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Original Research Article

Myoglobin elevation as an early prognostic index in stable patients with chronic kidney disease

ABSTRACT

Aim: To measure serum myoglobin concentration in stable patients with chronic kidney disease (CKD) who had not commenced hemodialysis and compare results with those of healthy control subjects.

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 June 2015.

Methodology

Serum myoglobin, HDL-cholesterol, total cholesterol, triglyceride, fasting plasma glucose, urine and serum albumin, urine and serum creatinine concentrations were analysed in 83 diagnosed chronic kidney disease patients attending the renal clinic and 83 age- and sex-matched healthy control subjects. 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 patients, myoglobin was associated positively with serum creatinine and UACR but inversely with eGFR.

Conclusion

Serum myoglobin elevation in CKD patients was associated with high UACR and low eGFR, which are indicative of progressive decline of renal function and increased risk of cardiovascular events. Despite the

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absence of cardiac specificity, myoglobin's high clinical sensitivity compared to other biomarkers enhances its usefulness as a marker of all-cause and cardiovascular mortality, including sudden cardiac death, among patients with CKD, in order to prompt early intervention.

Keywords: Myoglobin, chronic kidney disease, cardiovascular disease, UACR, albuminuria

1. INTRODUCTION

Chronic kidney disease (CKD) is an important, independent risk factor for cardiovascular disease (CVD) [1]. Multiple studies demonstrate a strong independent association between CKD and cardiovascular events including congestive heart failure (CHF), myocardial infarction (MI) and death [2]. Many patients with CKD die prematurely before or soon after beginning dialysis [1]. Twenty to sixty percent of all-cause mortality in CKD patients have been attributed to sudden cardiac death (SCD), which refers to unexpected death from a cardiovascular cause with or without structural heart disease [1-3]. It may result from small subclinical myocardial infarctions [4]. However, a substantial proportion of cardiac deaths is not directly linked to myocardial infarction, stroke, or heart failure, suggesting the presence of other processes contributing to cardiovascular mortality [2]. The most common causes of SCD include ventricular tachycardia, ventricular tachyarrhythmia, tachycardia torsade de pointes, sustained ventricular fibrillation and bradyarrhythmia [2]. Sudden cardiac death is sometimes the first manifestation of cardiovascular disease in CKD patients [4].

Increasing evidence demonstrates that pathology, manifestations, and complications of CVD differ in the presence of CKD [1]. These patients have a high prevalence of abnormal electrocardiographic (ECG) and echocardiographic changes, atypical cardiac symptoms and silent myocardial ischemia [1,5,6]. They are more likely to present with systolic or diastolic dysfunction causing heart failure symptoms, or with syncope. [1]. The diagnosis of acute coronary syndromes in CKD patients may thus be quite challenging [1,5,6].

Myoglobin is useful primarily as an early biomarker because of its high sensitivity and negative predictive value during the first hours after an acute myocardial infarction (AMI) [7,8]. However, it is not cardiac specific because of the large quantities of myoglobin found in skeletal muscle. Its release from the

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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 [7,9]. Myoglobin estimation is most useful for AMI rule-out, particularly in symptomatic patients [8]. Myoglobin is normally catabolized by endocytosis and proteolysis in the proximal tubules following glomerular filtration. Therefore, high serum levels are also found in patients with renal failure as a result of reduced clearance by the kidneys. Thus, myoglobin has low diagnostic efficiency in such patients at high risk for myocardial necrosis [7,9]**.**

However, despite the absence of cardiac specificity, an elevated myoglobin has been demonstrated to strongly predict mortality in patients with renal insufficiency [10]. Myoglobin may also have a role in cardiovascular risk prediction in CKD patients without symptoms of cardiovascular disease. The prevalence of asymptomatic coronary artery disease (CAD) is 20-40% among CKD patients [5]. One out of four myocardial infarctions resulting in Q-waves is asymptomatic, and smaller myocardial infarctions may be asymptomatic in as much as four out of five victims [4].

The aim of this study was to determine serum levels of myoglobin in CKD patients and compare with healthy control subjects.

2. METHODOLOGY

2.1 Subjects

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) of the renal unit of the University of Port Harcourt Teaching Hospital (UPTH) above the age of 18 years who were stable and ambulatory and had no symptoms of cardiac disease. Patients on dialysis and those with acute renal failure or other acute condition, symptoms or history of cardiovascular disease 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.

