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Original article

Assessment of Hematological Indices and Renal Function Parameters in Some Libyans with Chronic Kidney Disease

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Abstract
Chronic kidney disease (CKD) is a significant public health problem and is commonly associated with anemia. The prevalence of anemia increases as the estimated glomerular filtration rate (eGFR) decreases. This results from a complex interaction involving kidney impairment, erythropoietin deficiency, and an inflammatory state. The neutrophil/lymphocyte ratio (NLR) has been reported to reflect systemic inflammation in many diseases. The study aimed to assess hematological indices and kidney function parameters in CKD patients and to determine whether NLR can be used as a prognostic marker for disease progression. This study included 100 participants, including patients and healthy individuals aged 20-75 years, 30 of them are healthy controls, and 70 are CKD patients at stages 2-5. Blood samples from all participants were drawn to evaluate hematologic and kidney function parameters. An independent t-test was used for two-group comparisons, ANOVA for multiple comparisons, and Pearson's correlation coefficient for variable relationships. Progressive kidney dysfunction was observed, with decreasing eGFR and significantly elevated urea and creatinine as the CKD stage advanced. Additionally, anemia markers (HG, RBC, HCT) and lymphocytes were significantly decreased across CKD stages. However, WBC, neutrophils, and NLR were significantly increased in advanced stages compared with earlier stages and healthy controls. Furthermore, moderate negative correlations were found between HG, RBC, and HCT with both serum urea and creatinine, and positive associations with eGFR. This study also demonstrated a highly positive relationship between NLR and both urea and creatinine, and a significant inverse correlation with eGFR. Our findings show that HG and RBC are markedly decreased as disease severity progresses. At the same time, NLR is significantly higher in advanced stages, suggesting that NLR could be used as a marker for predicting the inflammatory state of CKD patients and checking disease progression.
Keywords. CKD, Anemia, Inflammation, NLR.

Introduction

Chronic kidney disease (CKD) has become a significant public health issue, with an estimated prevalence of approximately 9-10% of the worldwide population [1]. The increasing incidence of CKD is largely attributed to the rising prevalence of chronic diseases such as diabetes mellitus, hypertension, and obesity. These factors are the primary causes of kidney impairment in developed and developing countries [2, 3]. It has been suggested that diabetes mellitus is the main cause of CKD, followed by glomerulonephritis and hypertension [2]. On the other hand, other studies reported that hypertension is the most common cause of CKD [4, 5], followed by diabetes mellitus [5].

Anemia is one of the most common complications of CKD, with its incidence increasing progressively as the glomerular filtration rate declines [6]. One study reported that nearly 15.4 % of patients with CKD had anemia, compared to 7.6% in the general population. The prevalence of anemia was observed to be 8.4% in patients with CKD at stage 1 and 53.4% in those at stage 5 [7]. The pathophysiology of anemia in CKD involves a combination of factors, including deficiency of Erythropoietin (EPO) due to decreased renal production, reduced life span of red blood cells (RBC), dysfunction of bone marrow, chronic inflammation, and iron dysregulation [7-9]. CKD disrupts several biochemical markers necessary to maintain physiological balance, in addition to the abnormalities in hematological indices. Previous studies demonstrated substantial elevations in both serum urea and creatinine levels with concomitant alterations in electrolyte balance [3]. Additionally, a study conducted by Al-Ani and Al-Lami [10]. revealed a significant increase in the levels of creatinine and urea and a considerable decrease in eGFR

Chronic inflammation is responsible for the development of CKD. CKD patients frequently exhibit elevated levels of inflammatory parameters, which can contribute to fibrosis in the kidneys and progression of CKD, as well as increasing the risk of cardiovascular complications [11]. The neutrophil to lymphocyte ratio (NLR) has been identified as a prognostic indicator in CKD patients. A study by Kim et al [11] found that predialysis patients with high NLR had more advanced stages and lower eGFR values. In addition, another study emphasized that NLR is significantly increased with the progression of CKD stages [12]. Assessing hematological and biochemical parameters in CKD patients is essential for effective disease management and prognosis. Najat et al. [9] underscored that anemia in CKD is associated with poor outcomes and must be managed, as it adversely influences quality of life and increases the risk of cardiovascular disease and mortality. This study aimed to assess hematologic and kidney function parameters in some Libyan patients with CKD, and to determine whether NLR can be used as a prognostic marker in the advancement of the disease.

