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Study on hematological morphological and biochemical changes in patients with chronic kidney failure

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Abstract Chronic kidney failure is a worldwide health problem, affecting millions of people, it is a syndrome characterized by progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma, eventually terminating in death when sufficient numbers of nephrons have been damaged. This study was carried out to evaluate the hematological, morphological and biochemical changes in kidney failure patient and compared with normal group. 100 samples were collected from Sebha Medical Centre. Complete blood counts, kidney function (urea and creatinine) were measured. The results of this study indicate that there were significantly decreased values of Hg, RBC, PCV, RDW (p=0.0001) in chronic kidney failure patient when compared with normal group, On the other hand there no significantly different in the values of MCV, MCH, MCHC, WBC, Plt were observed in kidney failure patient when compared to normal group. In addition, kidney failure group showed significantly increased levels of urea and creatinine p=0.0001 when compared to normal group alone. The morphological changes of erythrocytes were observed under a light microscope. Kidney failure group showed morphological changes in the form of echinocytes, Target cell, Poikilocytosis, elliptocytes. In conclusion, based on the above finding these results indicate that the complications of chronic kidney failure may lead to hematological, morphological and biochemical changes of erythrocyte.

Keywords: Chronic kidney disease, Complete blood count, Erythrocyte morphology, Hematological changes, Kidney failure.

دراسة التغيرات في المقاييس الدموية و البيوكيميائية لدي مرضي الفشل الكلوي

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الملخص الفشل الكلوي المزمن هو مشكلة صحية في جميع أنحاء العالم، مما يؤثر على الملايين من الناس ويعد متلازمة تتميز بهبوط تدريجي وغير عكسي لتدهور وظائف الكلى والذي يؤدي في النهاية الي الفشل الكلوي في المرحلة النهائية تنتهي في نهاية المطاف الي الموت عند تضرر أعداد كافية من النيفرون. أجريت هذه الدراسة لتحديد التغيرات في المقاييس الدموية و وظائف الكلى لدى مرضى الفشل الكلوي ومقارنتها بالاشخاص الطبيعيين . تم تجميع عدد 100 عينة من مختبر مركز سبها الطبي، تم إجراء التحاليل التالية، اختبار عد الدم الكامل، الصورة الدموية، قياس مستوي الكرياتينين،قياس مستوي اليوريا، أظهرت نتائج هذه الدراسة انخفاض معنوي ملحوظ في قيم كلا من Hg, RBC, PCV, RDW لدي مرضي الفشل الكلوي مع وجود فروق معنوية P=0.0001 بالمقارنة مع الأشخاص الطبيعيين، بينما لم تكن هناك اي فروق معنوية تذكر في قيم كلا من MCV, MCH, MCHC, WBC, Plt بين مجموعة الأشخاص الطبيعيين و مجموعة مرضي الفشل الكلوي. كذلك أظهرت مجموعة الفشل الكلوي وجود ارتفاع معنوي إحصائي ملحوظ في مستويات كلا من اليوريا والكرياتينين، (P=0.0001) بالمقارنة مع الأشخاص الطبيعيين. بالأضافة الي ذلك بينت الصورة الدموية لمجموعة مرضي الفشل الكلوي وجود أشكال غير طبيعية لكريات الدم الحمراء فقد لوحظ وجود كريات دم حمراء مشوكة echinocytes ، كريات دم حمراء متباينة في الشكل Poikilocytosis، وأن أغلب الكريات المتباينة في الشكل كانت الخلايا الهدفية، (Target cell) والخلايا الأهليجية (elliptocytes). في الخلاصة، وطبقا لنتائج هذه الدراسة فأنها تشير إلى أن المضاعفات المرتبطة بالفشل الكلوي تؤدي الي حدوث تغيرات غير طبيعية في المقاييس الدموية والبيوكيميائية بالأضافة الي حدوث تغيرات مورفولوجية في اشكال الخلايا الدموية.

الكلمات المفتاحية: أمراض الكلي المزمنة، عد الدم الكامل، أشكال كريات الدم الحمراء، التغيرات في المقاييس الدموية، الفشل الكلوي.