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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 taken as a BP equal to or greater than 140/90 mmHg. 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 CK-MB. Plasma/serum was separated from blood cells after centrifugation at 2500g for 10 minutes, harvested with a clean Pasteur pipette and stored at -20^{0}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) [10]. Estimation of fasting plasma glucose was done using the colorimetric glucose oxidase method [11], urine and serum albumin by the BCG (Bromocresol Green) method [11], HDL-cholesterol by precipitation technique, total cholesterol and triglyceride by enzymatic method [11] and LDL-cholesterol was calculated using the Friedewald's formula [12]. 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

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categorical variables. 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 association between myoglobin and clinical 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, 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 [13].

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) [14].

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 (Figure 1).

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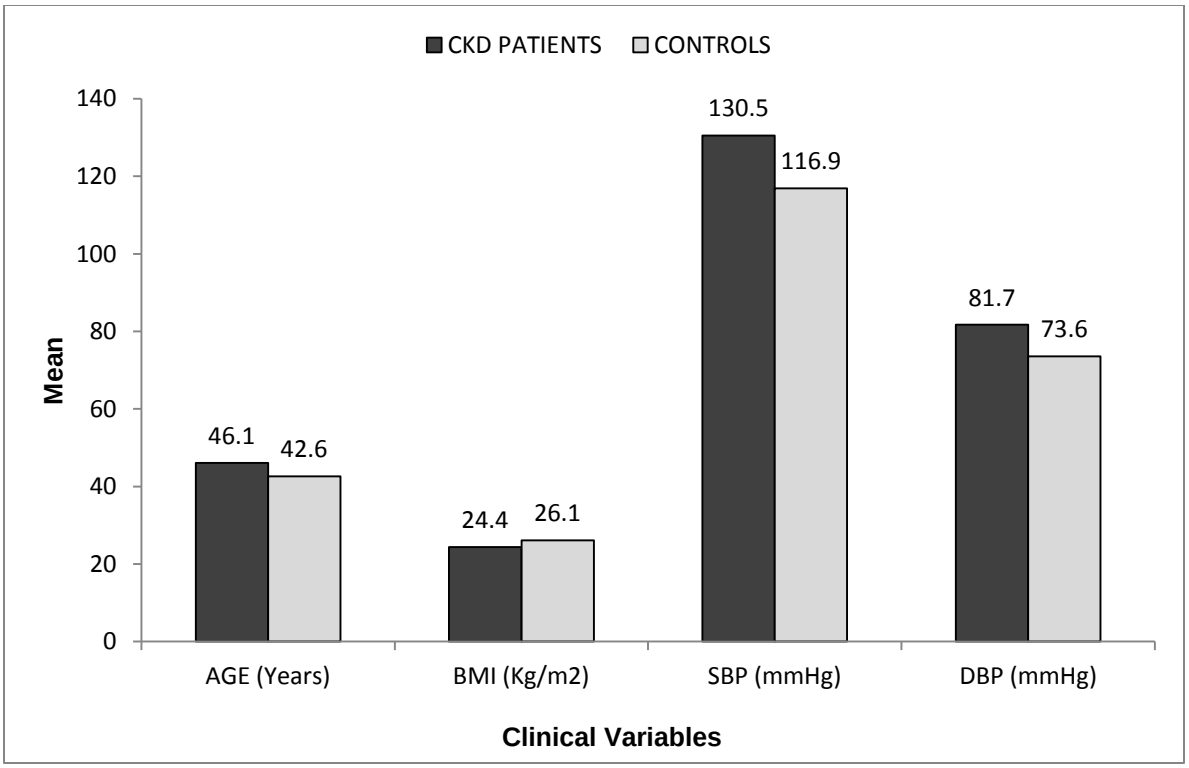

Figure 1: Clinical Variables of CKD Patients and Healthy Controls

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 (Figure 2). Prevalence of myoglobin elevation was 19 (22.9%) among CKD patients but zero (0%) among controls.

