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Assessment of red cell parameters and interleukin-6 level in chronic kidney disease patients in Sokoto, North-west Nigeria

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Abstract

Background: Anaemia is one of the conditions associated with chronic kidney disease which has contributed to the morbidity and mortality of patients. This study was undertaken to assess the red cell parameters and interleukin-6 level in patients with chronic kidney disease.

Materials and methods: One-hundred and twenty-two (122) male patients diagnosed with chronic kidney disease (CKD) and 50 control subjects were assessed for red cell parameters and interleukin-6 (IL-6) using standard techniques.

Results: The values of Hb, RBC and MCV of 10.25 ± 5.72g/dL, 3.40 ± 1.34 $x10^{12}$ /L and 71.17 ± 21.27 fl, respectively in CKD patients were significantly lower compared to 14.35 ± 1.56 g/dL, 5.44 ± 0.62 $x10^{12}$ /L and 82.5 ± 5.23 fl, respectively in control subjects ($p< 0.05$) while IL-6 level of 87.49 ± 36.46 ng/L in CKD patients was significantly higher compared to 44.88 ± 13.7 ng/L of control group ($p< 0.0001$). There were no statistically significant differences in the values of MCHC and MCH between CKD patients and control subjects ($p> 0.05$). However, stages and age of CKD patients had no effects on the values of RBC parameters ($p>0.05$) while the age showed significant differences with IL-6 levels ($p<0.05$).

Conclusion: Significantly lower values of Hb, RBC and MCV, and significantly higher value of IL-6 have been observed among the CKD patients and therefore, the patients are prone to anaemia, possibly microcytic hypochromic anaemia. However, advanced stage of CKD and increasing age of CKD patients could contribute to the development of severe anaemia.

Keywords: Red cell parameters, interleukin-6, chronic kidney disease

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Introduction

Chronic kidney disease (CKD) is one of the major health problems worldwide that has been associated with high levels of morbidity and mortality.[1, 2]

CKD is associated with progressive loss of kidney function and thereby requiring the need for renal replacement therapy such as dialysis or transplantation. Kidney damage has been linked to pathological abnormalities as revealed by imaging studies or renal biopsy, abnormalities in urinary sediment or increased urinary albumin excretion rates.[3]

CKD has been classified based on the cause, glomerular filtration rate (GFR) category and albuminuria category (CGA). However, based on GFR, CKD has been classified into 5 stages.[4] CKD stages were classified as follows: stage 1: estimated glomerular filtration rate (eGFR) ≥ 90ml/min/1.73m^2, stage 2: eGFR of 60-89 ml/min/1.73m^2, stage 3: eGFR of 30-59 ml/min/1.73m^2, stage 4: eGFR of 15-29 ml/min/1.73m^2 and stage 5: eGFR less than 15 ml/min/1.73m^2. [5]
Higher prevalence of CKD stages 1-5 has been associated with advancing age, ranging from 13.7% in 30–40-year-old group to 27.9% in patients above 70 years.[6] The haematological changes associated with chronic kidney disease have contributed immensely to the morbidity and mortality of the patients. [1] However, the most common haematological change in CKD patients is anaemia. [7]

The cause of anaemia in CKD patients are multifactorial, however, the primary defect is associated with decreased erythropoiesis due to inadequate erythropoietin (EPO) production from the kidneys while other causes have been associated with deficiencies of iron, vitamin B12, and folate due to nutritional insufficiency or increased blood loss. [8-10] Shortened red blood cell survival, chronic diseases and aluminium toxicity have also been implicated in the cause of anaemia in CKD patients. [9,11]
Anaemia in CKD is usually normocytic normochromic but sometimes, it may be microcytic hypochromic due to iron deficiency anaemia and hypoproliferative anaemia because of reduced EPO activity in the bone marrow. [12] CKD patients could experience macrocytic anaemia due to vitamin B12 / folate deficiency, dialysis-induced changes in red cell volume and bone marrow suppression. [13]
Interleukin-6 (IL-6) has both pro- and anti-inflammatory effects and it is produced by numerous immune cells which include monocytes, mesothelial cells, fibroblasts, adipocytes and lymphocytes in response to physiological stimuli such as tumor necrosis factor-α (TNF-α), IL-1β, bacterial endotoxins, physical exercise and oxidative stress. [14]