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Methods

Study population

This cross-sectional study was conducted on male and female patients diagnosed with CKD more than three months prior, as well as healthy individuals. The population of this study consists of 100 participants, including patients and healthy individuals aged 20-75 years, of whom 30 are healthy subjects. The patient group was composed of 70 individuals who attended the nephrology outpatient clinic of the Tripoli hospitals. The patients were divided into 4 groups according to the classification of the National Kidney Foundation 5, that consider eGFR< 60 ml/min/1.73m2 as decreased eGFR. eGFR was calculated using the Cockroft and Gault equation. Patients who are undergoing treatment with anti-inflammatory drugs, and patients with acute kidney injury, malignancy, on dialysis, or who have had kidney transplantation were excluded.

Questionnaire

The data for each case study were collected by using a special data collection form, a "questionnaire" to collect general information (Sex, age, height, weight), and medical history.

Blood sample collection and processing

A sample of 5 ml venous blood was drawn using sterile disposable plastic syringes for healthy individuals and patients. 2 ml of blood from each sample was transferred to special tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) to measure the hematology parameters. The other 3 ml of blood were placed in special tubes free of any anticoagulant and left at laboratory temperature for 30 minutes. After blood clotting, a centrifugation process was performed for 10 minutes at a speed of 3000 rpm to obtain blood serum, which is utilized for assessing biochemical markers.

Hematological studies

Hemoglobin, WBCs, RBCs, hematocrit (HCT), Neutrophils, and Lymphocytes were measured by Sysmex Solutions from China using a hematology analyzer (Sysmex KX.21N).

Biochemical parameters

Serum creatinine and urea levels were measured using a spectrophotometry-based automated routine chemistry analyzer (COBAS INTEGRA 400 plus, Germany) and were reported in mg/dL.

Statistical analysis

Results were expressed as means ± standard deviations, and data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23 for Windows. The Kolmogorov-Smirnov test was performed to assess normality. All variables in this study were normally distributed; therefore, parametric tests were used. A one-way analysis of variance (ANOVA) was applied to compare all parameters across multiple groups and identify significant differences. The independent t-test was used to compare two groups. Pearson's correlation coefficient (r) was used to evaluate the relationship between variables. Statistical significance was set at a p-value < 0.05.

Results

Demographic data and clinical characteristics of the study population

The study included 100 participants, consisting of 70 patients and 30 healthy individuals. Among the patients were 40 males (57.1 %) and 30 females (42.9 %). The healthy group comprised 10 males (33.3 %) and 20 females (66.7%). Demographic data and clinical characteristics of the control and patient groups are presented in (Table 1). In terms of age, patients were considerably older than the healthy individuals (P = 0.002). Additionally, the mean values for both weight and BMI were significantly higher in the patient group compared to the control group (P = 0.000 and P = 0.003, respectively). Our data showed that diabetes was present in 3 patients (4.29 %), hypertension in 8 patients (11.43 %), and 10 (14.29 %) patients had both diabetes and hypertension. Also, it was found that the cause of CKD was unknown for 70 % (49) of the patients. The patients were categorized into four groups (S2-S5) based on their eGFR (ml/min/1.73 m^2). Group 1 (S2) with an eGFR of 60-89, Group 2 (S3) with an eGFR of 30-59, Group 3 (S4) with an eGFR of 15-29, and Group 4 (S5) with an eGFR of less than 15 ml/min/1.73m^2. Statistical analysis revealed significant differences in weight (P = 0.000), BMI (P = 0.000), and period of disease (P = 0.008) among patients at different stages. However, there was no significant difference in sex and age between the stages (P = 0.740) and (P = 0.119), respectively, as illustrated in (Table 2).

