, TSAT, and TIBC). This was the first study to have analyzed the usefulness of Micro R% in dialysis patients receiving rHuEPO. Identification of iron deficiency is essential in patients with CKD as it mandates treatment with oral or IV iron therapy [15]. Anemia in CKD patients increases, the number of prescribed antibiotics, and the length of hospitalization [16]. Correction of anemia in CKD patients on hemodialysis and receiving EPO improves the quality of life [17].

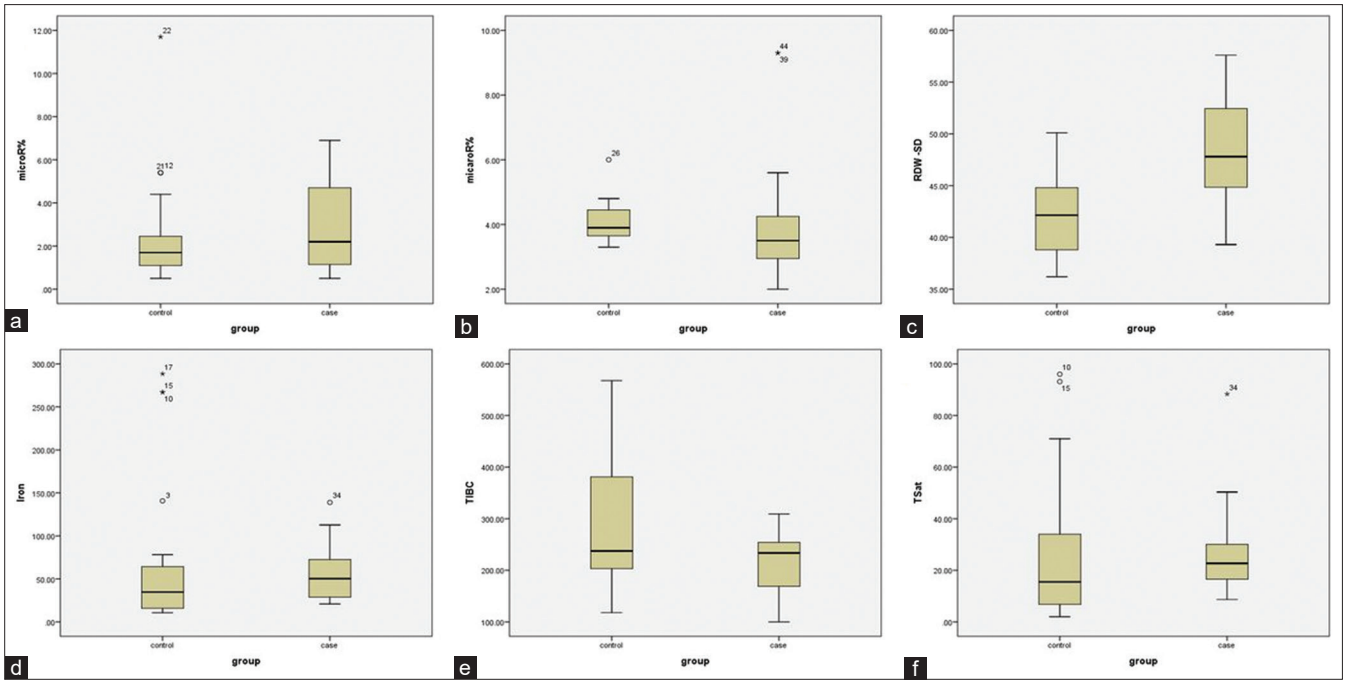

Fig. 2: Box and whisker plots of analysis of (a) Micro R%; (b) Macro R%; (c) red cell distribution width-coefficient of variation; (d) serum iron; (e) tissue iron binding capacity; (f) transferrin saturation among the cases and control groups

