ROLE OF NOVEL BIOMARKERS IN PREDICTING CHRONIC KIDNEY DISEASE PROGRESSION AMONG BLACK PATIENTS ATTENDING A TERTIARY HOSPITAL IN JOHANNESBURG, SOUTH AFRICA

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DECLARATION

I, Alfred Jackson Meremo declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other University.

Signed: 

Date: 10/11/2023

In memory of my lovely mother

Agness Bhoke Nyahiri Gimonge

1961 - 2018

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE STUDY

Publications:

Research articles:

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ABSTRACT

Background: Chronic kidney disease (CKD) is a leading health issue and its magnitude has been increasing globally; where the developing countries are the most affected and they are the least equipped to deal with its associated consequences. Chronic kidney disease can rapidly and quietly progress to late CKD stages in impoverished environments. Early recognition of patients who are likely to develop end-stage kidney disease (ESKD) is important.

Methodology: A prospective longitudinal study was conducted on CKD patients of black ethnicity attending at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) renal outpatient clinic in South Africa, as from September 2019 to March 2022. Patients provided blood and urine samples for investigations in the laboratory at study enrolment (0) and at the 24 months follow up. The concentrations of the transforming growth factor isoforms [(TGF)-β1, TGF-β2 and TGF-β3) were determined in serum and urine at baseline using the Human TGF-β duoset ELISA. Data were descriptively and inferentially processed by the REDcap and analyzed using STATA version 17 and multivariable logistic regression analysis was applied to find out the predictors of CKD progression.

Results: A total of 312 patients were recruited into the study; the median age was 58 (IQR 46 - 67) years and 162 (51.9 %) were male. Hypertension was present in majority (96.7 %) of the patients. Diabetes mellitus was present in 38.7 % of patients and 38.1 % of the study patients had both hypertension and diabetes mellitus. A total of 297 (95.2%) patients completed the study. Death was reported in 5 (1.6%) patients and 10 (3.2%) of patients were lost to follow up. The prevalence of CKD progression was 49.5%, 33% had CKD remission and 17.5% had CKD regression while the prevalence of CKD progression by change in uPCR > 30% was 51.9%. Almost half (47.8 %) had a sustained decline in eGFR of > 4 ml/min/1.73 m^2/year or more, 35.0% of the patients moved to a more severe stage of CKD and 19.9% had more than 30%

decline in eGFR in two years. For patients with CKD progression, 54.9% patients were men and at baseline, their median age was 59 (46 - 67) years, urine protein creatinine ratio (uPCR) increased at 0.039 (0.015-0.085) g/mmol, eGFR was 37 (32 -51) mL/min/1.73 m^2; the median serum TGF-β1 was 21210 (15915 – 25745) ng/L and the median urine TGF-β3 was 17.5 (5.4 – 76.2) ng/L. For those who had CKD progression, hypertension was present in the majority (95.2%) of the patients. Diabetes mellitus was present in 59 (40.1%) patients and 58 (39.5%) patients had both hypertension and diabetes mellitus; 48.3% had severely increased proteinuria, 45.6% patients had anaemia, 34.0% had hyperuricemia and 17.7% had hypocalcaemia at baseline. For those patients with CKD progression vs those without CKD progression, the baseline median serum TGF-β1 was 21210 (15915 – 25745) ng/L vs 24200 (17570 – 29560) ng/L, the baseline median urine TGF-β3 was 17.5 (5.4 – 76.2) ng/L vs 2.8 (1.8 – 15.3) ng/L; however, baseline serum and urine TGF-β isoforms did not predict progression of CKD on univariate and multivariable analyses. Regarding use of medications among patients with CKD progression, calcium channel blockers (amlodipine) were used by majority (85.2 %) of the patients. Diuretics were used by 63.4% of the patients and 31.7 % of the patients were using insulin. Variables associated with CKD progression after multivariable logistic regression analysis were moderately elevated proteinuria (OR 2.1, 95% CI (1.1 – 3.9), P= 0.019), severely elevated proteinuria (OR 6.1, 95 % CI (3.2 – 11.6), P = 0.001), hyponatraemia (OR 4.5, 95% CI 1.8 - 23.6, P= 0.042), hypocalcaemia (OR 3.8, 95 % CI 1.0 - 14.8, P = 0.047), anaemia (OR 2.1, 95% CI 1.0 - 4.3, P= 0.048), elevated HbA1c (OR 1.8, 95 % CI 1.2 - 2.8, P = 0.007), diabetes mellitus (OR 1.8, 95 % CI 1.9 - 3.6, P = 0.047), current smoking (OR 2.8, 95 % CI 1.9 - 8.6, P = 0.049), medications which were calcium channel blockers (OR 2.07, 95 % CI 1.04 – 4.12, P = 0.038), diuretics (OR 2.35, 95 % CI 1.37 – 4.00, P = 0.002), insulin (OR 1.96, 95 % CI 1.01 – 3.84, P = 0.048) and baseline serum calcium levels (OR 0.06, 95 % CI 0.01 -0.64, P = 0.019). An

increase in uPCR > 30% at two years identified most patients with CKD progression; clinicians and nephrologists should utilize change in uPCR > 30% at two years to identify those patients with CKD who are likely to progress more rapidly, who require closer surveillance and monitoring with emphasis on slowing or stopping progression of the CKD.

Conclusion: Our study has demonstrated a higher prevalence of CKD progression in a prospective longitudinal study among black patients than that reported in previous studies. CKD progression was associated with current smoking, hyponatremia, hypocalcemia, anaemia, elevated HbA1c, diabetes mellitus, and proteinuria. While patients with CKD progression had lower baseline concentrations of serum TGF-β1 and increased baseline urinary TGF-β3 concentrations, baseline serum and urine TGF-β isoforms did not predict progression of CKD. The roles of the various serum and urine TGF-β isoforms in CKD progression at baseline are still unclear and highlight the importance of further studies to determine their isoform specific effects.