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Table 1. Demographic data of healthy individuals and patients

Variables	Healthy Controls (n=30)	Patients (n=70)	P-value
Age (years)	45.30 ± 17.67	56.39 ± 14.58	0.002
Weight (kg)	69.43 ± 8.51	82.56 ± 17.87	<0.001
BMI (kg/m²)	25.10 ± 2.74	28.58 ± 5.91	0.003
Sex			
– Female	20 (66.7%)	30 (42.9%)	
– Male	10 (33.3%)	40 (57.1%)	
Diabetes, n (%)	0	3 (4.3%)	
Hypertension, n (%)	0	8 (11.4%)	
Diabetes + Hypertension, n (%)	0	10 (14.3%)	
Unknown, n (%)	0	49 (70.0%)	
Kidney disease, n (%)	0	70 (100%)	
Family history of:			
– Diabetes, n (%)	10 (33.3%)	16 (22.9%)	
– Hypertension, n (%)	5 (16.7%)	9 (12.9%)	
– CKD, n (%)	0	14 (20.0%)	

Data are presented as mean ± SD, and number (%) as appropriate. Abbreviations: CKD: chronic kidney disease

Table 2. Demographic data of patients with different stages of CKD

Variables	S2 (eGFR 60-89 ml/min/1.73m²)	S3(eGFR 30-59 ml/min/1.73m²)	S4 (eGFR 15-29 ml/min/1.73m²)	S5 (< 15 ml/min/1.73m²)	P-Value
Male Female	66.7% 33.3%	54.5% 45.5%	55.6% 44.4%	71.4% 28.6%	0.740
Age (years)	55.33 ±4.509	57.24±12.558	56.07 ±14.955	54 ±7.937	0.119
Weight (kg)	100 ±4.33	85.67 ±14.997	80.78 ±14.635	63 ±5.385	0.000
BMI (kg/m²)	32.33 ±4.043	29.068 ±4.767	28.4 ±4.977	22.44 ±2.011	0.000
Period of CKD (months)	7 ±4.583	19.7 ±22.015	38.26 ±43.155	60 ±24	0.008

Data are presented as mean ± SD, and number (%) as appropriate. Abbreviations: CKD: chronic kidney disease.

Comparison of hematologic and renal parameters between patients and healthy groups

Our findings demonstrated a highly significant decrease in HG (P=0.000), RBC (P=0.000), HCT (P=0.000), and Lymphocytes (P=0.001) in the CKD group compared to the healthy group. In addition, the mean values of WBC, Neutrophils, and NLR increased significantly (P=0.013), (P=0.002), and (P=0.000), respectively, in patients compared to healthy individuals (Table 3). However, there was no substantial difference (P=0.750) in platelet count between the two study groups. Furthermore, the results showed a highly significant difference between the two study groups for kidney function parameters, as shown in (Table 3). The average eGFR values for CKD patients were notably lower than those of the healthy group (P = 0.000). Additionally, the mean values of creatinine and urea assessment were statistically significantly higher (P=0.000) in the CKD patients compared to healthy individuals.

Table 3. Comparison of hematological and kidney function parameters between healthy and CKD patients

Parameters	Healthy group	Patients	P-value
HG (g/dl)	12.24±1.214	11.204±1.682	0.000
RBCs (10^6/ µl)	4.624±0.433	3.849±0.7354	0.000
HCT (%)	39.036±3.388	33.54±5.567	0.000
WBCs (10^3/ µl)	6.848±1.791	7.195±1.701	0.013
Neutrophils (10^3/ µl)	3.34±1.016	4.33±1.39	0.002
Lymphocytes (10^3/ µl)	2.38±0.603	1.6±0.53	0.001
NLR	1.426±0.385	3.098±1.651	0.000
Platelets	230.93±67.897	236.04±75.449	0.750
eGFR (ml/min/1.73m²)	116.294±25.697	32.347±15.573	0.000
Creatinine (mg/dl)	0.7942±0.1659	3.190 ± 1.859	0.000
Urea (mg/dl)	22.645±4.923	93.743±44.229	0.000

Data are presented as mean ± SD. Abbreviations: HG: Hemoglobin; RBCs: Red Blood Cells; WBCs: White Blood Cells; HCT: Hematocrit; NLR: Neutrophils- Lymphocytes ratio; eGFR: estimated Glomerular Filtration Rate.