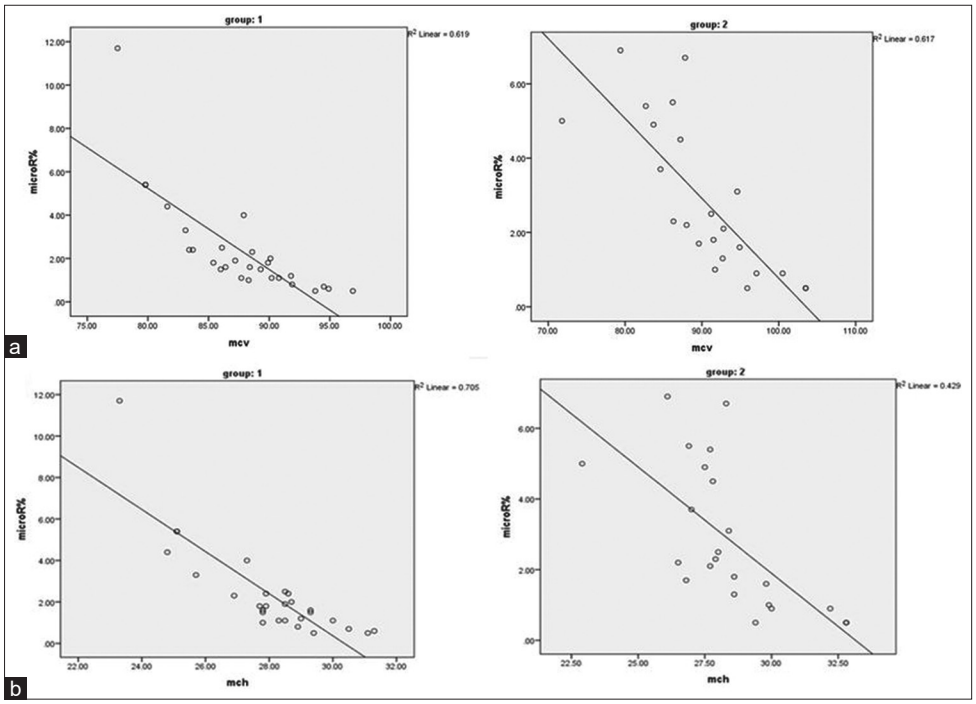

Fig. 3: Correlation of Micro R% with (a) mean corpuscular volume in both controls (group 1: r-value: 0.619) and patients (group 2: r-value: 0.617); (b) mean corpuscular hemoglobin: in controls (group 1: r-value:0.709) and patients (group 2: r-value: 0.429)

CONCLUSION

Anemia in CKD is a complex disease and there are many factors associated including nephrotoxins [18]. To treat anemia, CKD patients are put on EPO therapy. Iron supplementation after assessing iron indices is necessary for an effective response. In our study, we tried to study if Micro R% done using a hematology analyzer could be an alternative to studying iron indices in a resource-poor setting. However, we conclude that Micro R% alone cannot replace the role of iron studies in CKD patients with anemia.

AUTHOR'S CONTRIBUTION

Dr. Divya Kannan: Conducted research, collected and organized data. Dr. Sri Gayathri: conceived and designed the study, and edited the initial and final draft of the article. Dr. Pavithra Sankar: Wrote the initial and final draft of the article. Dr. UmaLakshmi Krishnan: Provided research materials. Dr. Gayathri Thiruvengadam: Analyzed and interpreted data with statistical analysis. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

CONFLICT OF INTEREST

Nil.

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Nil.