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LIST OF ABBREVIATIONS

ACEIs	Angiotensin Converting Enzyme Inhibitors
AI	Artificial Intelligence
ARBs	Aldosterone Receptors Blockers
BMI	Body Mass Index
CCBs	Calcium Channel Blockers
CKD	Chronic Kidney Diseases
CKD-EPI	Chronic Kidney Disease Epidemiology collaboration
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DBP	Diastolic Blood Pressure
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immunosorbent Assay
ESKD	End Stage Kidney Disease
FBG	Fasting Blood Glucose
FSGS	Focal Segmental Glomerulosclerosis
GN	Glomerulonephritis
HbA1C	Glycosylated haemoglobin A1C
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
IDMS	Isotope Dilution Mass Spectroscopy
ISN	International Society of Nephrology
IQR	Interquartile Range
KDIGO	Kidney Disease Improving Global Outcomes
KIM-1	Kidney Injury Molecule-1 (KIM-1

KRT	Kidney Replacement Therapies
LFABP	Liver-type Fatty Acid Binding Protein
MCP	Monocyte Chemoattractant Protein
MMP	Matrix Metalloproteinase
NAG	N-acetyl-beta-D-glucosaminidase
NCDs	Non - Communicable Diseases
NGAL	Neutrophil Gelatinase-Associated Lipocalin (NGAL)
NHLS	National Health Laboratory Services
REDcap	Research Electronic Data Capture
ROC	Receiver operating characteristic
SBP	Systolic Blood Pressure
SLE	Systemic Lupus Erythematosus
SSA	Sub – Saharan Africa
STATA	Statistics and data
TGF-β	Transforming Growth Factor-beta
TNF	Tumor Necrosis Factor
T2DM	Type 2 Diabetes Mellitus
UK	United Kingdom
uPCR	urine Protein Creatinine Ratio
USA	United States of America
WBC	White Blood Cells

PREFACE

Breakthroughs in care of CKD patients are delayed by unavailability of biomarkers in clinical practice that can diagnose CKD early, and predict its progression for timely and appropriate interventions. This study on TGF-β isomers is essential as it will allow more accurate prediction of CKD progression in its earlier stages and correlation with the decline of eGFR and change in uPCR. We need to know the roles of TGF-β isomers in predicting CKD progression among black CKD patients in South Africa; and those who are at increased risk for CKD and its progression. The findings from this study will eventually lead to earlier detection of those patients at increased risk for CKD progression by risk stratification and thus improve patient outcomes by adjusting their treatment plans including more frequent follow ups.

This PhD thesis is presented in an integrative format consisting of 6 chapters:

Chapters 1 – 2: Literature review and methodology

Chapter 3: Demographic and clinical profile of black patients with chronic kidney disease attending the kidney outpatient clinic at a tertiary hospital in Johannesburg, South Africa (Chapter 3 was ***published in the PLOS ONE Journal on 19.09.2022;*** Appendix D).

Chapter 4: Progression of chronic kidney disease among black patients attending a tertiary hospital in Johannesburg, South Africa. (Chapter 4 was ***published in the PLOS ONE Journal on 13.02.2023;*** Appendix E).

Chapter 5: Baseline transforming growth factor-ß isoforms were not associated with chronic kidney disease progression among black patients attending a tertiary hospital in Johannesburg, South Africa. A submissible manuscript is included as Chapter 5.

Chapter 6: Summary of the study findings, discussion, recommendations and limitations of the study.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Chronic kidney disease (CKD) is a decline in kidney function recognised by markers of kidney damage or estimated glomerular filtration rate (eGFR) of less than 60 mL/min per 1·73 m^2 or both, of at least 3 months, irrespective of the underlying cause (1, 2). The burden of CKD is increasing globally; more than 850 million individuals were reported to have CKD worldwide (3). Hypertension and diabetes mellitus account for the major causes of CKD worldwide; hypertension accounts for and is the leading cause of CKD in sub-Saharan Africa (4, 5). Use of traditional herbal medicines, remote living, low socio-economic status and unemployment are all associated with the rising CKD among black patients (6-8).

Chronic kidney disease contributes massively to the global non-communicable disease burden (9, 10) and data from several sources have shown that CKD usually and frequently contributes to end stage kidney disease (ESKD) and death (11, 12). In December 2021, the total number of patients with ESKD on chronic dialysis or transplantation in South Africa stood at 8 866, with an overall prevalence of 147 per million people (13). Individuals of black ethnicity succumb and are at increased risk of death from CKD due to socioeconomic issues including access to health care and affordability of treatments (14, 15).

Studies have reported elevated serum creatinine levels and reduced eGFR to be associated with African ancestry, thus recalculation of eGFR based on genetic ancestry affects CKD staging among Africans (16, 17). Also, while techniques exist for the measurement of GFR in few African countries, early evidence shows that their availability is inadequate (18). Serum cystatin C based equations underestimated the GFR, reporting lower prevalence estimates of CKD (19, 20) while β-Trace protein (BTP) and β2-microglobulin (B2M) provided less correct GFR estimates than the cystatin C equations and CKD-EPI creatinine equations (21, 22). This has

brought considerable research into novel biomarkers that would help in such prediction beyond the frequently used legacy biomarkers such as albuminuria, cystatin C and serum creatinine (23, 24). In this regard, studies have highlighted changes in a number of novel biomarkers that occur before changes in albuminuria, serum creatinine and can diagnose CKD early and predict its progression (25, 26).

1.2 Burden of Chronic Kidney Disease (CKD)

Chronic kidney disease globally has a prevalence of between 11 to 13%; with the prevalence by stages as follows: stage 1 is 3.5%, stage 2 is 3.9%, stage 3 is 7.6%, stage 4 is 0.4% and stage 5 is 0.1%; thus, greater number of patients is in stage 3 (27, 28). The prevalence of CKD is highest in the developed countries when compared to developing countries including SSA where the numbers of patients with CKD are increasing rapidly (9, 29). With regard to CKD prevalence by gender, in stages 3–5, 4.7% occur in men and 5.8% in women with a range of variability within and between specific geographic locations (11, 30).

The global all-age mortality rate from CKD has risen by 41·5% between 1990 and 2017; about 697·5 million cases of all-stage CKD were reported in 2017 with a global prevalence of 9·1%; all-age prevalence of CKD rose by 29·3% since 1990 (31). However, exact prevalence statistics are not available in many regions of the world and sub-Saharan Africa is not an exception (32). The prevalence of CKD in Africa from recent studies is reported to be 15.8%, which has a resemblance to other regions, thus constituting a true global health burden with significant economic consequences to healthcare systems (2, 33). It has been found that the developing countries are most disadvantaged due to inadequate resources (5, 29) and consequently the demand for kidney replacement therapies (KRT), such as dialysis and transplantation is high. The incidence of cardiovascular events is also increasing (12, 34).

Population-based studies report the prevalence of CKD in South Africa to range between 6.7 % to 14·0% with regional differences in prevalence and risks of CKD due in part to different levels of epidemiological and sociodemographic health transitions (35, 36). Sub-Saharan Africa tends to have younger populations mostly not more aware of their underlying CKD than the developed countries, and despite the high demand, a small number receive KRT, mainly because of resource limitations (14, 34). Therefore, early diagnosis of CKD is critical for initiating timely interventions in an attempt to prevent progression of CKD.