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Comparison of blood and kidney parameters between different stages of CKD

Hematological parameters

Our findings regarding hematological parameters indicated that the levels of HG, RBC, and HCT were significantly decreased as the severity of CKD progressed in patients (P=0.002), (P=0.001), (P=0.01), respectively. Similarly, the mean values of lymphocytes show a stepwise decrease from stage 2 to stage 5, and it was highly statistically significant between groups (P=0.008). However, regarding neutrophil count and NLR, the lowest mean was found in stage 2, and then the mean values tend to increase toward later stages. This increase is particularly noticeable in stages 4 and 5, and it was significant (P=0.006) and (P=0.001), respectively, as illustrated in (Table 4). Moreover, the WBC count (10^3/µl) was significantly higher at stage 5 than in the earlier stages, P = 0.006. However, the results indicated no significant difference in the mean levels of platelets among the different CKD stages, with P = 0.221, as shown in (Table 4).

Renal function parameters

The average eGFR (ml/min/1.73m^2) declined progressively with increasing severity of kidney disease; it was highest in stage 2 when compared to stages 3, 4, and 5 (P = 0.000) (Table 4). On the other hand, mean creatinine (mg/dl) and urea levels (mg/dl) were lower in stage 2 compared to those in stages 3, 4, and 5, with a P-value of 0.000 as presented in (Table 4).

Table 4. Comparison of blood indices and kidney parameters between different stages of CKD

Parameters	Stage 2	Stage 3	Stage 4	Stage 5	P-value
HG (g/dl)	11.667±1.193	11.933±1.658	10.596±1.425	9.914±1.398	0.002
RBCs (10^6 /µl)	3.980±0.2536	4.145±0.643	3.684±0.533	3.037±1.174	0.001
HCT (%)	33.223±2.382	35.752±5.919	31.819±4.859	29.886±3.0689	0.01
WBCs (10^3 /µl)	7.6967±1.466	6.4661±1.574	7.7726±1.573	8.1857±1.702	0.006
Neutrophils (10^3 /µl)	3.5±0.173	3.79±1.21	4.69±1.31	5.81±1.29	0.006
Lymphocytes (10^3 /µl)	2.44±0.21	1.66±0.56	1.5±0.399	1.31±0.52	0.008
NLR	1.667±0.249	2.566±1.419	3.434±1.571	4.925±1.701	0.001
Platelets (10^3 /µl)	269.33±21.962	219.091±83.227	245.444±66.717	271.429±75.377	0.221
eGFR (ml/min/1.73m^2)	74.223±2.259	41.9197±9.009	22.098±5.028	10.660±1.825	0.000
Creatinine (mg/dl)	1.463±0.3099	2.085±0.540	3.726±1.407	6.989±1.866	0.000
Urea (mg/dl)	40.400±9.374	72.392±27.243	108.456±32.293	159.081±65.044	0.000

Data are presented as mean ± SD. Abbreviations: HG: Hemoglobin; RBCs: Red Blood Cells; WBCs: White Blood Cells; HCT: Hematocrit; NLR: Neutrophils – Lymphocytes ratio; eGFR: estimated Glomerular Filtration Rate.

Correlation of hematological indices with kidney parameters

The WBC count showed a negative correlation with the eGFR and a positive association with both serum creatinine and urea. Conversely, HG, HCT, and RBC counts were found to be positively correlated with eGFR, and negatively correlated with both serum creatinine and urea, as illustrated in (Table 5). Additionally, the present results found a significant positive correlation between NLR and both creatinine and urea. In contrast, a significant negative correlation was found between NLR and eGFR. On the other hand, platelets show very weak correlations between the three kidney function parameters (urea, creatinine, and eGFR). Likewise, the correlations of kidney parameters with both age and BMI were not statistically significant.