REFERENCES

1. Alzain MA. Assessment of Iron Profile, Hemoglobin Level and Reticulocyte Count among Sudanese Dialysis Patients Receiving Erythropoietin Injection, Khartoum State. Sudan University of Science and Technology; 2020. Available from: https://repository.sustech.edu/handle/123456789/25341
2. Buttarello M. Laboratory diagnosis of anemia: Are the old and new red cell parameters useful in classification and treatment, how? Int J Lab Hematol. 2016;38(Suppl 1):123-32. doi: 10.1111/ijlh.12500, PMID: 27195903
3. Gafter-Gvili A, Schechter A, Rozen-Zvi B. Iron deficiency anemia in chronic kidney disease. Acta Haematol. 2019;142(1):44-50. doi: 10.1159/000496492, PMID: 30970355
4. Naeem MH, Ali J, Naeem M, Ashraf A, Muhammad H, Safdar Z, *et al*. Biochemical changes in patients with chronic kidney failure in relation to complete blood count and anemia. Int J Biol Sci. 2020;16(1):267-71. doi: 10.12692/ijb/16.1.267-271
5. Prasad N, Barai S, Gambhir S, Parasar DS, Ora M, Gupta A, *et al*. Comparison of glomerular filtration rate estimated by plasma clearance method with modification of diet in renal disease prediction equation and Gates method. Indian J Nephrol. 2012;22(2):103-7. doi: 10.4103/0971-4065.97123, PMID: 22787311
6. Reddy GC, Devaki R, Rao P. Iron indices in patients with functional anemia in chronic kidney disease. EJIFCC. 2013;24(3):129-36. PMID: 27683448
7. Schapkaitz E. Stability of new erythrocyte and reticulocyte parameters in testing for anemia on the Sysmex XN 9000. Lab Med. 2018;49(3):219-25. doi: 10.1093/labmed/lmx095, PMID: 29365192
8. Yilmaz F, Sozel H. Red blood cell distribution width is a predictor of chronic kidney disease progression and all-cause mortality. Bratisl Lek Listy. 2021;122(1):49-55. doi: 10.4149/BLL_2021_006, PMID: 33393321
9. SYSMEX. New Blood Cell Counting Technology in the VCUHS Hematology Laboratory; 2017. p. 1-16. Available from: https://www.pathology.vcu.edu/media/pathology/clinical/hem/bloodcellcountingatvcuhs.pdf
10. Huebers HA, Eng MJ, Josephson BM, Ekpoom N, Rettmer RL, Labbé RF, *et al*. Plasma iron and transferrin iron-binding capacity evaluated by colorimetric and immunoprecipitation methods. Clin Chem. 1987;33(2 Pt. 1):273-7. doi: 10.1093/clinchem/33.2.273, PMID: 3542299
11. Gaweda AE, Goldsmith LJ, Brier ME, Aronoff GR. Iron, inflammation, dialysis adequacy, nutritional status, and hyperparathyroidism modify erythropoietic response. Clin J Am Soc Nephrol. 2010;5(4):576-81. doi: 10.2215/CJN.04710709, PMID: 20110344
12. Kalantar-Zadeh K, Lee GH, Miller JE, Streja E, Jing J, Robertson JA, *et al*. Predictors of hyporesponsiveness to erythropoiesis-stimulating agents in hemodialysis patients. Am J Kidney Dis. 2009;53(5):823-34. doi: 10.1053/j.ajkd.2008.12.040, PMID: 19339087
13. Gaweda AE, Bhat P, Maglinte GA, Chang CL, Hill J, Park GS, *et al*. TSAT is a better predictor than ferritin of hemoglobin response to epoetin alfa in US dialysis patients. Hemodial Int. 2014;18(1):38-46. doi: 10.1111/hdi.12078, PMID: 23968235
14. Cody JD, Hodson EM. Recombinant human erythropoietin versus placebo or no treatment for the anaemia of chronic kidney disease in people not requiring dialysis. Cochrane Database Syst Rev. 2016;1:CD003266. doi: 10.1002/14651858
15. Dean S. An overview of iron deficiency anaemia and its management options. Int J Adv Pract. 2023;1(3):123-8.
16. Chalivendra P. Umamaheswara Rao K, Sucharitha J, Chandra VB, Nelavala AD. Prescription pattern of antibiotics and their appropriateness in patients with chronic kidney disease-an observational study in a tertiary care teaching hospital in South India. Int J Curr Pharm Res. 2023;15(1):63-7. doi: 10.22159/ijcpr.2023v15i1.2077
17. Sihombing J, Hakim L, Andayani TM, Irijanto F. Quality of life of chronic kidney disease patients with routine hemodialysis in general hospitals in Sleman Yogyakarta. Int J Pharm Pharm Sci. 2017;9(2):213-6. doi: 10.22159/ijpps.2017v9i2.15844
18. Idacahyati K, Nurdianti L, Husni SS, Gustaman F, Wulandari WT. Nephroprotective activity of ethanol extract of kirinyuh (*Chromolaena odorata* L) in gentamicin induced nephrotoxicity in Wistar rats. Int J Appl Pharm. 2021;13(3):53-6. doi: 10.22159/ijap.2021.v13s3.1