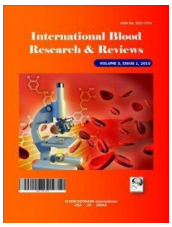

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Myoglobin Status in Stable Patients with Chronic Kidney Disease

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Authors' contributions

This work was carried out in collaboration between both authors. Author VCW designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors VCW and EPO managed the analyses of the study. Author EPO managed the literature searches and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To measure serum myoglobin concentration in stable patients with chronic kidney disease (CKD) who had not commenced hemodialysis and determine its relationship with cardiovascular risk factors.
Study Design: Cross-sectional study.
Place and Duration of Study: Renal Unit, Department of Internal Medicine, University of Port Harcourt Teaching Hospital, Rivers State, Nigeria from January 2014 to December 2015.
Methodology: Blood pressure, serum myoglobin, HDL-cholesterol, total cholesterol, triglyceride, fasting plasma glucose, urine and serum albumin, urine and serum creatinine concentrations were measured in 83 diagnosed chronic kidney disease patients attending the renal clinic and 83 age- and sex-matched healthy control subjects. Body mass index (BMI), estimated glomerular filtration rate (eGFR), urinary albumin-creatinine ratio (UACR) and LDL-cholesterol were calculated.
Results: CKD patients had higher myoglobin, higher blood pressure, higher serum creatinine, higher triglyceride, higher UACR, lower serum albumin, lower HDL and lower eGFR compared to controls. Nineteen (22.9%) patients versus zero (0%) controls had elevated myoglobin. Among

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patients, myoglobin was associated positively with serum creatinine and UACR but inversely with eGFR. Apart from obesity, high myoglobin levels were not significantly associated with cardiovascular risk factors.

Conclusion: Serum myoglobin elevation in CKD patients was associated with high UACR and low eGFR, which are indicative of progressive decline of renal function. Myoglobin levels are influenced by impaired renal status and not primarily related to cardiovascular risk factors in stable patients with CKD. Thus, it may not be useful as a biomarker of myocardial injury in CKD patients.

Keywords: Myoglobin; chronic kidney disease; cardiovascular risk factors; UACR; albuminuria.

1. INTRODUCTION

Chronic kidney disease (CKD) is diagnosed based on the presence of kidney damage, manifested by abnormal albumin excretion or reduced kidney function lasting for a minimum of three months [1,2]. The most common indicators of kidney damage are proteins in the urine (proteinuria or albuminuria), blood in the urine (hematuria), and raised levels of creatinine in the blood, and these can be confirmed by abnormalities on imaging studies or kidney biopsy [2,3]. Chronic kidney disease is characterized by multiple comorbidities and the commonest complication and leading cause of death is cardiovascular disease [4]. Cardiovascular risk factors are more prevalent in CKD patients than in the general population and include traditional risk factors such as hypertension, diabetes mellitus, dyslipidemia, obesity and advanced age, as well as uremia-related factors such as anemia, calcium/phosphate product, markers of inflammation, albuminuria and hypoalbuminemia [4,5]. In patients with renal failure, metabolism and diagnostic accuracy of several markers of cardiac injury have been found to be altered and their serum levels falsely elevated in the absence of symptoms of cardiac disease [5-7].

Myoglobin is a heme-containing, oxygen-binding protein found in all striated muscles [8,9]. It has a low-molecular weight and is normally catabolized by endocytosis and proteolysis in the proximal tubules following glomerular filtration. Therefore, high serum levels are found in patients with renal failure as a result of reduced clearance by the kidneys [9]. Myoglobin has been recognized as a biomarker of acute myocardial ischemia and rhabdomyolysis, and high concentrations in the blood or urine are indicative of cardiac or skeletal muscle injury [10]. In recent times, it has found clinical utility as an adjunct to highly cardiac-specific biomarkers such as cardiac troponin, in the diagnosis, risk stratification and prognosis of acute coronary syndromes because it appears in the circulation after myocardial injury earlier than any other marker available [11-13]. However, it is not cardiac specific because of the large quantities of myoglobin found in skeletal muscle. Its release from the skeletal muscles cannot be distinguished from that released due to cardiac injury because no structural differences exist between the molecules expressed in myocardial and skeletal muscles [11,13].

The aim of this study was to measure serum levels of myoglobin and determine its relationship with cardiovascular risk factors in stable CKD patients.