1.3 Factors associated with chronic kidney disease (CKD)

Factors associated with CKD include: increasing age, female gender, low income, low level of education, alcohol consumption, current smoking, hypertension, overweight, obesity, diabetes mellitus, quality of drinking water, consumption of fatty food and dyslipidaemia (37-39). Also, studies have reported other common risk factors for developing CKD which include glomerular diseases and infections especially among the poor (40, 41). Proteinuria and black ancestry are known risk factors for CKD and other modifiable factors including anaemia, heart failure, elevated phosphate levels, hyperuricaemia, hyperlipidaemia and medications (traditional and herbal) are common in advanced CKD (42, 43). CKD is associated with hyperuricaemia; high serum uric acid levels are associated with inflammation, endothelial cell injury, crystalluria which eventually lead to activation of the renin-angiotensin system (44-46).

Studies have reported that majority (80%) of the black individuals use traditional herbal medicines; these can cause crystalluria or hypertension eventually leading to CKD (6, 7). The combination of remote living, low socio-economic status and unemployment are associated with CKD among black patients (8, 47, 48).

1.4 CKD progression

Chronic kidney disease (CKD) progression is defined as a change in GFR or markers of kidney damage or both, over a set time period (49, 50). It is important for clinicians to identify patients with CKD at higher risk of rapid progression and those for whom close monitoring is needed (51, 52). CKD progression can be assessed by (i) continued decrease in eGFR of > 4 ml/min/1.73 m^2/year or more (53) (ii) change to a more severe stage of CKD (51) (iii) more than 30% reduction of eGFR in two years, whereby percent change in eGFR is calculated as follow up eGFR – baseline eGFR/ baseline eGFR x 100 (54) (iv) more than 30% increase in uPCR in two years, whereby change in uPCR is calculated as follow up uPCR – baseline uPCR / baseline uPCR (55).

1.4.1 Pathophysiology of CKD progression

Most kidney diseases advance to ESKD because of functional adaptations in the kidney after an initial depletion of nephron units (56, 57). Such changes include glomerular hypertension and hyperperfusion which result in increasing the filtration capacity of single nephrons (58, 59). These changes initially reduce the functional consequences of nephron loss but ultimately lead to the impairment of the glomerular barrier's selective properties promoting protein ultrafiltration resulting in proteinuria, tubular atrophy which eventually leads to glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis (60, 61). The underlying glomerular impairment, the unusual passage of plasma proteins across the filtration barrier causes accelerated injury to the tubules which promotes the vicious cycle of tubular epithelial degeneration and glomerular scarring. Figure 1.1 (57, 62) highlights these findings.

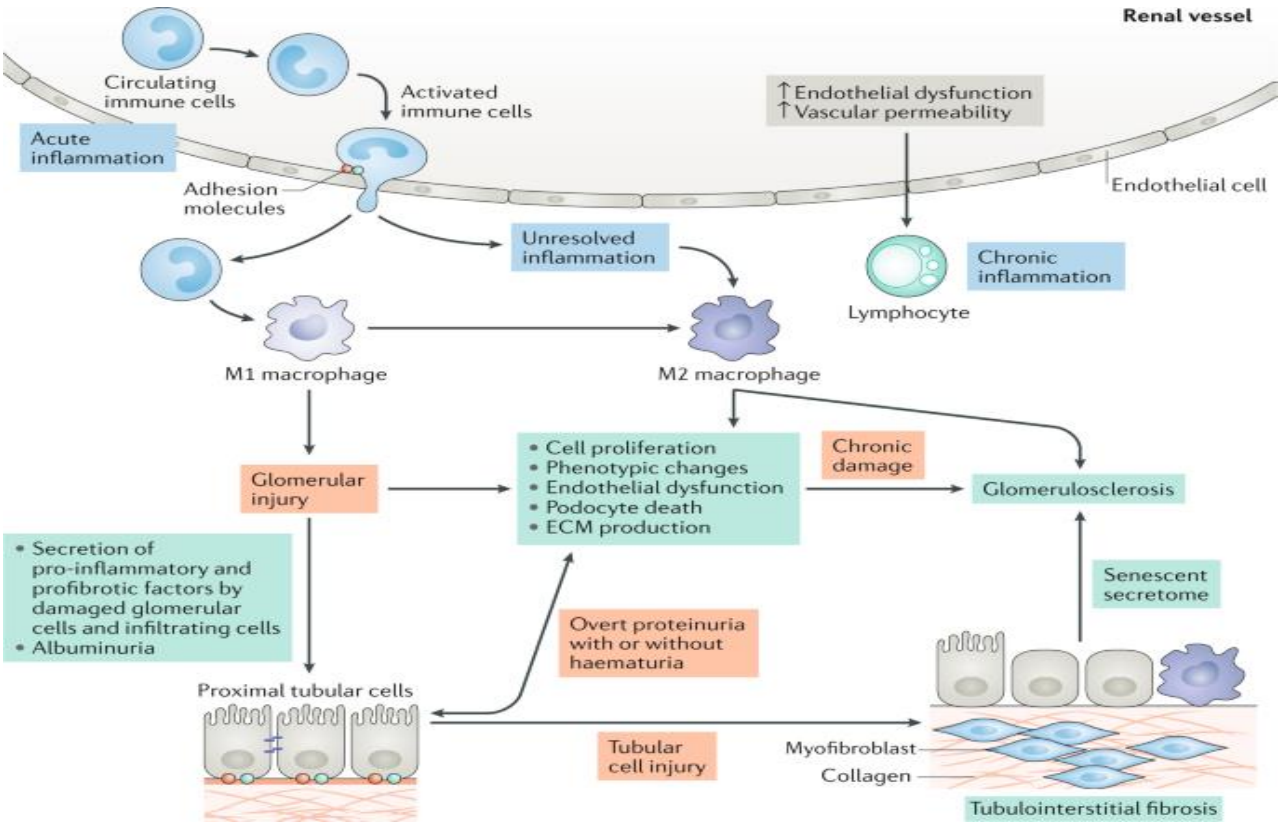

Figure1.1: Events causing progressive glomerular and tubulointerstitial damage in CKD (62) .