Introduction

Normal renal function is very important for homeostasis, so much so, that situations in which renal functions are impaired can be life threatening. Kidney diseases are among the most important causes of death and disability in many countries throughout the world [1]. Chronic kidney disease (CKD) is a worldwide public health problem. Chronic kidney failure is defined by the National Kidney Foundation (NKF) as either damage or a glomerular filtration rate less than 60ml/minute/1.73m² of body surface area for more than 3 months [2]. Previous studies have

shown that hematological alterations are a common finding in patients with kidney disease. Hematological abnormalities in CKD are Anemia, Leukocytopenia, Bleeding diathesis, Hypocellular bone marrow, Shortened red blood cell lifespan, and Splenomegaly/ hypersplenism [3].

Anemia was first linked to CKD over 170 years ago by Richard Bright.[4] As kidney disease progresses, anemia increases in prevalence, affecting nearly all patients with stage 5 CKD [4] Anemia in CKD is associated with reduced quality of life and increased cardiovascular disease, hospitalizations, cognitive impairment, and mortality [5].

The primary cause of anemia in patients with chronic kidney failure is insufficient production of erythropoietin by the diseased kidneys [6]. Other factors include iron, folate and vitamin B12 deficiency due to nutritional insufficiency or increased blood loss [7], acute and chronic inflammation with impaired iron utilization, severe hyperparathyroidism with consequent bone marrow fibrosis and shortened red cell survival in the uremic environment [8], [9].

Etiopathogenesis of kidney disease anemia is multifactorial. One of the reasons is shortened life of red blood cells (RBCs) bound directly and indirectly to the shape, which is a consequence of cell's reological possibility, metabolic processes and environment influence. Physiological RBC is biconcave, regular shaped discs-discocythes. The electron microscopy analysis of RBCs revealed the loss of their typical biconcave shapes in chronic kidney disease (CKD) [10]. Clinical studies on the Sebha people are limited and generally restricted to analysis of classical markers due to Libya's modern political instability, thus this study was conducted to evaluate the hematological, morphological and biochemical changes in chronic kidney failure patient and compared with normal group.

MATERIALES and METHDOS

This cross-sectional study was performed over a period of three months (March to June 2016), samples were collected from Sebha medical center. A total of 100 samples which include 50 patients (36 males, 14 females) and 50 healthy individuals (18 males, 32 females) from 20-70 years of age were studied. Freshly collected blood samples were used to measure complete blood count (CBC), for urea and creatinine the blood samples were centrifuged at 4000 rpm for 10 minutes, then the plasma samples stored at freeze until assayed

1. Hematological study

Hematological parameters (complete blood count) like Total Red Blood Cell count (RBC), Haemoglobin concentration (Hb), Packed cell volume (PCV), Mean cell volume (MCV), Mean cell hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Red blood cells distribution width (RDW), Total White Blood Cells (WBC) and Platelet count (Plt) were measured on the same day by using SYSMEX Bc 3600 Germany hematology automatic analyzer.

2. Measurement of plasma creatinine

Plasma creatinine level was estimated by Creatinine Kinetic test (BIOMAGHREB Cat No 20151). The reading for samples and standard were determined using a spectrophotometer (shemadzu UV-160 A) at 492 nm against blank without samples, level of creatinine were calculated as mg/dl.

3. Measurement of plasma Urea

Plasma urea level was measured by UREA Enzymatic Colorimetric method (BIOMAGHREB Cat No 20141). The reading for samples and standard were determined using a spectrophotometer (shemadzu UV-160 A) at 590 nm against blank without samples, level of urea were calculated as mg/dl

Statistical Analysis:

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) IBM version 20.0. All results were expressed as mean ±standard deviation (SD) in the tables and mean ± standard error (SEM) in the graphs. Shapiro- Wilk test was used to check the normality of the variable. Accordingly t-test was used to analyze data follow normal behaviour of distribution pattern. The difference between groups was considered significant when $p< 0.05$.

RESULTS

In the current *in vivo* cross-sectional study, we investigated the hematological, morphological and biochemical changes in kidney failure patient.

1. Hematological parameters

Table (1) shows the complete blood count of chronic kidney failure patient and normal group. The kidney failure patient group had a significantly decreased of Hb concentration, RBC, PCV, and RDW compared to the control group, and p values were p= 0.0001. On the other hand, there were no significant differences in WBCs, Plt, MCV, MCH, and MCHC values between the chronic kidney failure patient and control groups.