2. METHODOLOGY

2.1Subjects

The target population included diagnosed chronic kidney disease patients (those with symptoms and signs of renal disease and/or glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 for ≥ 3 months, with laboratory or radiological evidence) above the age of 18 years who were stable and ambulatory and attending the Renal Clinic of the University of Port Harcourt Teaching Hospital (UPTH). Patients on dialysis and those with acute renal failure or other acute illness were excluded. A corresponding number of age- and sex-matched control subjects with normal renal function and no history of cardiovascular disease, diabetes, hypertension, or other acute or chronic condition were drawn from the general population. Approval was obtained from the Ethical Committee of UPTH and informed consent was obtained from all participants. Demographic, social and medical data of participants were assessed with the use of questionnaires.

2.2 Physical Examination

Blood pressure (BP) of each participant was measured with a mercury sphygmomanometer

after ten minutes of rest on two occasions and hypertension was defined as a BP equal to or greater than 140/90 mmHg or the use of antihypertensive drugs. Participants were weighed bare footed and wearing light clothing on a weighing balance placed on a flat surface. Their heights were measured on a portable collapsible stadiometer and body mass index (BMI = weight/height^2) was calculated.

2.3 Specimen Collection

After 10-12 hours overnight fast and observing aseptic procedure, 10ml of venous blood was drawn from the antecubital fossa of each participant into a fluoride oxalate bottle for fasting plasma glucose analysis, an EDTA bottle for analysis of lipids and a plain bottle for the estimation of serum creatinine, albumin and myoglobin. Plasma/serum was separated from blood cells after centrifugation at 2500 g for 10 minutes, harvested with a clean Pasteur pipette and stored at -20°C. Freshly voided spot mid-stream urine was also collected from each participant in a plain bottle for determination of urinary albumin-creatinine ratio (UACR).

2.4 Laboratory Analysis

Urine and serum creatinine concentrations were analysed using the modified Jaffe method and the serum value obtained was used to calculate the estimated glomerular filtration rate (eGFR) of each participant using the Abbreviated Modification of Diet in Renal Disease (MDRD) formula: 32788 x (serum creatinine in µmol/L)$^{-1.154}$ x (Age)$^{-0.203}$ x 1.210 (if black) x 0.742 (if female) [14]. Estimation of fasting plasma glucose was done using the colorimetric glucose oxidase method [15], urine and serum albumin by the BCG (Bromocresol Green) method [15], HDL-cholesterol by precipitation technique, total cholesterol and triglyceride by enzymatic method [15] and LDL-cholesterol was calculated using the Friedewald's formula: (Total cholesterol) – (HDL-C) – (Triglyceride/2.2) in mmol/L [16]. Myoglobin was determined using ELISA technique (Calbiotech). Reference ranges provided by the manufacturer for myoglobin was ≤ 100 ng/ml.

2.5 Statistical Analysis

Data obtained from this study was analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc. Chicago, Illinois, U.S.A.). Frequencies and percentages were obtained for categorical variables. Differences in proportions were analysed using the Chi-squared test. The means of continuous variables were compared using unpaired students t test and one way analysis of variance (ANOVA) and expressed as mean ± standard deviation (SD). Pearson correlation statistics was used to determine associations between myoglobin and biochemical variables. *P*-values less than or equal to 0.05 were taken to be significant in all analyses.

2.6 Definition of Variables

CKD Stages: Stage 1: eGFR ≥ 90 ml/min/1.73 m^2 with kidney damage (persistent albuminuria), stage 2: eGFR = 60 – 89.9 ml/min/1.73 m^2 with kidney damage, stage 3: eGFR = 30 – 59.9 ml/min/1.73 m^2, stage 4: eGFR = 15 – 29.9 ml/min/1.73 m^2, stage 5: eGFR < 15 ml/min/1.73 m^2 [1,17].

Albuminuria: UACR of < 30 mg albumin/g creatinine (3.4 mg albumin/mmol creatinine) was regarded as normal, UACR of 30 – 300 mg/g (3.4-33.9 mg/mmol) as microalbuminuria and UACR of > 300 mg/g creatinine (> 33.9 mg/mmol) as overt albuminuria (macroalbuminuria) [2].

Obesity: Defined as BMI ≥ 30 Kg/m^2 [18].

3. RESULTS

There were 83 CKD patients made up of 41 (49.4%) males and 42 (50.6%) females and 83 age- (*P* = .09) and sex-matched control subjects consisting of 41 (49.4%) males and 42 (50.6%) females. CKD patients had higher systolic (*P* < .001) and diastolic blood pressure values (*P* < .001) but similar BMI (*P* = .06) compared to controls (Fig. 1).

Plasma triglyceride (*P* < .001) of CKD patients was higher and their HDL (*P* =.001) was lower than that of controls but there was no significant difference in their total cholesterol (*P* = .63), LDL (*P* = .09) and fasting glucose (*P* = .14) levels (Fig. 2).