Advanced CKD has been associated with a state of chronic inflammation, as evidenced by either elevated levels of various pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) or altered levels of acute phase proteins (C-reactive proteins, albumin and fetuin-A). [15-17] It has been proposed that increased circulating level of IL-6 in CKD induces hepcidin overproduction, resulting in decreased duodenal iron absorption and in the release of iron from macrophages, which leads to decreased iron availability for erythropoiesis and then anaemia. [17,18]

Hepcidin is expressed on enterocytes, hepatocytes and macrophages. It binds to iron exporter, ferroportin, and promotes the internalization and degradation of ferroportin and therefore prevents iron egress from the cells, which leads to reduction in iron supply to the bone marrow. [17,18]

Red cell indices and IL-6 level among CKD patients have been scantily documented in Northern Nigeria; therefore, the assessment of red cell indices and IL-6 level among CKD patients as well as the effects of age and CKD stage on these parameters were studied in Sokoto, Northwest Nigeria to provide more information that could be used in the management of CKD patients.

Materials and methods

Setting

The study was carried out in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto and Specialist Hospital, Sokoto (SHS).

Study design

A cross- sectional study was performed on samples collected from CKD patients to assess the red cell parameters and interleukin-6 between October 2019 and September, 2020.

Population

One hundred and twenty-two (122) male patients with CKD, aged 20-84 years were recruited for the study from Usmanu Danfodiyo University Teaching Hospital, Sokoto and Specialist Hospital, Sokoto while 50, age- and sex- matched apparently healthy subjects recruited from Sokoto metropolis served as control group.

Inclusion criteria

1. Diagnosed and consented patients with CKD.
2. CKD patients whose ages were ≥20 years.

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Exclusion criteria

1. Non-consenting CKD patients.
2. Patients on renal replacement therapy.
3. CKD patients on erythropoiesis stimulating agents or had blood transfusion 4 weeks prior to the study.
4. CKD patients with tuberculosis, HIV, haemoglobinopathies and malignancies.

Sample size determination

Based on the prevalence range of 1.6%-12.4% of CKD patients in Nigeria.[19] The average of 7% was used in this study.

Sample size (n) $=Z^2 P (1-P)/e^2$. [20]

$Z= 1.96$, $P=7\%$, $e= 0.05$

$n=1.96^2 \times 0.07(1-0.07)/ 0.05^2$

$n = 3.8416 \times 0.0651/0.0025$

$n = 100.04$

Therefore, the sample size = 100.

However, sample size of 122 was used for the study.

Informed consent

Informed consent was obtained from every participant for the study.

Ethical approval

Ethics and research committees of Usmanu Danfodiyo University Teaching Hospital, Sokoto and Specialist Hospital, Sokoto gave the approval letters UDUTH/HREC/2019/No. 877 and SHS/SUB/133/VOL 1, respectively, for the study.

Sample collection

Five milliliters (5ml) of venous blood was collected from each participant aseptically and 2ml of blood was dispensed into EDTA bottle for the determination of Hb, MCV, MCH, MCHC while the remaining 3ml was put in a plain bottle to obtain serum sample for the estimation of IL-6 levels.

Study instruments

Haemoglobin concentration, MCV, MCH, MCHC were determined from EDTA blood samples using a quality controlled calibrated 3-part differentiation Mythic-18 haematology analyser manufactured in Geneva, Switzerland while the estimation of IL-6 level was carried out from the serum sample using ELISA kit with catalogue number PRS-00871hu, manufactured by Nanjing Pars Biochec Co. Ltd, China.