Table 5. Correlation of hematological indices, age, and BMI with kidney parameters

Variables	Pearson correlations (r) and P-value	eGFR (ml/min/1.73m^2)	Creatinine (mg/dl)	Urea (mg/dl)
HG (g/dl)	r P	0.474 0.000	-0.417 0.001	-0.509 0.000
RBCs (10^6 /µl)	r P	0.48 0.000	-0.45 0.000	-0.488 0.000
HCT (%)	r P	0.419 0.001	-0.410 0.001	-0.499 0.000
WBCs (10^3 /µl)	r P	-0.327 0.006	0.455 0.000	0.259 0.012
NLR (10^3 /µl)	r P	-0.522 <0.0001	0.527 <0.0001	0.56 <0.0001
Platelets (10^3 /µl)	r P	0.101 0.32	-0.065 0.52	-0.131 0.19

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Age (years)	r P	0.116 0.25	0.151 0.13	0.014 0.89
BMI (Kg/m²)	r P	-0.053 0.6	0.156 0.12	0.157 0.12

Data are presented as mean ± SD. Abbreviations: HG: Hemoglobin; RBCs: Red Blood Cells; WBCs: White Blood Cells; HCT: Hematocrit; NLR: Neutrophils – Lymphocytes ratio; eGFR: estimated Glomerular Filtration Rate; BMI: body mass index.

Discussion

Kidney disease poses a significant global health issue, primarily due to its late diagnosis, which typically occurs only in advanced stages of the disease. This delay is largely attributed to the absence of clinical symptoms in the early stages [13]. The early detection and treatment of CKD are essential for slowing disease progression and preventing complications [14]. The current study evaluated hematologic and kidney function parameters in Libyan patients with CKD across different disease stages. It showed that the risk of anemia increases as the disease progresses and highlighted the potential use of the NLR for predicting the inflammatory state of CKD patients.

The current study revealed that patients with CKD were older than healthy controls, and they also had higher weight and BMI (Table 1). This suggests an association between these factors and CKD. Significant differences in weight, BMI, and disease duration were observed when the patients were stratified by disease stages, while age and gender showed no significant variation (Table 2). A recent study demonstrated a substantial association between advancing age and the risk of developing CKD [15]. This finding aligns with our results, indicating that older individuals are more likely to have the disease. Our findings regarding body weight align with previous research indicating that overweight and obesity are associated with an increased risk of CKD, regardless of the presence of metabolic syndrome [16]. Reporting that obese individuals faced a greater risk compared to those of normal weight [17]. Research has identified BMI as an independent risk factor for CKD, particularly among individuals without preexisting conditions such as diabetes, hypertension, coronary heart disease, or stroke [18]. Another study found a strong positive relationship between higher BMI and an increased risk of advanced stages of CKD, with the risk progressively rising for individuals with a BMI over 25 kg/m² [19].