The fact is that a massive protein load can be a factor resulting in progressive damage of glomerular cells, including podocytes; resultant release of transforming growth factor-β1 eventually result in myofibroblast differentiation of mesangial cells (63, 64). Uncontrolled protein reabsorption by proximal tubules results in further intrinsic injury to this nephron hence leading to increased production of inflammatory and vasoactive mediators such as endothelin-1, interleukin-8, monocyte chemoattractant protein-1 and RANTES (65, 66). A number of cytokines and pro-inflammatory mediators are actively released including growth factors and vasoactive substances thus leading to irregular piling up of extracellular matrix collagen, local recruitment of macrophages, fibronectin and lymphocytes in the interstitium; this activates the transformation of interstitial cells into myofibroblasts hence causing interstitial fibrosis and inflammation (67-69).

1.4.2 Prevalence of CKD progression

The prevalence of CKD progression among patients with diabetes was 23% and 15.3% in patients without diabetes, while other studies reported that about 30% of the patients with diabetes had CKD progression (53, 70). The prevalence of CKD is higher in patients with hypertension than with diabetes. However, the number of diabetic patients with albuminuria is higher (71, 72). Diabetic patients progress to ESKD about twice as rapidly as those without diabetes (53, 72). As CKD progresses, many patients present with poorly controlled hypertension therefore resulting in more rapid CKD progression (73, 74). The prevalence of CKD progression was reported by a continued decrease in eGFR of > 4 ml/min/1.73 m^2/year). The prevalence of CKD progression in a UK cohort study was reported to be 46.7% and 38% in a study that was conducted in the USA (53, 75). The prevalence of CKD progression by a change in uPCR > 30% in two years was 59% in Australia while a lower prevalence of 26.1% was reported in a study that was conducted in Japan (76, 77).

1.4.3 Factors associated with CKD progression

Age, male sex, black race, hypertension, diabetes mellitus and proteinuria are known risk factors for CKD progression (78, 79). Younger age, lower eGFR, diabetes mellitus, tobacco smoking and elevated albuminuria are associated with increased odds for CKD progression (80, 81). Men, when compared to women, have increased risks for CKD progression with a rapid decline in eGFR, higher rates of all-cause and cardiovascular mortality (82, 83).

Black African ancestry, as a consequence of ethnic/racial disparities, are at increased odds for CKD progression (84, 85) . There is a 2- to 4-fold higher risk for ESKD in African-Americans thus necessitating life supporting treatment compared to their white counterparts (86, 87). Elevated serum creatinine levels, reduced eGFR have been associated with African ancestry and more rapid CKD progression (16, 88). Due to their genes, black individuals who include the

APOL1 high-risk genotypes, have increased risk of death due to CKD and its related complications (15, 89). Due to disparities in healthcare, socioeconomic and cultural factors, black individuals have higher prevalence of obesity/overweight, diabetes mellitus, hypertension and have 2-fold odds for albuminuria (86, 90, 91). Unemployment, lower levels of income and combined socioeconomic status (SES) were significantly associated with poorer health characteristics and CKD progression (92, 93). Poor socioeconomic status has been reported as the most important contributor to CKD progression especially in Africa (94, 95).

Hypertension is strongly associated with CKD progression (96, 97) and both elevated diastolic and systolic blood pressure are associated with rapid CKD progression (98, 99). Smoking is associated with increased rates of CKD progression and there is evidence that stopping smoking may help in halting CKD progression (100, 101). Obesity and/or hyperlipidaemia were found to be associated with CKD progression (102, 103) and weight loss and reducing dysregulated lipids is associated with reducing CKD progression (104, 105).

Among patients with diabetes, regardless of their status, proteinuria, elevated systolic blood pressure, heart failure, male sex and anaemia were strong independent predictors of rapid CKD progression (30, 106). Diabetes mellitus and elevated HbA1c were associated with CKD progression and studies have reported increasing HbA1c to be strongly associated with diabetic nephropathy and CKD progression among patients who were diagnosed with T2DM (107, 108). Studies have shown that CKD progression is highly prevalent in patients who present with proteinuria (77, 109) and using ARBs or ACEIs was successful in decreasing proteinuria when were compared to other antihypertensive drugs thus highlighting the effectiveness of ARBs/ACEIs in blocking RAAS which aims at decreasing proteinuria and halting CKD progression (110, 111).

Anaemia is frequent in late CKD stages which therefore results in poor outcomes including frequent hospitalization, higher cardiovascular complications, decline in quality of life and higher rates of death (112, 113). Anaemia in CKD results from impaired erythropoietin production by the diseased kidneys. Anaemia has been found to be strongly associated with CKD, CKD progression and worse clinical outcomes (113, 114). Elevated serum uric acid levels are frequently observed with reduced eGFR and hyperuricaemia which tends to occur before CKD, predicts CKD and is positively associated with CKD progression (79, 115, 116). Reduced serum calcium levels tend to occur as a result of elevated serum phosphate and decreased manufacture of 1,25(OH)2 vitamin D by the kidney due to hyperparathyroidism. Hypocalcemia is associated with rapid CKD progression (117, 118) and elevated serum phosphate levels were associated with CKD progression (119, 120).

Hyponatraemia as a result of excess fluid and water intake or diuretic use is common in CKD and it eventually results in increased odds of cardiovascular events, CKD progression, hospitalisation and increased mortality (121, 122). Hyperkalemia is common in advanced CKD; its prevalence rises with declining eGFR and it is associated with rapid CKD progression (123, 124). Metabolic acidosis is frequently found in advanced CKD and it is associated with poor outcomes including muscle mass loss, bone demineralization and impaired renal function(125, 126). The prevalence of metabolic acidosis rises with declining eGFR; studies have reported a prevalence of 40% in patients with advanced CKD stage 4, with greater rates of CKD progression (127, 128). Metabolic acidosis has increased odds for rapid decline in eGFR, CKD progression and worse consequences including death (129, 130).

1.4.4 Outcomes of CKD progression

CKD progression results in disease progression to a more advanced stage of CKD, ESKD requiring KRT and death (131, 132). All CKD stages are associated with higher risk of

cardiovascular complications, premature mortality, cognitive dysfunction, frequent hospitalisation and decreased quality of life (132, 133). Older age, reduced eGFR, reduced systolic blood pressure, anaemia and baseline diabetes are significant predictors of mortality among CKD patients (134, 135). Individuals with CKD progression and hypertension had four times higher mortality than those without hypertension (58, 134). CKD progression has increased risks of ESKD or death for patients with decreased eGFR at baseline, male sex, diabetes mellitus and those with uncontrolled blood pressure. Increased risk of ESKD is associated with high levels of albuminuria and increased risk of death among those with older age or cardiovascular comorbidity(136, 137).

