Morphological Types of Anemia Associated with Chronic Renal Diseases

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Abstract

BACKGROUND: Anemia develops early in the course of renal disease and worsens as kidney function declines, a typical consequence of CKD is caused by a disruption in erythropoietin production as well as caused by iron and vitamin shortages, blood loss, shortened erythrocyte life span, chronic inflammation, and the uremic environment.

AIM: Our study aimed to identify and classify different types of anemia that complicates patients with chronic kidney disease.

PATIENTS AND METHODS: Forty patients were included in this study, 32 males (80%) and eight females (20%), the age range was 40−70 for males and 48−68 for females. Depending on clinical signs, symptoms, and laboratory diagnostic tests, all those patients are diagnosed previously as cases of chronic renal failure by clinicians in our city and developed anemia during the course of their diseases, so clinicians referred patients to our laboratory to find the cause of anemia. Depending on data collected from peripheral hematological parameters, blood film examination, different types of anemia have been detected in patients involved on this study.

RESULTS: The most common types of anemia were normochromic anemia (62.5%), which develop in 25 patients followed by hypochromic microcytic anemia (8%) which develops in eight patients, and followed by macrocytic anemia (12.5 %) which develops in five patients and the less common type of anemia was hemolytic anemia (5%) which was founded in two patients.

CONCLUSION: All types of anemia can be detected in a patient with different types of renal diseases and it's important to use different types of laboratory tests for the diagnosed type of anemia to treat it.

Introduction

Anemia is defined as a low hemoglobin level or hematocrit level, it is a common complication of chronic kidney disease, and it is linked to a lot of morbidities. Renal failure anemia appears early on in the progression of kidney disease [1]. There is a link between Hb levels and renal function, according to numerous research [2]. Anemia develops early in the course of renal disease and worsens as kidney function declines, a typical consequence of CKD is caused by a disruption in erythropoietin production as well as caused by iron and vitamin shortages, blood loss, shortened erythrocyte life span, chronic inflammation, and the uremic environment [3]. Hb concentrations have been observed to be reduced as a result of malnutrition and iron, folate, and vitamin B12 deficits [4]. Other's factor includes platelet dysfunction, which increases the risk of gastrointestinal bleeding, a shorter erythrocyte survival time (30–60% of the typical 120 days), and hemolysis due to uremic toxin buildup. Chronic blood loss from repeated phlebotomy for laboratory studies and blood loss in the dialysis tubing and dialyzer after each hemodialysis treatment may further contribute to falling Hb values in dialysis patients, particularly those on hemodialysis, so we should use all the diagnostic tools to a diagnosed different type of anemia to treat it perfectly [5]. Hence, studying RBC morphology plays important role in understanding and diagnosing the different type of anemia in patients with renal diseases.

Patients and Methods

During the period of 8 months from January to August 2021, a total of 40 patients who Attended Al-Shams medical laboratory in IRAQ-Diayla were included in this study, 32 males (80%) and eight females (20%), the age range was 40−70 for male and 48−68 for female. Depending on clinical signs, symptoms, and laboratory diagnostic tests, all those patients are diagnosed previously as a case of chronic renal failure by clinicians in our city and develop anemia during the course of the disease, so clinicians referred patients to our laboratory to find the cause of anemia. Hence, all the laboratory test which include

complete blood pictures, blood film, reticulocytes count, erythrocytes sedimentation rate, serum iron, total iron-binding capacity, serum ferritin, serum B12, and folate assessment were done an attempt to identify of the type of anemia in this cases. Written consent take from all the patients for sharing them in this study. Moreover, the following patient excluded from our study, patients on erythropoietin therapy, patients with tonics therapy, patients with repeated blood transfusion, and patients with positive inflammatory markers as all of these factors could affect our results.

After good cleaning of the patient's venipuncture site, 5.0 ml of venous blood was collected using standard collecting techniques and the collected blood sample proceeded as follows:

1. A 2.5 ml of venous blood is added into a disposable tube containing EDTA K2 anticoagulant, which follows by gentile repeated inversion of tubes several times to allow good mixing of blood with anticoagulant and it is used to perform complete blood pictures, blood film, and reticulocytes count using fully automated hematology auto-analyzer Sysmex XN-350.