CKD patients were divided into 5 CKD stages. There were 17 (20.5%), 19 (22.9%), 24 (28.9%), 9 (10.8%) and 14 (16.9%) patients in CKD stages 1, 2, 3, 4 and 5 respectively. Serum myoglobin (*P* < .001), creatinine (*P* < .001) and UACR (*P* = .001) of CKD patients were higher while serum albumin (*P* = .001) and estimated GFR (*P* < .001) were lower than that of controls (Table 1).

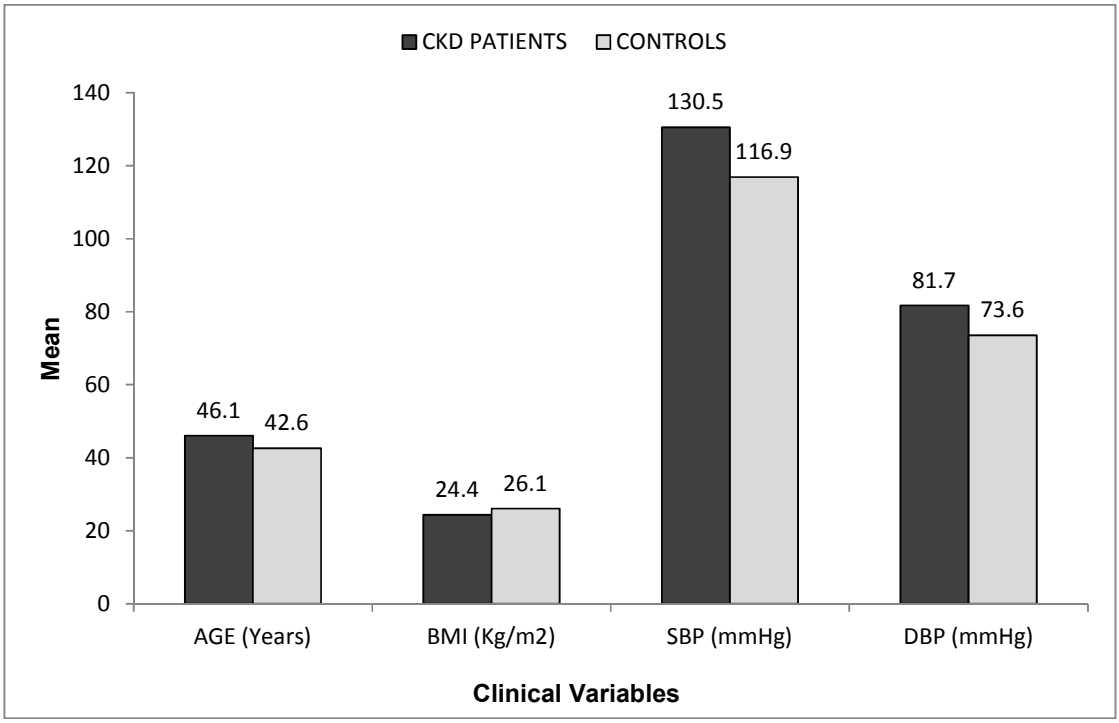

Fig. 1. Clinical variables of CKD patients and healthy controls

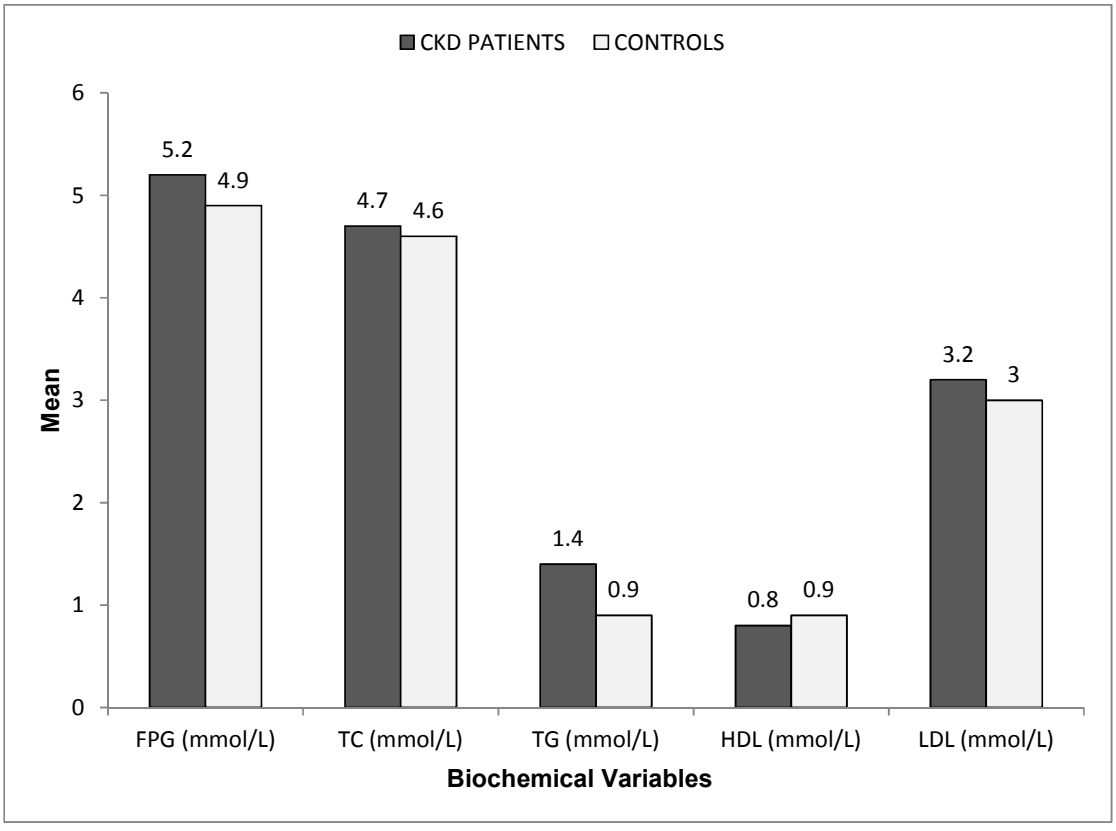

Fig. 2. Biochemical variables of CKD patients and healthy controls

Table 1. Variables of controls and patients that are related to renal function

Variables mean (SD)	Controls	Patients according to CKD stages (GFR in ml/min/1.73m^2)					*P*
		CKD1 (≥90)	CKD2 (60-89.9)	CKD3 (30-59.9)	CKD4 (15-29.9)	CKD5 (<15)	