In the current study, we found a significant reduction in HG, HCT levels, and RBC count among patients with CKD compared to healthy controls (Table 3). Additionally, these hematological indices exhibited a progressive decline across the different stages of CKD, with the lowest levels observed in the more advanced stages. These findings align with previous studies [20-22], which also reported a decline in these parameters as CKD advances. However, they contradict the findings of Kaze et al. [23], who found no significant association between complete blood count and the stages of CKD. Several factors can cause anemia in patients with CKD. One primary factor is the decreased renal production of erythropoietin, which is crucial for stimulating RBC production. Additionally, the accumulation of uremic toxins can lead to RBC deformity and hemolysis. Other contributing factors may include deficiencies in folate and vitamin B12 [24], inflammation, and iron deficiency [8]. Additionally, our results demonstrated a significant progressive increase in WBC, neutrophil counts, and NLR values as CKD developed from earlier to more advanced stages. These findings are in agreement with the growing body of evidence [25, 26], suggesting that systemic inflammation has a vital role in the progression of CKD. The increase in WBC counts among patients with CKD compared to healthy individuals supports the hypothesis of a chronic low-grade inflammatory state associated with the disease. This observation is consistent with previous studies indicating that WBC levels are significantly higher in CKD patients than in healthy individuals [28, 29]. Shrestha et al. [30] also found that WBC counts increased with the severity of CKD, with the highest levels observed in dialysis patients compared to non-dialysis patients. However, these findings contrast with those of Sarabandi et al.[31], who reported that elevated uremia levels do not affect leukocyte counts. The advancing rise of NLR across stages of CKD found in this study is also consistent with the results of Kim et al. [11], who reported that patients with the highest NLR had substantially lower eGFR values, higher creatinine levels, and a higher frequency of advanced stages compared to those who had the lowest NLR. Additionally, Uduagbamen et al. [26] found that the mean of NLR elevated from 2.3 in stages 1-2 to 5.3 in stages 3-4 of CKD. Moreover, our findings confirmed a significant decrease in lymphocyte counts with the progression of CKD stages. The reduction in lymphocyte counts observed in this study is consistent with the study of Uduagbamen et al. [26], who found a marked decrease in lymphocyte counts in CKD patients at stages 3 and 4 compared to healthy subjects and patients at stages 1 and 2.

Creatinine and urea are the most important biomarkers for assessing renal function. CKD is typically diagnosed by measuring GFR levels, evaluating biomarkers, or using both methods. These toxic waste products need to be filtered out by the kidneys; otherwise, their increased levels can lead to the accumulation of various nitrogenous metabolites in the blood, including urea and creatinine [32, 33]. The results of this study showed that the eGFR in patients with CKD was significantly lower than that of the control group (Table 3). Additionally, there is a continuous progressive decline in GFR, a crucial clinical indicator of kidney function, as it decreases with the progression of CKD stages (Table 4). In contrast, the serum levels of

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creatinine and urea were significantly higher in CKD patients compared to healthy individuals (Table 3), and significantly increased with advancing stages of CKD (Table 4). These results are consistent with previous studies reporting a decrease in eGFR and increased levels of urea and creatinine in CKD patients [3, 10, 15, 26], supporting the association between disease progression and the deterioration of kidney function markers.

Our results demonstrated a significant negative correlation of HG, RBC, and HCT with both urea and creatinine, while a significant positive association was found between these indices and eGFR. These results are in line with the study of Rehmat et al. [34], who found a negative correlation between HG and both urea and creatinine and a positive association with eGFR. These associations highlight the importance of regularly monitoring hematologic and kidney markers to facilitate early detection and management of anemia, thus improving patient status. Furthermore, the findings of this study revealed a statistically significant inverse correlation between WBC count and eGFR (Table 5), indicating that higher WBC levels are associated with declining kidney function. These results are in agreement with a study by Fan et al.[35] which showed a significant relationship between WBC count and the decline in eGFR. In our study, WBCs exhibited a significant positive correlation with serum creatinine and urea levels. This is consistent with a previous study [36], which demonstrated a positive correlation between WBCs and CKD. The significant positive correlation between NLR and both urea and creatinine levels identified in this study is particularly noteworthy and aligns with the findings of Latiwesh et al. [4], who reported a significant association between kidney function parameters and hematological indices. This association strongly indicates a link between systemic inflammation and the severity of kidney dysfunction. Furthermore, this study found a significant negative correlation between eGFR and NLR, indicating that as kidney function decreases, NLR increases. This inverse association between NLR and eGFR is in accord with several earlier studies in similar populations [25, 26]. These correlations suggest that systemic inflammation, reflected by higher NLR, may significantly contribute to the kidney impairment [37].

Conclusion

The current investigation demonstrated that HG, HCT, and RBC are markedly decreased as disease severity progresses. At the same time, NLR is significantly higher in advanced stages, suggesting that NLR could be used as a valuable marker for predicting the inflammatory state of CKD patients and monitoring disease progression.

Conflicts of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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