Hematological parameters	Normal group	Chronic Kidney failure group
RBC $\times 10^{12}$L	4.4± 0.5	3.1± 0.9 ***
Hb (g/L)	13±2.1	9.6± 2.0 ***
PCV (%)	41.4± 4.1	27.9±6.6 ***
MCV (fl)	87.6.2± 5.5	88.0± 9.2
MCH (Pg)	30.1± 2.2	31.4±2.9
MCHC (g/dl)	33.5±3.0	34.5± 2.6
RDW (fl)	14.0±1.5	6.9± 1.2 ***
WBC× 10^9 L^{-1})	6.8± 2.0	7.0± 2.3
PLT $\times 10^9$ L^{-1}	241.9±48	219.8±101

*** Significantly different as compared to the control group. Results expressed as means ±SD ($P<0.05$).

1. Plasma creatinine and urea levels

Plasma creatinine level was significantly higher in kidney failure patient group compared to the normal group (9.44 ± 0.54 *vs* 0.67 ± 0.01 mg\dl) $p < 0.0001$. In addition, the findings of the present study showed that the levels of plasma urea was significantly higher in kidney failure patient group compared to the normal group (149.42 ± 7.2 *vs* ± 22.82 ± 1.5 mg\dl) $p < 0.0001$ Fig.1 and 2).

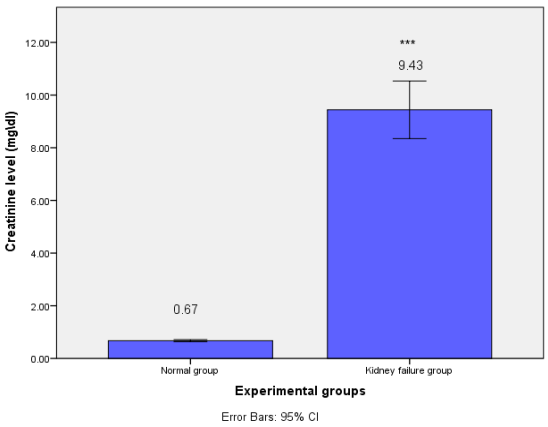

Fig. 1: Plasma creatinine level from normal and kidney failure patient group.

Results are expressed as the mean ± S.E.M.

(*******) significantly different from the normal group ($p < 0.0001$)

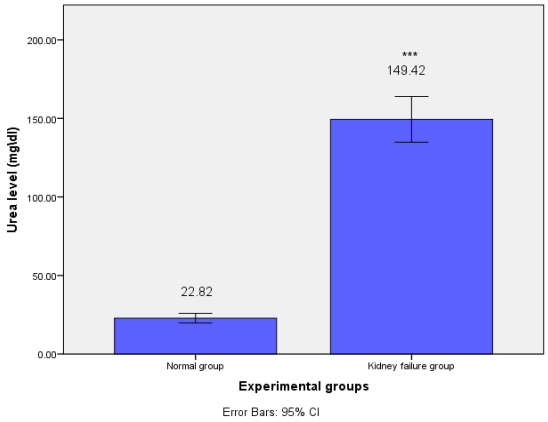

Fig. 2: Plasma urea level from normal and kidney failure patient group.

Results are expressed as the mean ± S.E.M.

(*******) significantly different from the normal group ($p < 0.0001$)

2. Erythrocyte morphology

The morphological changes of erythrocytes were observed under a light microscope. The kidney failure patient group showed morphological changes in the form of echinocytes. The results of present study showed a higher percentage of echinocytes among the kidney failure patient group compared to the control group (Fig. 3). In addition, the finding of current study showed that there were abnormal shapes of erythrocyte in kidney failure patient group as most of erythrocyte displayed morphological alteration which were in the form of Burr cell (echinocytes), target cell, acanthocyte, Poikilocytosis (erythrocyte show variation in shape), elliptocytos is (the affected cells appear oval shape) (Fig. 4 and 5).