Myoglobin (ng/ml)	28.2 (16.7)	52.4 (47.0)	53.7 (35.5)	65.8 (113.6)	102.5 (25.9)	230.8 (166.2)	<.001*
Creatinine (µmol/L)	95.2 (22.8)	82.3 (9.7)	94.3 (20.2)	165.8 (46.1)	325.7 (92.1)	669.5 (255.3)	<.001*
eGFR (ml/min/1.73m^2)	91.6 (32.4)	108.6 (16.6)	76.2 (8.3)	47.6 (8.3)	20.7 (2.1)	11.1 (3.9)	<.001*
Albumin (g/L)	42.9 (2.0)	35.2 (10.9)	42.5 (5.1)	40.3 (7.0)	40.0 (10.0)	40.1 (3.9)	.001*
UACR (mg/g)	13.0 (26.3)	151.0 (201.8)	239.7 (251.3)	403.2 (710.5)	472.1 (753.0)	634.1 (1191.6)	.001*

** P-values ≤ .05 significant; eGFR – Estimated Glomerular Filtration Rate; UACR - Urinary Albumin: Creatinine Ratio*

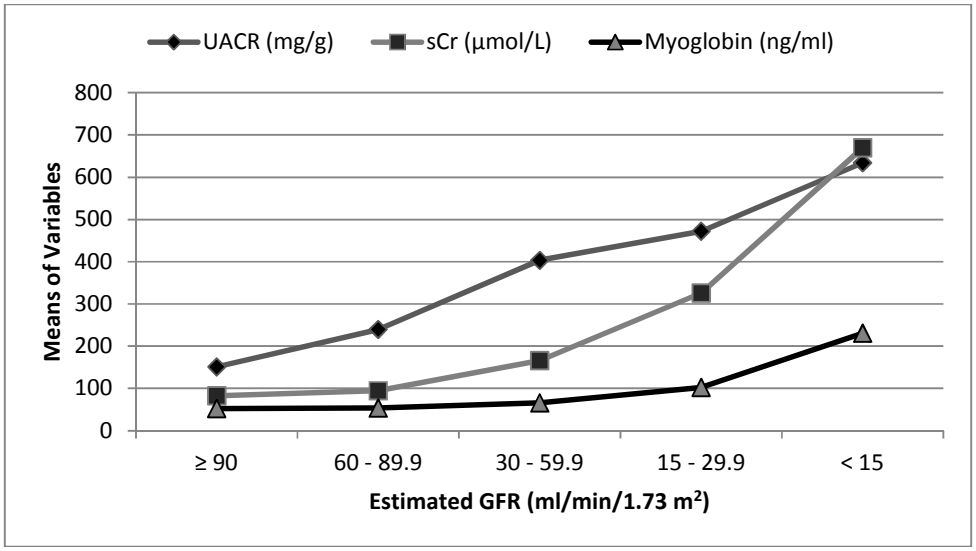

Fig. 3. Means of variables of patients according to CKD stages

Table 2. Prevalence of cardiovascular risk factors in patients with high and normal myoglobin