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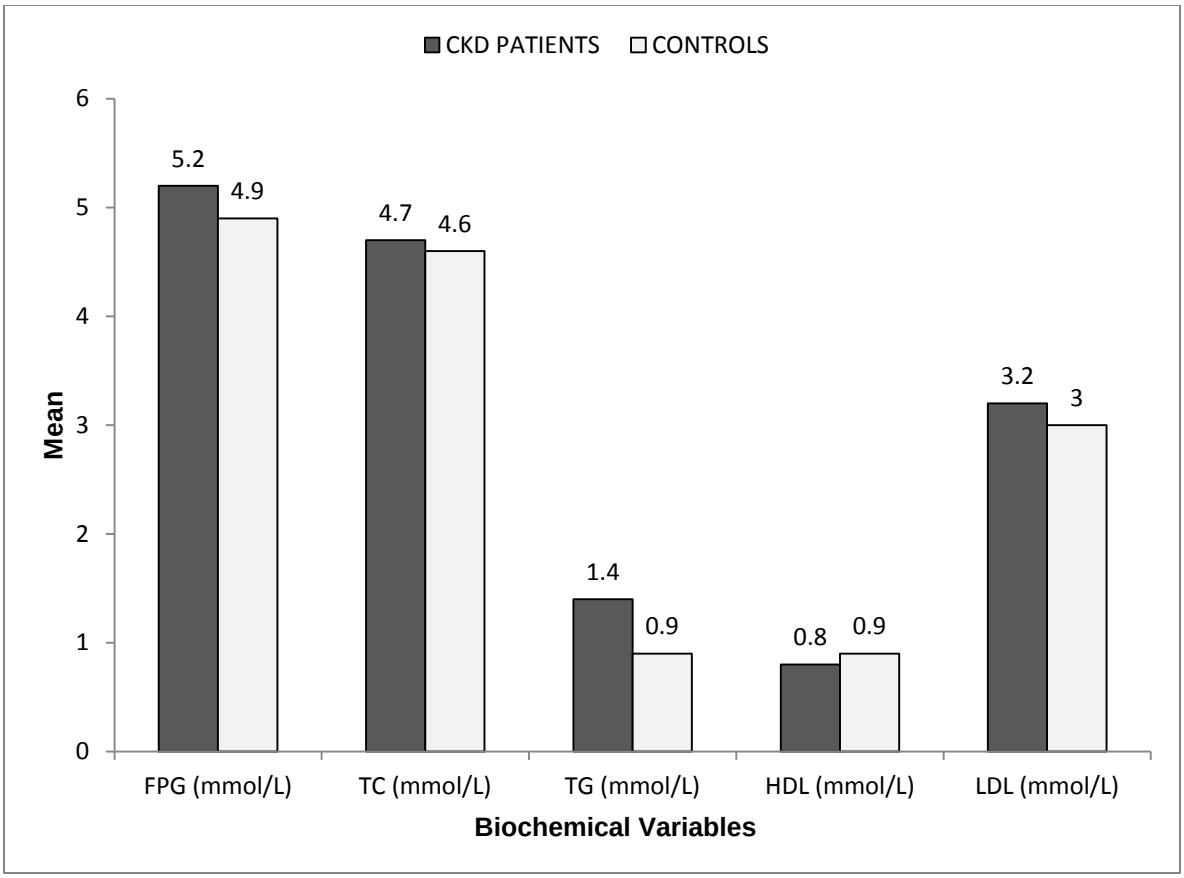

Figure 2: Biochemical Variables of CKD Patients and Healthy Controls

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).

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

Variables	**Controls**	**Patients according to CKD stages (GFR in ml/min/1.73m^2)**					***P***
Mean (SD)		**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*

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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*

Among patients, CKD stage 5 had higher myoglobin concentration than each of the other four CKD stages (*P* < .001 for all) (Figure 3). Serum creatinine was higher in CKD stages 4 (*P* < .001) and 5 (*P* < .001) compared to stages 1, 2 and 3 respectively (Figure 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).