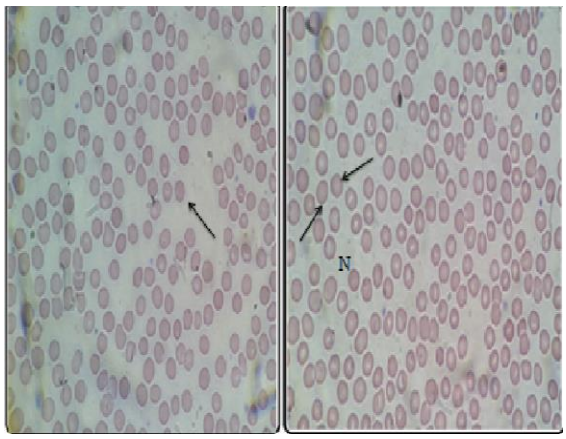

Fig. 3: The morphology of erythrocyte as observed using light microscopy (100X immersion lens).Images of the erythrocyte from the Normal group. Normal erythrocyte show little variation in cell size appearing as 7 μm discs with central pallor occupying the inner one-third to one-half of the cell diameter surrounded by a rim of pink staining hemoglobin. N: Normal erythrocyte

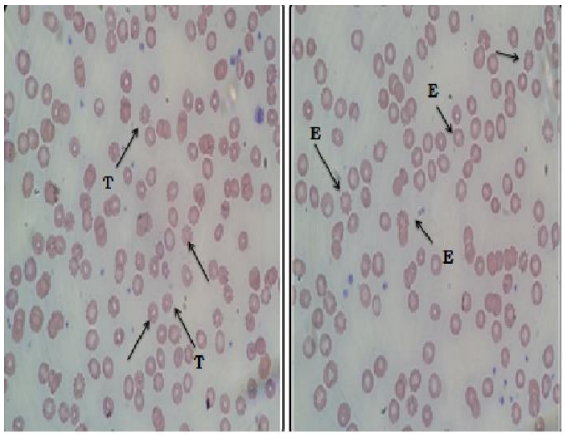

Fig. 4: The morphology of erythrocyte as observed using light microscopy (100X immersion lens). Images of the erythrocyte from the kidney failure group. Burr cell (echinocytes) (E), target cell (T)

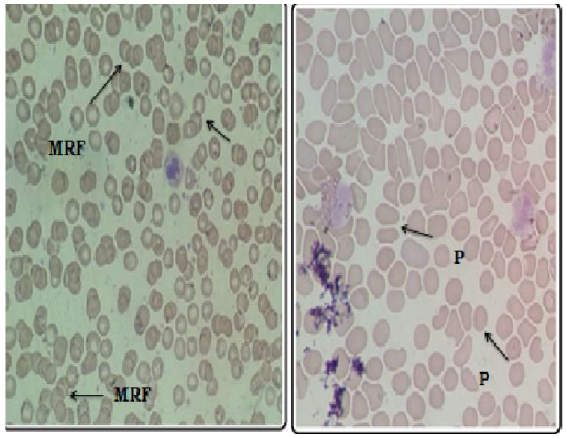

Fig. 5: The morphology of erythrocyte as observed using light microscopy (100X immersion lens) Images of the erythrocyte from

the kidney failure patient group. Moderate rouleaux formation (MRF) Poikilocytosis (P)

DISSCUTION

Previous studies have shown that chronic kidney disease causes alteration in various biochemical and hematological Parameters [3], [11]. Several studies have shown an increased level of creatinine and urea in clinical and experimental kidney disease. Chronic kidney failure is a gradual, progressive and irreversible loss of normal functioning of kidneys. As the excretory function of kidney is impaired, urea and creatinine excretion is hampered leading to its increased levels in blood, high significant elevation in blood urea and creatinine levels are observed in chronic kidney failure group. These results are compatibles with the previous studies [12-14], which explained that the continued decreased in renal clearance or glomerular filtration rate, leads to the gathering of urea, creatinine and other chemicals in the blood and chronic kidney failure which applies to the process of containing significant irreversible reduction in the nephron reduced after hemodialysis. In chronic kidney failure, the increase of blood urea is proportional to the progression of the disease, but it is highly influenced by catabolic state or excessive protein ingestion, leading to a higher production of other waste substances of protein catabolism [15].