Data analysis

Data were analysed using SPSS, version 22 (Chicago, IL, USA) and presented as mean ± standard deviation. Student's t-test and one-way analysis of variance with post-hoc Tukey test were employed to compare the groups. P-value of <0.05 was considered statistically significant

Results

Table 1 shows significantly lower values of Hb, RBC and MCV of 10.25 ± 5.72 g/dL, 3.40 ± 1.34 x 10^{12}/L and 71.17 ± 21.27 fl, respectively in CKD patients compared to 14.35 ± 1.56 g/dL, 5.44 ± 0.62 x 10^{12}/L and 82.5 ± 5.23 fl, respectively in control group ($p<0.05$). However, there was significantly higher value of IL-6 of 87.49 ± 36.46 ng/L in CKD patients compared to 44.88 ± 13.74 ng/L in control subjects ($p=0.0001$). The differences in the values of MCH and MCHC between CKD patients and controls were not statistically significant ($p>0.05$)

Red cell indices and interleukin-6 level with CKD stages are shown in Table 2. The values of Hb of 12.8 ± 10.09 g/dL, 10.47 ± 2.01 g/dL, 10.31 ± 5.34 g/dL, 9.91±3.20 g/dL and 10.94±7.52 g/dL, respectively; RBC of 3.16 ± 0.75 x 10^{12}/L, 3.81 ± 0.84 x 10^{12}/L, 3.36 ± 0.73 x 10^{12}/L, 3.31 ± 0.89 x 10^{12}/L and 3.45 ± 1.95 x 10^{12}/L, respectively; MCV of 72.5 ± 30.35 fl, 74.27 ± 22.56 fl, 71.95 ± 20.57 fl, 69.83 ± 22.46 fl and 72.5 ± 30.35 fl, respectively; MCH of 24.7±2.48 pg, 26.34±2.24 pg, 25.66±3.26 pg, 26.11±11.37 pg and 25.74±4.82 pg, respectively; MCHC of 22.98 ± 17.19 g/dL, 30.4 ± 7.05 g/dL, 37.78 ± 19.68 g/dL, 30.59 ± 10.29 g/dL and 36.0 ± 23.69 g/dL, respectively; and IL-6 of 64.12 ± 16.31 ng/L, 69.4 ± 13.28 ng/L, 81.25 ± 41.43 ng/L, 84.02 ± 21.21 ng/L and 99.34 ± 45.03 ng/mL, respectively for stage 1, stage 2, stage 3, stage 4 and stage 5, respectively showed no statistically significant differences ($p>0.05$).

Table 3 demonstrates red cell indices and interleukin-6 level according to age of CKD patients. The levels of Hb of 10.64 ± 5.55 g/dL, 9.33 ± 4.12 g/dL, 11.59 ± 7.99 g/dL, 9.04 ± 3.68 g/dL, respectively; RBC of 3.33 ± 0.97 x 10^{12}/L, 3.27 ± 0.75 x 10^{12}/L, 3.71 ± 2.23 x 10^{12}/L and 3.17 ± 0.91 x 10^{12}/L, respectively; MCV of 68.41 ± 25.08 fl, 72.37 ± 18.79 fl, 69.32 ± 22.32 fl and 84.14 ± 4.71 fl, respectively; MCH of 25.2 ± 5.23 pg, 26.3 ± 9.89 pg, 25.74 ± 5.53 pg and 26.33 ± 2.9 pg, respectively; and MCHC of 29.02 ± 15.4 g/dL, 34.16 ± 15.85 g/dL, 38.89 ± 25.43 g/dL and 31.13 ± 10.72 g/dL, respectively for age ranges of 21-40 years, 41-60 years, 61-80 years and 81-100 years of CKD patients, respectively showed no statistically significant differences ($p>0.05$). However, IL-6 levels of 71.58 ± 18.02 ng/L, 93.3 ± 43.62 ng/L, 95.29 ± 37.77 ng/L and 88.66 ± 17.69 ng/L, respectively for age ranges of 21-40 years, 41-60 years, 61-80 years and 81-100 years of CKD patients,