2. Another 2.5 ml of venous blood was added to the gel tube and was immediately centrifuged at 4000 r.p.m for 15 min to obtain serum which was used to assess serum iron, total iron-binding capacity, serum ferritin, serum B12, and folate assessment using Cobas C311 and Vida's instrument.

Data analysis was done by using SPSS 20 Software, and expressed as the mean ± standard error. ANOVA was done to evaluate the differences in these variables. $p \leq 0.05$ was measured to indicate a statistically significant difference.

Results

The results obtained from this study were based on analysis of 40 patient's complain of chronic renal failures and develop anemia during the course of disease. The frequency of distribution of sex and age in those patients is shown in Table 1. There were 32 males (80%) and eight females (20 %), the age range was 40−70 for male and 48−68 for female. Majority of patients included in this study between 60 and 70 years of age.

Table 1: Characterization of the patients

Sex	Number (%)	Age range (years)
Male	32 (80)	40–70
Female	8 (20)	48–68

Depending's on hematological laboratory data, peripheral blood smear examination from these 40 patients, which showed different types of anemia

is shown below in Table 2, the most common types of anemia were normochromic anemia (62.5%) which develops in 25 patients followed by hypochromic microcytic anemia (8 %) which develops in eight patients, and followed by macrocytic anemia (12.5%) which develops in five patients and the less common type of anemia was hemolytic anemia (5%) which was founded in two patients. The determination of types of anemia in those patients depending on the results of hematological test which done for all the patients.

Table 2: The percentage of different types of anemia in renal disease

Types of anemia	Number total (40) (%)
Normochromic	25 (62.5)
Hypochromic	8 (20)
Macrocytic	5 (12.5)
Hemolytic	2 (5)

The result of different hematological parameters, which includes hemoglobin, white blood cells, platelets, and reticulocytes count among different types of anemia are shown in Table 3 show the following. Regarding the hemoglobin level, the mean hemoglobin level tends to be low in hemolytic anemia (4.15) than in the other three types of anemia (i.e., normochromic, hypochromic, and macrocytic) which was 10.05, 9.28, and 6.84, respectively, and the differences were statistically significant. The mean of white blood cells tends to be higher in hemolytic anemia (14.95) than in the other three types of anemia (i.e., normochromic, hypochromic, and macrocytic) which was 10.42, 9.97, and 3.26, respectively, and the differences were statistically significant. The mean platelet count tends to be higher in hemolytic anemia (519.50) than in the other three types of anemia (i.e., hypochromic normochromic and macrocytic) which was 386.25, 3.62.00, and 68.80, respectively, and the differences were statistically significant. The mean reticulocyte count tends to be higher in hemolytic anemia (5.95) than in the other three types of anemia (i.e., normochromic, hypochromic, and macrocytic) which was (1.48, 1.87, and 0.66), respectively, and the differences were statistically significant.

Table 3: Hematological parameters among different types of anemia

parameters	Types of anemia	Mean ± SE	Significant
Hb	Normochromic	10.05 ± 0.18	Hemolytic versus normochromic <0.000
	Hypochromic	9.28 ± 0.21	Hemolytic versus hypochromic <0.000
	Macrocytic	6.84 ± 0.45	Hemolytic versus macrocytic 0.001
	Hemolytic	4.15 ± 0.15	
RETC	Normochromic	1.48 ± 0.09	Hemolytic versus normochromic <0.000
	Hypochromic	1.87 ± 0.92	Hemolytic versus hypochromic <0.000
	Macrocytic	0.66 ± 0.11	Hemolytic versus macrocytic <0.000
	Hemolytic	5.95 ± 0.55	
WBC	Normochromic	10.42 ± 0.72	Hemolytic versus normochromic 0.052
	Hypochromic	8.97 ± 0.59	Hemolytic versus hypochromic 0.019
	Macrocytic	3.26 ± 0.26	Hemolytic versus macrocytic <0.000
	Hemolytic	14.95 ± 1.35	
PLT	Normochromic	362.00 ± 23.25	Hemolytic versus normochromic 0.042
	Hypochromic	361.25 ± 22.44	Hemolytic versus hypochromic 0.041
	Macrocytic	68.80 ± 12.99	Hemolytic versus macrocytic 0.001
	Hemolytic	519.50 ± 9.50	