Anemia is a major consequence of CKD that develops early in the course of illness and affects most patients who exhibit some degree of reduced renal function. Pooled study data consistently show that lower hemoglobin levels are associated with lower levels of GFR , that anemia can be seen even at GFR levels as high as 60 ml\min, and that severity of anemia in CKD correlates with duration and extent of renal disease [16].

The hematological parameters in 50 chronic kidney failure patients compared with 50 controls were investigated. In our study, it has been observed that the RBC count is decreased in chronic kidney failures ($P<0.0001$, highly significant). These findings agree with other research conducted by Suresh et al. 2011 [11].

Previous study demonstrated that the Primary cause of decrease RBC count in chronic kidney failure is impaired erythropoietin production and other factors which suppress marrow erythropoiesis and shortened red cell survival. Erythropoietin is the hormone which is the major humoral regulator of red cell production and helps to maintain the viability of RBC by retarding the cleavage of DNA that occurs normally in Colony Forming Unit Erythrocytes (CFU-Es). In the absence of EPO, DNA cleavage is rapid and leads to cell death [12].

Several studies have shown that RBC survival is decreased in uremic patient's in proportion to the blood urea nitrogen concentration and, it improves significantly after intensive hemodialysis. Uremic plasma increases the expression of phosphatidylserine on the outer cell surface in red blood cells. This enhances the recognition of damaged red blood cells by macrophage, leading to their subsequent destruction and decreased survival [17].

The hemolytic factor implicated in decreased red blood cells survival is presumed to be a toxic substance normally excreted or metabolized by the kidneys, one such substance is guanidine and its derivatives which appear to be a subset of the many retained metabolites, adversely affect erythrocyte survival [18].

The hemoglobin concentration and hematocrit are decreased (Table-1) in chronic kidney failure patients

($P<0.0001$ highly significant) These results are in agreement with a previous study [19], Conducted by Arun et al 2012. The hemoglobin concentration and hematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced. In chronic kidney disease because of impaired erythropoietin secretion, increased destruction of RBC, leads to a fall in red blood cell count, which reduces the hemoglobin concentration and hematocrit. A decrease in hematocrit is apparent even among patients with mild to moderate renal insufficiency [20].

The morphological changes in erythrocytes during the course of the disease reflect the effect of the altered environment, which is measured from the microscopic images by vibration in shape descriptors and application of wavelet transforms [21]. The normal mammalian RBC is a flexible biconcave disk. The present study shows that chronic kidney disease induced a morphological change in the erythrocytes from the normal discoid shape to an echinocytic form. The chronic kidney failure group had a lower percentage of discocytes and a significant increase in the altered erythrocyte forms (echinocytes) compared to the normal group. Other morphological change observed in the red series was represented by poikilocytosis, Anisocytosis. (Poikilocytosis is a specific phenomenon, but occurs in many anemia, especially in severe anemia. Anisocytosis was the generic term used to describe an abnormally wide distribution of the sizes of the erythrocytes in the blood, such as macrocytes, which were larger than mature normal red blood cells, or microcyte (the presence of smaller red blood cells such as microcytes).

Conclusion

In conclusion, the findings of this study may conclude that patients with chronic kidney failure show various hematological abnormalities of which anemia being the commonest. It has been proposed that in chronic kidney failure, impaired production of erythropoietin is the main reason for the decrease in red blood cell count, hemoglobin concentration, hematocrit, even though the distribution of the creatinine and urea values is significantly wider compared to the control group. Furthermore the results also show that the morphological changes of the red blood cells are highly present in the chronic kidney failure patients compared to control; the patients were generally characterized by, echinocytes, target cell, anisocytosis and poikilocytosis, but the frequency of these modifications was reduced in the control group. These changes may have a

direct impact on erythrocyte function and may contribute to the patient complex pathology. A limitation of our study is represented by the relatively small number of patients enrolled. Future clinical and experimental studies should explore potential causal mechanisms linking hematological alterations and chronic kidney disease

Abbreviations

CKD: Chronic kidney disease; NKF: National Kidney Foundation; GFR: glomerular filtration rate; CBC: complete blood count; RBC: red blood cells; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; RDW: Red blood cells distribution width; WBC: White Blood Cells; Plt: Platelet count; SPSS: Statistical Package for Social Sciences; CFU-Es: Colony Forming Unit Erythrocytes.

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