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respectively showed statistically significant differences (p=0.0251

Table 1: Red cell indices and interleukin-6 level in CKD patients

Parameter	Control subjects (n=50)	CKD patients (n=122)	P-value
Hb (g/dL)	14.35 ± 1.56	10.25 ± 5.72	0.0001
RBC (x10^{12}/L)	5.44 ± 0.62	3.40 ± 1.34	0.0001
MCV (fl)	82.5 ± 5.23	71.17 ± 21.27	0.0003
MCH (pg)	27.5 ± 3.16	25.85 ± 7.41	0.1311
MCHC (g/dL)	30.57 ± 2.15	33.76 ± 30.75	0.4656
IL-6 (ng/L)	44.88 ± 13.74	87.49 ± 36.46	0.0001

Table 2: Red cell indices and interleukin-6 level with CKD stages

Parameter	CKD Stages					P-value
	1 (n=4)	2 (n=7)	3 (n=25)	4 (n=42)	5 (n=44)	
Hb (g/dL)	12.8 ± 10.09	10.47± 2.01	10.31±5.34	9.91±3.2	10.94±7.52	0.849
RBC (x10^{12}/L)	3.16 ± 0.75	3.81 ± 0.84	3.36±0.73	3.31±0.89	3.45±1.95	0.899
MCV (fl)	72.5 ± 30.35	74.27± 22.56	71.95±20.57	69.83±22.46	72.5±30.350	0.986
MCH (pg)	24.7 ± 2.48	26.34± 2.24	25.66±3.26	26.11±11.37	25.74±4.82	0.995
MCHC (g/dL)	22.98±17.19	30. 4±7.05	37.78±19.68	30.59±10.29	36.0±23.69	0.317
IL-6 (ng/L)	64.12±16.31	69.4±13.28	81.25±41.43	84.02±21.21	99.34±45.03	0.055

Table 3: Red cell indices and interleukin-6 level according to age of CKD patients

Parameter	21-40 years (n=34)	41-60 years (n=50)	61-80 years (n=31)	81-100 years (n=7)	P-value
Hb (g/dL)	10.64 ± 5.55	9.33 ± 4.12	11.59±7.99	9.04±3.68	0.329
RBC (x10^{12}/L)	3.33 ± 0.97	3.27 ± 0.75	3.71±2.23	3.17±0.91	0.499
MCV (fl)	68.41 ± 25.08	72.37 ± 18.79	69.32±22.32	84.14±4.71	0.315
MCH (pg)	25.21 ± 5.23	26.3 ± 9.89	25.74±5.53	26.33±2.9	0.928
MCHC (g/dL)	29.02±15.4	34.16±15.85	38.89±25.43	31.13±10.72	0.193
IL-6 (ng/L)	71.58±18.02	93.3±43.63*	95.29±37.77*	88.66±17.69	0.0251

* P<0.05, IL-6 levels in 21-40 years versus 41-60 years and 61-80 years.

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Discussion

The study has shown significantly lower values of haemoglobin concentration, red blood cell count, mean cell volume (MCV) and significantly higher value of interleukin-6 among the CKD patients. Age and stages of CKD patients had little or no significant effects on red cell indices and interleukin-6 level.

Our study has revealed significantly lower values of haemoglobin and red blood cell count in CKD patients compared to control subjects. These findings are consistent with the previous reports. [1, 21,22] Anaemia in CKD patients has been primarily linked to deficiency of erythropoietin (EPO) but other causes include haemolysis, shortened red cell survival, iron deficiency, vitamin B12 and folate deficiency, anaemia of chronic disease due to infection among others. [9]

The study further showed that age and stage of CKD had no significant effects on haemoglobin concentration and RBC count in CKD patients, however, haemoglobin values appeared to have reduced insignificantly with stage of CKD. This is in line with report of Khadayate et al. [2] which showed that severity of anaemia correlated with the stages of CKD.