SE: Standard error, Hb: Hemoglobin, PLT: Platelet, WBC: White blood cell, RETC: Reticulocytes count.

The result of body iron assessment, which includes serum iron, total iron binding capacity, and serum ferritin among different type of anemia, as shown in Table 4, shows the following, regarding the level of

serum iron, the mean of serum iron level tends to be low in hypochromic anemia (5.56) than in other three types of anemia (i.e., macrocytic, normochromic, and hemolytic anemia) which was 23.60, 19.04, and 18.0, respectively, and the differences were statistically significant. The mean of TIBC tended to be high in hypochromic anemia (93.0) when compared with macrocytic, normochromic, and hemolytic anemia which was (65.20, 62.69, and 58.0) respectively and the differences were statistically significant. Regarding the level of serum ferritin, the mean of serum ferritin level tends to be low in hypochromic anemia (117.08) than in other three types of anemia (i.e., macrocytic, normochromic, and hemolytic anemia) which was 491.20, 493.20, and 607.50, respectively, and the differences were statistically significant.

Table 4: Difference in body iron assessment among different types of anemia

Parameters	Types of anemia	Mean ± SE	Significant
Iron	Normochromic	19.04 ± 0.98	Hypochromic versus normochromic <0.000
	Hypochromic	6.56 ± 0.90	Hypochromic versus macrocytic <0.000
	Macrocytic	23.60 ± 1.96	Hypochromic versus hemolytic 0.002
	Hemolytic	18.00 ± 2.00	
TIBC	Normochromic	62.69 ± 2.29	Hypochromic versus normochromic <0.000
	Hypochromic	93.00 ± 7.67	Hypochromic versus macrocytic 0.002
	Macrocytic	65.20 ± 8.44	Hypochromic versus hemolytic 0.005
	Hemolytic	58.00 ± 2.00	
Ferritin	Normochromic	493.20 ± 40.75	Hypochromic versus normochromic <0.000
	Hypochromic	117.08 ± 78.81	Hypochromic versus macrocytic 0.003
	Macrocytic	491.20 ± 80.20	Hypochromic versus hemolytic 0.004
	Hemolytic	607.50 ± 24.50	

TIBC: Total iron-binding capacity, SE: Standard error.

The result of vitamin B12 and folate assessment, as shown in Table 5, shows the following: Regarding the level of serum folate level, the mean of serum folate level tends to be low in macrocytic anemia (3.41) than in other three types of anemia (i.e., hemolytic, normochromic, and hypochromic anemia) which was 5.44, 10.54, and 12.50, respectively, and the differences were statistically significant. Regarding the level of serum B12 level, the mean of serum B12 level tends to be low in macrocytic anemia (71.18) than in other three types of anemia (i.e., hemolytic, hypochromic, and normochromic anemia) which was 101.80, 482.21, and 544.62, respectively, and the differences were statistically significant.

Discussion

Anemia refers to decrease hemoglobin level below normal physiological level take in consideration patient age and sex. Anemia is a frequent complication of many chronic diseases and chronic kidney diseases (CKD) and its often called anemia of renal disease, it is usually associated with a reduced quality of life and increased mortality and morbidity in this patients. It was firstly linked to CKD over 170 years ago by Richard Bright, it is less commonly encountered in early phase of kidney disease and it often get worse as kidney functions deteriorated and more kidney function is lost [6], [7], [8].