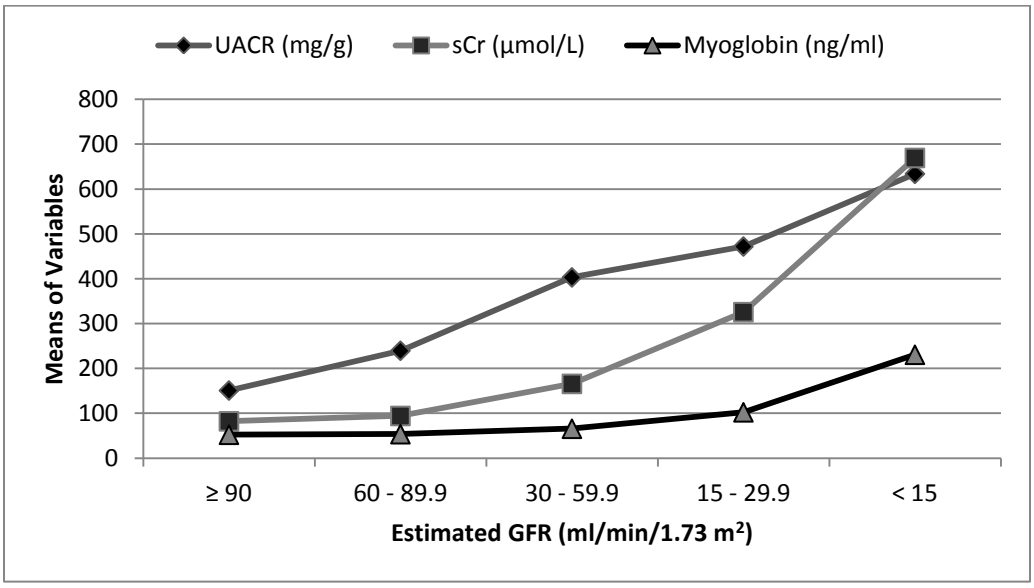

Figure 3: Means of variables of patients according to CKD Stages

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. It is an established fact that myoglobin elevations are often present in patients with renal insufficiency [8,10]. This finding is not unexpected since myoglobin is a small protein

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normally cleared by the kidneys, but retained in the circulation of renal failure patients [8,16]. Kontos et al reported a higher frequency of myoglobin elevation in 43% of patients in their study [10]. The high levels of myoglobin observed may be secondary to reduced renal clearance due to renal insufficiency. Both hypotension and reduced renal perfusion may also result in skeletal muscle release of myoglobin [17]. However, it is possible for elevated myoglobin levels to also predict an increased risk of adverse cardiovascular events and identify some high-risk patients early [10].

Myoglobin is a heme-containing, oxygen-binding protein found in all striated muscles [18]. Myoglobin's sensitivity for myocardial necrosis combined with its unique release and clearance properties may render it particularly attractive as a risk marker either alone or in combination with other markers [18]. 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 [8,9]. Myoglobin has been shown to predict adverse events with higher sensitivity than more cardiac-specific biomarkers, though with lower specificity [8,19]. Wu et al demonstrated that myoglobin is released from damaged cells earlier than creatine kinase MB (CK-MB) and cardiac troponins, and had the highest sensitivity during the initial hours after AMI, allowing for earlier detection of myocardial injury [8]. The addition of myoglobin to biomarker panels that include CK-MB and cardiac troponins I or T improves sensitivity for the detection of AMI, particularly in patients presenting early after symptom onset [17].

In contrast with cardiac troponins, myoglobin appears to be more predictive of mortality than of nonfatal ischemic end points and myocardial infarction [18]. Myoglobin has been shown to be the strongest multivariate predictor of mortality among patients with renal insufficiency and it discriminates patients at high and low risk for mortality better than cardiac troponins and CK-MB, independent of baseline clinical variables and ECG changes [10,17,18]. This is particularly useful in asymptomatic patients because unrecognized or undertreated cardiovascular disease may predispose to a higher risk of death that occurs suddenly and unexpectedly [20].

Sudden cardiac death (SCD) is relatively common in pre-dialysis patients, particularly those in advanced CKD stages [20]. Caravaca et al reported a frequency of SCD of 3% among pre-dialysis CKD patients, which accounted for 16% of total deaths and more than 29% of cardiovascular mortality. Patients who

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died from SCD were observed to have a high burden of co-morbidities and were not adequately treated [20] CKD is associated with increased prevalence of concomitant CHF, ischemic heart disease, cardiac arrhythmias (most commonly atrial fibrillation), and valvular calcification [1]. CHF is the leading cardiovascular condition in CKD patients, with mortality slightly higher for diastolic than for systolic CHF. Terminal events in CHF are pump failure and sudden arrhythmic death [1].

Proteinuria, most often assessed as albuminuria (microalbuminuria and macroalbuminuria), is a strong predictor of renal, cardiovascular, and mortality risk, independent of traditional cardiovascular risk factors [21,22]. An increasing level of urinary albumin to creatinine ratio (UACR) is independently associated with higher incidence and severity of cardiovascular events, premature mortality, progressive decline in glomerular filtration rate and progression to end stage renal disease (ESRD) [21,23]. The presence of proteinuria increases the incidence of ESRD approximately 100-fold higher and puts a patient with stage 1 or 2 CKD with proteinuria but normal eGFR at a greater risk for progression to ESRD compared to a patient with stage 3 or 4 CKD without proteinuria [23]. Microalbuminuria may also serve as a marker for subclinical cardiovascular organ damage such as left ventricular hypertrophy and peripheral atherosclerosis in asymptomatic patients with traditional CV risk factors such as diabetes and hypertension [22]. Existing data indicate that angiotensin-converting enzyme inhibition in patients with microalbuminuria or macroalbuminuria results in a lowering of albuminuria, a better cardiovascular prognosis, prevention of accelerated loss of renal function and potential prevention of ESRD [23].