The study has further shown significantly lower value of MCV in CKD patients compared to control group. This is in agreement with the earlier study, [21] however, Shastry and Belurkar [1] reported that 70% of CKD patients had normal MCV level, 27.6% had low MCV with microcytes in peripheral smear and 2.1% had high MCV with 2 patients having macro-ovalocytes and decreased serum B12 and folate levels. Decreased MCV level in CKD patients in this study could be associated with reduced haemoglobin concentration of the red cell, affecting the average size of the red cell, and ultimately resulting in the presence of microcytes.

Changes in the red blood cell indices have been associated with vitamin B12, iron and folic acid deficiencies, which are consequences of dietary insufficiency or blood or by decreased lifespan of erythrocytes. [10,11]

Age and stage of CKD patients had no significant effects on the values of MCV in this study. This finding on the stage of CKD is in support of earlier study. [1]
The study further displayed that the values of MCH and MCHC in CKD patients were not significantly affected, and these agree with previous studies. [1,21]
Age and stage of CKD patients showed no influences on the levels of MCH and MCHC in this study. Although, limited studies are available on these parameters but Shastry and Belurkar [1] observed similar findings of no significant differences in the MCH and MCHC values with respect to stage of CKD.

Interleukin-6 (IL-6) level was significantly higher in patients with CKD compared to the control group in this study and this agrees with the reports of earlier researchers. [23,24] However, IL-6 and TNF-α have been associated with increase in hepcidin production, which reduces iron release from macrophages and reduces availability of iron for erythropoiesis. [25] IL-6 has therefore been linked to development of anaemia in CKD due to mechanisms that include induction of hypoferremia, aggravation of renal fibrosis and alteration of the erythropoietin axis. [18]

The study has further revealed that IL-6 increased insignificantly with increasing stage of CKD while it increased significantly with age. This shows that aging and increasing stage of CKD could contribute to the development of anaemia.

Strengths and limitations of the study

The study was not limited to CKD patients with end-stage renal disease requiring haemodialysis but included the non-dialysis patients.

The study was limited to 122 male CKD patients and therefore, the report obtained may not represent the entire CKD patients in Sokoto State as other local government areas and females were not included.

The selection of the CKD patients was not through random sampling because they were few in number.
The study did not assess erythropoietin, vitamin B12 and iron levels which could be helpful in determining the cause of anaemia.

The study did not consider the demographic characteristics of the CKD patients.

Implications of the findings of the study

The findings of reduced red cell parameters and increased IL-6 level are associated with chronic kidney disease. This information would serve as guide to the physicians in the management of patients with CKD and thereby reduce the morbidity and mortality rates.

Conclusion

CKD patients had significantly lower values of haemoglobin, red blood cell count, MCV, and significantly higher value of IL-6, which make the

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patients to be prone to anaemia, most especially microcytic hypochromic anaemia, however, advanced age of CKD patients and increasing stage of CKD could contribute to the vulnerability of the patients to the development of severe anaemia.

It is therefore recommended that IL-6 level and red cell indices, especially MCV be determined among other laboratory tests for CKD patients to support their management.

Declarations

Ethical Consideration: Ethical approval was granted by the ethics and research committees of Specialist Hospital, Sokoto and Usman Danfodiyo University Teaching Hospital, Sokoto through letters SHS/SUB/122/VOL 1 and UDUTH/HREC/2019/NO. 877, respectively.
Authors' Contribution: IM and IZI conceived the study design, IZI collected the data while IM, IZI and LHM analysed the data. IM and LHM wrote the manuscript while all authors reviewed and approved the final manuscript for publication.
Conflict of interest: None
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