Table 5: Folate and B12 assessment among different types of anemia

Parameters	Types of anemia	Mean ± SE	Significant
Folate	Normochromic	10.54 ± 0.50	Macrocytic versus normochromic <0.000
	Hypochromic	12.50 ± 0.51	Macrocytic versus hypochromic <0.000
	Macrocytic	3.41 ± 0.45	Macrocytic versus hemolytic <0.000
	Hemolytic	5.44 ± 0.35	
B12	Normochromic	554.62 ± 1.20	Macrocytic versus normochromic <0.000
	Hypochromic	482.21 ± 1.59	Macrocytic versus hypochromic <0.000
	Macrocytic	71.18 ± 0.73	Macrocytic versus hemolytic <0.000
	Hemolytic	101.80 ± 2.05	

SE: Standard error.

Anemia that develops in renal disease is usually multifactorial. Although progressive reduction of erythropoietin levels plays an essential role, other factors also play a role in anemia development, such as the impaired response of bone marrow to erythropoietin caused by uremic toxins, infection and inflammations, an iron deficiency which may be caused by malnutrition, blood loss during dialysis, repeated blood sampling, from bleeding due to abnormal platelets function and due to decreased iron absorption. Other associated causes of anemia are vitamin B12, folic acid deficiency, ineffective use of iron store due to increased hepcidin level, reduced red cells life span, and possibilities of hemolysis which may accompany some type of acute and Chronic kidney disease [9]. For all the above reasons, morphologically many types of anemia could see in CKD such as normochromic normocytic anemia, hypochromic microcytic anemia, macrocytic anemia, and hemolytic anemia [10].

In the present study, most of patients 62% including in study develop normochromic anemia and this result is agree with result of the previous study which conducted by Shastry (I) which found that more than 94 % of patient suffer from CKD develop normochromic normocytic anemia [11]. This goes with fact that most patient with chronic renal failures have this type of anemia due to impaired red cells production that occurs due to defect in erythropoietin secretion; also, it has been founded that uremic serum may contain some factors that suppress the proliferation of erythroid series in the marrow also aluminum excess in patients on continuous dialysis also inhibits erythropoiesis. And generally, there is a 2 g/dl fall in hemoglobin level for every 10 mmol/l rise in blood urea [12]. In other hand, 20% of patient included in study develop hypochromic microcytic anemia as the second most common types of anemia in patients suffering from renal failures, this result also agrees with result of the previous study which conducted by Shastry I which found that more than 3.4% of patient suffer from CKD develop hypochromic microcytic anemia [13] and this can be explain by facts that iron deficiency could occur in this patient due to many factors which include malnutrition, blood loos during dialysis procedures, and chronic bleeding due to defective platelets function and from repeated blood sampling [12]. Other 12.5% of patient included in the study that develop macrocytic anemia which occurs due to vitamin B12 and folate deficiency, which are lower than results obtain by the

previous study conducted by Rohan G Patil *et al.* which found that about 56% of patients involved in this study, which develop macrocytic anemia due to vitamin B12 deficiency [13], [14], the differences may be due to different in stage of renal failures in patients involved by two study. Macrocytic anemia develops in this patient which can be explain by facts that folate and B12 deficiency could occur in this patient playing additional role for inadequate hematopoiesis in uremic patients and macrocytic anemia also can occur in patients undergoes chronic dialysis without folate replacement therapy, also malnutrition specially in patients who survive for long period, suffering from renal failures, and defects in protein reabsorption in proximal tubules which lead to a loss of biologically active transcobalamin 2 in the urine [10], [13], [14]. Finally, 5.0% of patient in the present study develop hemolytic anemia, and this observation can be explain by variable shorting of red blood cells survival which could occur in this patient as many other systemic disorders, although the etiology tell now is not entirely clear, but many metabolic, mechanical, and morphological abnormalities which detected in examine peripheral blood smear in patients with renal failure including spicules (spurs) and burr cells could play role [9], [10].

Conclusion

All types of anemia can be detected in-patient with different types of renal diseases and it's important to used different type of laboratory test to diagnosed type of anemia to treat it.

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