1.4.5 Prevention of CKD progression

Prevention of CKD progression entails cardiovascular risk reduction using statins, good glycaemic control, blood pressure control, treatment of albuminuria, avoiding acute kidney injury (AKI) including avoidance of nephrotoxins (51, 96, 138). Massive proteinuria is associated with rapid CKD progression with decreased eGFR; when combined with hypertension, they both accelerate the rate of eGFR decline and death from ESKD (71, 139). Renoprotection using angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), direct renin inhibitors, SGLT2 inhibitors and mineralocorticoids aimed to halt or reverse the process of progressive renal damage, and studies have demonstrated that it is possible to stabilize or even reverse CKD (58, 140, 141).

Studies have also reported that stopping smoking, weight loss and reducing lipids may help in halting CKD progression (100, 101, 104, 105). Intensive treatment of modifiable risk factors like smoking, obesity, hypertension, diabetes mellitus, elevated proteinuria and hyperlipidaemia could be of value in preventing CKD progression (142, 143). If CKD progression is predicted earlier (stages 1 to 3a), disease progression may be halted with few complications (131, 144).

Diagnosis, staging and appropriate management of CKD across primary care practices including role of diet, lifestyle modifications and referral of CKD patients by primary care clinicians are key in decreasing CKD progression worldwide (51, 145-147).

1.5 Biomarkers of CKD Progression

1.5.1 Routinely used Biomarkers

Albuminuria and serum creatinine still form the core of most predictive models of CKD and risk of progression. However, both of these biomarkers exhibit alterations relatively late in the disease trajectory and thus are not sensitive for early diagnosis of CKD (148, 149). Albuminuria is a biomarker of early-stage CKD but excludes many patients who already have significant kidney damage eventually presenting with proteinuria and decrease in eGFR (150, 151). Albuminuria is also used as a marker of risk for CKD progression. However, strategies that reduce albuminuria do not always prevent CKD progression (152, 153). Urine albumin-to-creatinine ratio (ACR) and protein-to-creatinine ratio (PCR) are used to measure urine protein (154, 155). Recent guidelines recommend urine ACR to predict risk of ESKD but in areas where ACR is not available, urine PCR can be used (156, 157). Analyses of alterations in proteinuria as a predictor of CKD progression are heterogeneous and not complete thus highlighting the need for further studies on novel biomarkers (139, 158).

Measurement of the glomerular filtration rate (GFR) is the most recommended index of kidney function and is the most preferred common method to measure kidney function. However, it is very complex and has higher costs, and it is not done routinely in clinical practice (19, 159). The formulae for estimating GFR were developed to identify patients with CKD, with eGFR ≤60 mL/min/1.73 m^2 defined as stage 3 CKD; they are not sensitive for earlier stages of CKD (20, 134). Serum creatinine can be altered by a number of factors that are particularly changeable in CKD patients, including muscle mass, dietary protein; and exercise may over- or underestimate

eGFR (160, 161). Therefore, the estimating equations may over- or underestimate GFR; thus, serum creatinine does not have high predictive value (162, 163).

Addition of cystatin C tends to improve the sensitivity of creatinine-based equations (164, 165). Serum cystatin C, though not routinely used in clinical practice, is sensitive in mild GFR decline between 60 and 90 mL/min/1.73 m (2). However, there is no agreed method and no constant calibration material for cystatin C. Further limitations are the role of thyroid dysfunction, of extreme glucocorticoid doses and potentially the effect of cardiovascular diseases on cystatin C levels (166, 167). β-Trace protein (BTP) and β2-microglobulin (B2M) are novel glomerular filtration markers that are rarely influenced by age, sex, and race than creatinine, and rarely influenced by race than cystatin C, but provide inaccurate GFR estimates compared with cystatin C and CKD-EPI creatinine equations (168, 169).

Up to 20% of CKD patients with diabetes would have other secondary or alternative aetiologies if biopsies were performed (170, 171) and about 50% of the patients with diabetes and reduced eGFR had no proteinuria (172, 173). To patients who had no proteinuria and those with early CKD disease, albuminuria and serum creatinine did not predict their CKD progression (174, 175). In predicting progression of CKD to ESKD, the kidney failure risk equation (KFRE) has been used. The eGFR and serum calcium levels were strongly associated with kidney failure in the measurement models unlike male sex, phosphate, albuminuria, and bicarbonate levels (176, 177). The KFRE is accurate for eGFR < 45 ml/min per 1.73 m^2 when using the CKD-EPI 2021 equation (154, 178). In this regard, studies have demonstrated a variety of novel biomarkers that occur prior to changes in albuminuria, cystatin-C and serum creatinine (148, 179).

1.5.2 Novel biomarkers

Novel biomarkers may help in predicting CKD progression earlier compared with the routinely used biomarkers of albuminuria, cystatin-C and serum creatinine (180, 181). A good biomarker should be rapid, non-invasive and specific and correlate well with kidney pathology (144, 182).

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine produced mainly by T-lymphocytes (183, 184). TNF-α-induced changes in renal haemodynamics and excretory function are mediated by two receptors (TNFR1 and TNFR2) (183, 185). Serum TNFRs were found to be significantly increased in early CKD and were associated with rapid CKD progression (184, 186). Elevated levels of serum TNFRs were associated with decline in eGFR and rapid CKD progression, and hence may be useful for early detection of CKD progression (185, 187, 188).

Matrix metalloproteinase (MMPs) play an important role in the degradation of the extracellular matrix (ECM), development CKD and CKD progression (189, 190). During the process of renal fibrosis, levels of MMP-2 and 9 are upregulated rapidly which is important for the development of kidney fibrosis (191, 192). Increased circulating MMP-9 levels are associated with resistant albuminuria thus contributing to decline in eGFR and CKD progression (193, 194). Matrix metalloproteinase (MMPs) have been linked to kidney fibrosis and ESKD, suggesting that they may be used as novel biomarkers for predicting early CKD progression (195, 196).