5. CONCLUSION

Serum myoglobin elevation in CKD patients was associated with high UACR and low eGFR, which are indicative of progressive decline of renal function and increased risk of cardiovascular events. Despite the absence of cardiac specificity, myoglobin's high clinical sensitivity compared to other biomarkers enhances its usefulness as a marker of all-cause and cardiovascular mortality, including sudden cardiac death, among patients with CKD. This will prompt early detection and treatment of patients at risk.

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CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for this study. A copy of the written consent is available for review by the editorial office/chief editor/editorial board members of this journal.

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

None exists.

REFERENCES

1. Herzorg CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, et al. Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2011; 80:572-586.
2. Whitman IS, Feldman HI, Deo R. CKD and sudden cardiac death: epidemiology, mechanisms and therapeutic approaches. J Am Soc Nephrol 2012;23:1929-1939.
3. Shamseddin MK, Parfrey PS. Sudden cardiac death in chronic kidney disease: epidemiology and prevention. Nat Rev Nephrol 2011;7:145-154.
4. Sundstrom J. Myocardial biomarkers for prediction of cardiovascular disease. Disease Markers 2009;26:234-246.
5. Alam GK, Lieb DB. Biochemical markers of myocardial ischemia in renal failure. Hosp Physician 2002;1:27-31.
6. Kiranmayi B, Lakshmi N, Tagore R, Kambar C. Evaluation of cardiac markers in chronic renal failure. IOSR-JDMS 2015;14:46-52.

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7. Bel MS, Soldevila JG, Lianos JO. Biological markers of myocardial necrosis. Rev Esp Cardiol 2003;56:703-720.
8. Wu AH, Feng YJ, Contois JH, Pervaiz S. Comparison of myoglobin, creatine kinase-MB and cardiac troponin I for diagnosis of acute myocardial infarction. Ann Clin Lab Sci 1996;26:291-300.
9. Nigam PK. Biochemical markers of myocardial injury. Indian Journal of Clinical Biochemistry 2007;22:10-17.
10. Kontos MC, Garg R, Anderson FP, Roberts CS, Ornato JP, Tatum JL, et al. Ability of myoglobin to predict mortality in patients admitted for exclusion of myocardial infarction. Am J Emerg Med 2007;25:873-879.
11. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-470.
12. Randox Laboratories Ltd, Antrum, BT294QY, UK. Manual Procedures, 4th Edition. 1996: 126-189.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of preparative ultracentrifugation. Clin Chem. 1972;18:499-502.
14. Ulasi II, Arodiwe EB, Ijoma CK. Left Ventricular Hypertrophy in African Black Patients with Chronic Renal Failure at First Evaluation. Ethn Dis 2006;16:859-864.
15. Afolabi MO, Abioye-Kuteyi EA, Arogundade FA, Bello IS. Prevalence of chronic kidney disease in a Nigerian family practice population. SA Fam Pract 2009;51:132-137.
16. Davis CP, Barret K, Torre P, Wacasey K. Serial myoglobin levels for patients with possible myocardial infarction. Acad Emerg Med 1996;3:590-597.
17. Lemos JA, Morrow DA, Gibson M, Murphy SA, Sabatine MS, Rifai N, et al. The prognostic value of serum myoglobin in patients with non-ST-segment elevation of acute coronary syndromes. Results from the TIMI 11B and TACTICS-TIMI 18 studies. J Am Coll Cardiol 2002;40:238-244.
18. Newby KL. The emerging role of myoglobin for risk stratification. Am Heart J 2001;142:4-6.

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19. Green GB, Skarbek-Borowski GW, Chan DW, Kelen GB. Myoglobin for early risk stratification of emergency department patients with possible myocardial ischemia. Acad Emerg Med 2000;7:625-636.
20. Caravaca F, Chavez E, Alvarado R, Garcia-Pino G, Luna E. Sudden cardiac death in non-dialysis chronic kidney disease patients. Nefrologia 2016;36:404-409.
21. Fraser SD, Roderick PJ, McIntyre NJ, Harris S, McIntyre C, Fluck R, Taal MW. Assessment of proteinuria in patients with chronic kidney disease stage 3: Albuminuria and non-albumin proteinuria. PLoS ONE 2014;9:e98261.doi:10.1371/journal.pone.0098261.
22. Sarafidis PA, Bakris GL. Microalbuminuria and chronic kidney disease as risk factors for cardiovascular disease. Nephrol Dial Transplant 2006;21:2366-2374.
23. Gansevoort RT, Jong PE. The case for using albuminuria in staging chronic kidney disease. J Am Soc Nephrol 2009;20:465-468.