Risk factor frequency (%)	High myoglobin (n=19)	Normal myoglobin (n=64)	*P*
Hypertension	12 (63.2)	34 (53.1)	.76
Diabetes Mellitus	2 (10.5)	14 (21.9)	.31
Obesity	19 (100.0)	12 (18.8)	.05*
Dyslipidemia	6 (31.6)	36 (56.3)	.16
Hypercholesterolemia	4 (21.1)	17 (26.6)	.78
Hypertriglyceridemia	4 (21.1)	20 (31.3)	.52
Low HDL	3 (15.8)	37 (57.8)	.02*
High LDL	6 (31.6)	19 (29.7)	1.0

** P-values ≤ .05 significant*

Among patients, CKD stage 5 had higher myoglobin concentration than each of the other four CKD stages ($P < .001$ for all) (Fig. 3). Serum creatinine was higher in CKD stages 4 ($P < .001$) and 5 ($P < .001$) compared to stages 1, 2 and 3 respectively (Fig. 3). Myoglobin correlated positively with serum creatinine ($r = .58$, $P < .001$) and UACR ($r = .37$, $P = .005$) but inversely with eGFR ($r = -.32$, $P = .016$).

Nineteen (22.9%) CKD patients had elevated myoglobin levels compared to zero (0%) controls. Prevalence of cardiovascular risk factors was similar among patients with high and normal myoglobin (Table 2) except for obesity which had a higher prevalence among patients with high myoglobin ($P = .05$) and low HDL which had a higher prevalence among patients with normal myoglobin levels ($P = .02$).

4. DISCUSSION

Serum myoglobin was observed to be higher in patients than in controls and was associated positively with serum creatinine and UACR but inversely with eGFR among CKD patients. Nineteen (22.9%) CKD patients had high myoglobin levels, which was associated with obesity as defined by body mass index. It is an established fact that myoglobin elevations are often present in patients with renal insufficiency [19,20]. This finding is not unexpected since myoglobin is a small protein normally cleared by the kidneys, but retained in the circulation of renal failure patients [19,21]. Kontos et al. reported a higher frequency of myoglobin elevation in 43% of patients in their study [20]. Lenglet et al. recorded a very high prevalence of 81% among predialysis CKD patients [10]. Mutluay and colleagues also demonstrated significant elevations of myoglobin among asymptomatic end-stage renal disease patients [6]. The exact mechanisms for elevation of serum myoglobin levels in CKD patients have not been clearly elucidated. Though high levels of myoglobin observed may be secondary to reduced renal clearance due to renal insufficiency, several studies have indicated that this might not be the sole explanation [5,10]. Hypotension, reduced renal perfusion and systemic hypoxia may also result in skeletal muscle release of myoglobin [10,22]. Subclinical myocyte damage and apoptosis due to uremia have also been proposed as a likely mechanism [6].

In this study, high myoglobin levels were not significantly associated with cardiovascular risk factors apart from obesity. This corroborates similar findings from previous literature [6,10]. Lenglet et al. observed that there was no relationship between high myoglobin levels and diabetes, blood pressure, BMI, or presence of cardiovascular disease among CKD patients [10]. Longitudinal studies have indicated that myoglobin is not a predictor of myocardial injury, overall and cardiovascular mortality in these patients [6,10].

We observed that myoglobin increased progressively with increasing serum creatinine, increasing UACR and decreasing eGFR, which are all indices of renal dysfunction. This is similar to the data from the study by Lenglet et al. which showed that myoglobin levels increased significantly and progressively with increasing CKD stage [10]. These findings indicate that myoglobin levels are significantly influenced by renal status.

5. CONCLUSION

Serum myoglobin elevation in CKD patients was associated primarily with high serum creatinine, high UACR and low eGFR, which are indicative of progressive decline of renal function. It was

not significantly related to cardiovascular risk factors apart from obesity. These findings suggest that myoglobin may not be suitable as a biomarker of myocardial injury in patients with CKD.

6. LIMITATION

This study was a cross-sectional study, so patients were not followed up over a period of time to ascertain cause and effect. Longitudinal studies are required to determine outcome.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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