Glomerular function and injury are detected by eGFR decline and albuminuria whereas CKD progression mainly occurs as a result of tubular cell atrophy and interstitial fibrosis (197-199). Uromodulin (also known as the Tamm–Horsfall protein) is a glycoprotein produced in the tubular cells of the thick ascending limb and the early distal tubule (200, 201). Uromodulin is a good marker for intact renal mass and can be used for identification of early CKD progression (202, 203). The combined analysis of serum and urinary uromodulin gives new insights into the

role of uromodulin in CKD and suggests that uromodulin may be an active player in CKD progression (203, 204). Serum and urinary uromodulin can be used as a biomarker for predicting early CKD progression (205-207). Urinary IL-18 was associated with eGFR decline and CKD progression beyond serum creatinine, serum cystatin C and urine albumin (208, 209). Decreased urinary epidermal growth factor (EGF) concentration and elevated urinary monocyte chemoattractant protein 1 (MCP-1), kidney injury molecule-1 (KIM-1), and α-1 microglobulin concentrations were each associated with CKD progression (198, 210, 211). Elevated plasma KIM-1 levels were independently associated with decreased eGFR and CKD progression (188, 212). There is a significant association between higher urinary MCP-1 and risks of incident CKD and CKD progression (207, 213). Among patients with CKD, urinary neutrophil gelatinase-associated lipocalin (NGAL) was independently associated with CKD progression, ESKD or death (214-216). Urinary N-acetyl-beta-d-glucosaminidase (NAG) can be used as an early marker in the diagnosis of diabetic nephropathy since it increases first before the other markers (211, 217). Changes in urinary liver-type fatty acid-binding protein (L-FABP) levels occurred earlier than in urinary albumin hence making it a potential novel biomarker for detection of all stages of diabetic nephropathy and for early prediction of CKD progression (218-220).

Transforming growth factor-beta (TGF-β) has been regarded as the master cytokine in kidney inflammation and fibrosis through the TGF-β/Smad signalling. Kidney fibrosis is the common pathway that leads to CKD progression and ESKD (221, 222).

1.5.3 Transforming growth factor-beta (TGF-β)

Transforming growth factor-beta (TGF-β) proteins belong to the TGF-β super family which is made up of 33 members that are further sub classified into several subfamilies, including the TGF-βs (TGF-ß1, -β2, and -β3) (223, 224). TGF-ß1, -β2, and -β3 are encoded by genes on

different chromosomes. Their isomers share 60–80% homology and their expression has been confirmed in the human kidney. TGF-β2 and TGF-β3 are found in podocytes, whereas TGF-β1 is chiefly found in mesangial cells and tubules (225, 226).

Transforming growth factor-β has contrasting roles in kidney fibrosis and inflammation. TGF-β commonly modulates kidney fibrosis and inflammation via the Smad-dependent mechanisms requiring Smad2, Smad3, Smad4, Smad7 and specifically through the Smad3-dependent non-coding RNAs (227, 228). Smad2 is protective, while Smad3 is pathogenic in fibrosis thus in CKD, TGF-β/Smad signalling is down regulated, thus leading into excessive activation of Smad3 and Smad4 with low Smad7. This imbalance may account for the kidney inflammation and fibrosis culminating into ESKD (31, 229). Figure 1.2 highlights these findings.

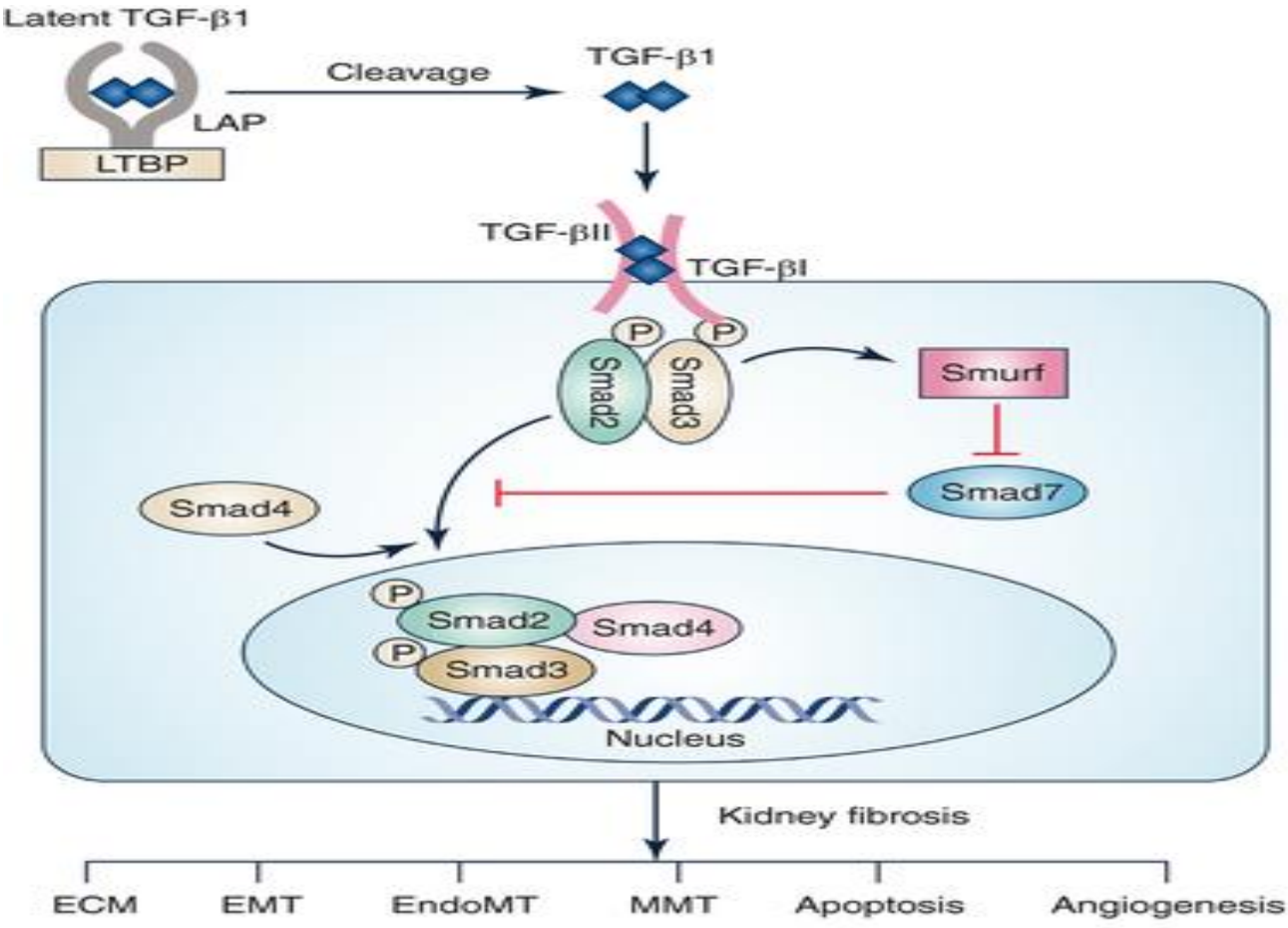

Figure 1.2: The TGF-β/Smad signalling and kidney fibrosis (229).

Numerous stress molecules such as TGF-β1, EGF, TGF-α, Ang II, ROS and AGEs can turn on individual pathways and associate with the TGF-β/Smad signalling pathway to control renal fibrosis and inflammation (31, 230). Most studies have been done extensively on TGF-β1. TGF-β1 is regarded as a profibrotic mediator in most kidney diseases (231, 232). It has anti-inflammatory and antiproliferative properties as it inhibits production of tumor necrosis factor-α (TNF-α) or it reduces the proinflammatory effects of IL-1β and interferon-γ in the heart and blood vessels (233, 234). Through its effects on mesangial cells, podocytes, endothelial and tubular cells, TGF-β1 play a critical role in the pathogenesis of glomerular and tubulointerstitial renal diseases (235, 236).

TGF-β1 plays an extremely important role in kidney fibrosis and its signalling is finely regulated by miRNAs (237, 238). Expression of miRNAs involved in regulatory mechanisms is key to increasing levels of TGF-β1 in CKD progression (239, 240). TGF-β1 incites changes in the expression of genes in kidney mesangial cells causing inflammation and it is highly expressed in most kidney diseases that are associated with fibrosis (241, 242). It accounts for the pathological alterations, which induce changes in the glomerular filtration barrier, glomerulosclerosis and fibrosis, eventually causing degeneration of tubules leading to permanent renal dysfunction (243, 244).

In studies among patients with type 2 diabetes mellitus and those with albuminuria, the levels of TGF-β1 were significantly increased making it a potential biomarker of CKD progression (238, 245). Similar findings were reported in another study where TGF-β1 levels were found to be increased before documented decline of renal function in healthy cats, that were subsequently established to have CKD and predicted CKD progression. It corresponded with histopathological confirmation of renal inflammation and interstitial fibrosis (240, 246). These findings advocate that both serum and urinary TGF-β1 may be used as biomarkers for prediction of early CKD

progression and its outcomes. Studies have reported that TGF-β2 plays a role in the fracture healing process (247, 248) while TGF-β3 has a role in immune mediated responses (249, 250); however, there is a paucity of data on TGF-β2 and TGF-β3 as biomarkers that can predict CKD progression.

1.5.4 Interaction of transforming growth factor-beta (TGF-β) and MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that behave as post-transcriptional repressors which are gaining momentum as biomarkers in a number of disease areas (251, 252). The miRNAs prevent protein synthesis due to mRNA destabilization, initially by arresting translation at an early step which ultimately is followed by deadenylation of target mRNAs, eventually resulting in mRNA degradation (253-255)**.**

MiRNAs act on cellular metabolism, differentiation, and proliferation of the kidney, which directly or indirectly have the capacity of controlling the pathophysiology of an array of kidney diseases (256, 257). TGF-β1 cause changes in the expression of genes in kidney mesangial cells resulting in inflammation and fibrosis. Expression of miRNAs entailed in regulatory mechanisms is central to high levels of TGF-β1 in CKD progression (239, 258). TGF-β1 and miRNAs play a crucial role in the initiation and development of tubulointerstitial sclerosis and end-stage glomerular lesions that are found in CKD (237, 259-261). Figure 1.3 highlights these findings.

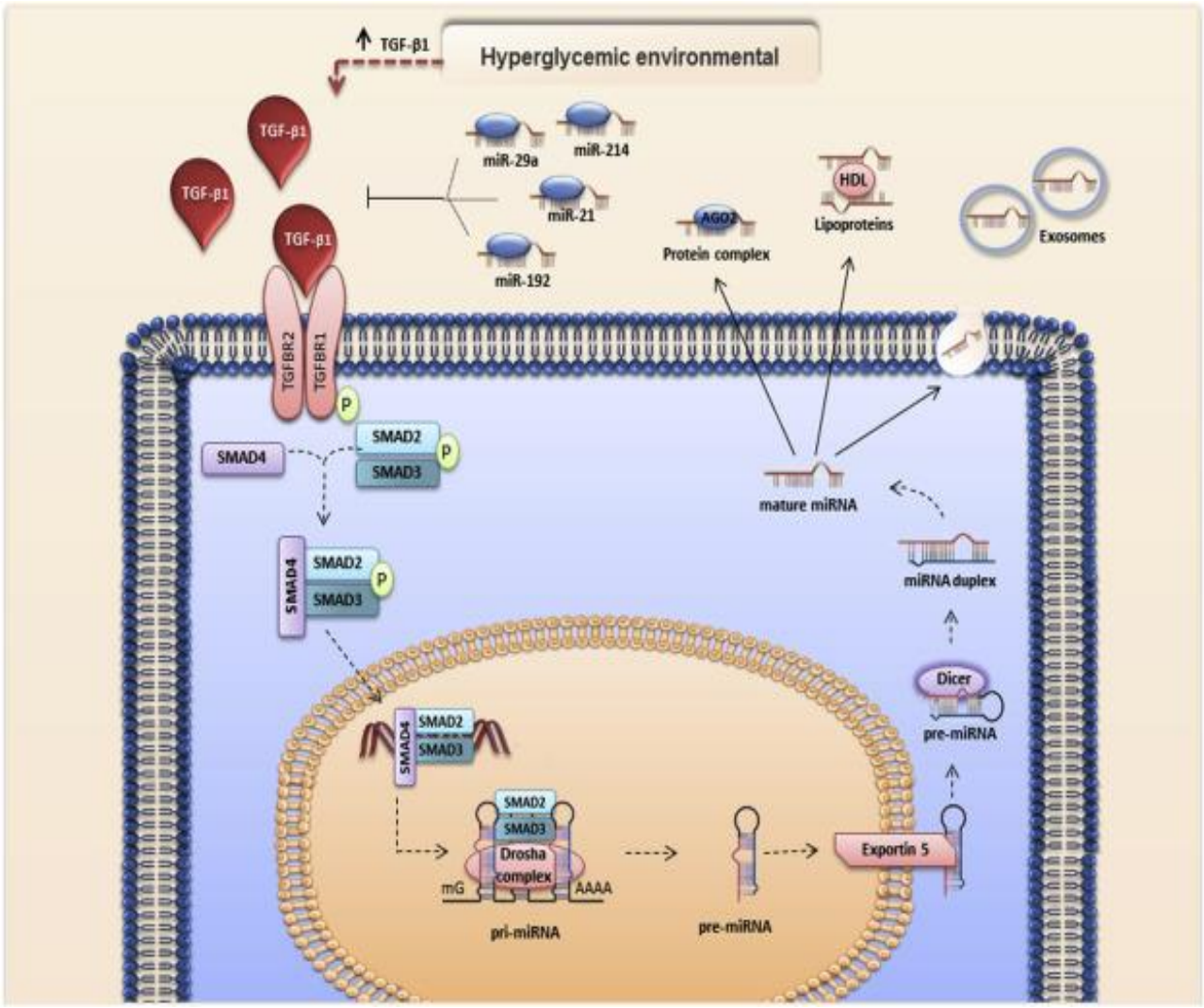

Fig. 1.3: Interactions between TFG-β1/Smad signalling and miRNAs (237).

MiRNAs have a critical role in the progression of CKD; miRNAs can be utilized as potential and effective biomarkers for early CKD diagnosis and in monitoring of CKD progression (257, 262, 263). It is evident that miRNAs are involved in renal fibrosis and some of the miRNAs, such as Let-7, miR-21, and -192 play a vital role in renal fibrosis (264-266). The miRNAs signal TGF-β mediated kidney fibrosis; the miRNAs whose levels are increased by TGF-β signaling include miR-21, the miR-181 family, miR-155 and miR-192 (257, 267-269). Plasma miR-155-5p was elevated among CKD patients and further elevated among individuals presenting with both CKD and hypertension (270, 271).

In a study on non-coding RNAs in the urine of CKD patients as suitable biomarkers for disease progression, miRNA-181a was seen to be the predominant robust biomarker for CKD progression (272, 273). Studies have also shown that miRNA-21 both in serum and urine may be a special, sensitive, specific, reliable, noninvasive biomarker for early detection of hypertensive renal injury, fibrosis and most CKD diseases (182, 274). TGF-β and miRNAs are associated with kidney fibrosis, and both levels in urine and plasma may be used for diagnosis and monitoring progression of CKD (275, 276).

1.6 Summary of the study and the research gaps

Chronic kidney disease (CKD) progression is defined as a change in GFR or markers of kidney damage or both, over a set time period (49, 50). Screening of patients at risk for CKD by serum creatinine/eGFR and urine examination will benefit from point-of-care measurements, so that there are no delays in diagnosis or patients lost to care (277, 278). Detection of early CKD progression aims to help clinicians identify patients with CKD at higher risk of rapid progression and those for whom close monitoring is needed (51, 52). When patients with CKD progression are diagnosed in early CKD (stages 1 to 3), disease progression may be reversed with minimal complications (10, 78). Clinicians and nephrologists should identify CKD patients who are likely to deteriorate quickly and predict their CKD progression at various stages (163, 174). Early recognition of the CKD patients at risk of more rapid CKD progression would improve risk stratification and better outcomes (24, 279).

In developing countries like South Africa, as a result of the rising prevalence of CKD, black patients have higher chances of dying due to CKD and its related complications (280, 281). Elevated serum creatinine levels and reduced eGFR have been associated with African ancestry, thus recalculation of eGFR basing on genetic ancestry has affected CKD staging (15, 16). This has brought studies in developing novel biomarkers that would help in such prediction far above

the commonly utilized legacy biomarkers such as serum creatinine, albuminuria, and cystatin-C (24, 28). There have been intensive efforts made in the discovery of novel biomarkers in the past two decades, but their application in the clinical setting is an area that requires further studies (19, 239).

Studies on novel biomarker discovery are essential to allow more accurate prediction of CKD progression in its earlier stages and correlate better with the decline of eGFR (23, 282). Breakthrough in CKD care is delayed by the unavailability of biomarkers that can diagnose and predict CKD progression early for timely and appropriate interventions (25, 283). There is scarce data on novel biomarkers that can predict early progression of CKD among black patients. Therefore, the aim of this study was to identify novel biomarkers that could predict CKD progression among black CKD patients in South Africa.

1.6.1 Research hypotheses

Our research hypotheses were as follows:

- Novel biomarkers may be more accurate in prognosticating kidney disease compared with routinely used biomarkers among black CKD patients in South Africa.
- Urinary novel biomarkers may be more accurate in prognosticating kidney disease compared with serum novel biomarkers among black CKD patients in South Africa.

1.6.2 AIMS:

To study novel biomarkers that predict CKD progression among black patients attending a tertiary hospital in Johannesburg, South Africa.

1.6.3 Specific objectives

- To determine the prevalence of CKD progression using routinely used biomarkers among black patients in South Africa.

- To determine the risk factors associated with CKD progression among black patients in South Africa.
- To determine the most predictive novel biomarkers for CKD progression among black patients in South Africa.
- To compare urinary novel biomarkers and serum novel biomarkers in predicting CKD progression among black patients in South Africa.

CHAPTER 2. MATERIALS AND METHODS

2.1 Study design, setting and population

To study novel biomarkers that predict CKD progression among black patients attending the kidney outpatient clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a prospective longitudinal study was conducted from September 2019 to March 2022. The enrollment period was done from September 2019 to March 2020 and follow up was conducted from September 2021 to March 2023. The CMJAH is an accredited public hospital with a capacity of 1088 beds serving patients in Gauteng province and close by provinces in South Africa. CMJAH is also one of the primary teaching and tertiary hospitals for the Faculty of Health Sciences of the University of the Witwatersrand.

Johannesburg is the largest city in South Africa and the capital of Gauteng province. Johannesburg had an estimated population of around 5.9 million in 2021. The ethnic groups include black Africans (76.4%), Whites (12.3%), mixed ethnicity (5.6%) and Indian/Asians (4.9%). The study population comprised of black patients with CKD stages 1 – 4 according to the K/DOQI CKD Guidelines (284), attending the kidney outpatient clinic at CMJAH, in which approximately 120-150 patients attend per clinic visit weekly, of whom more than 75% are of black ethnicity. Personnel skilled in all the techniques required to conduct this project including biomarker evaluation and a well-equipped laboratory, were available in the Department of Internal Medicine.

2.1.1 Inclusion and exclusion criteria

Inclusion criteria:	Exclusion criteria:
Age ≥ 18 years	Infections including HIV, Hepatitis B & C, FSGS, other GN, SLE, other autoimmune conditions & patients on immunosuppressants
Black ethnicity	White, Indian, Asian
Informed consent	Active malignancy
CKD (stages 1- 4)	ESKD (CKD stage 5)
Controlled hypertension (BP<140/90 mmHg) Controlled diabetes mellitus (Hb A_{1c} <7%) (285, 286).	Uncontrolled hypertension Uncontrolled diabetes mellitus

2.1.2 Study outcomes

The primary outcome measure was CKD progression which was determined by; (i) continued decrease in eGFR of > 4 ml/min/1.73 m^2/year or more (53) (ii) change to a more severe stage of CKD (51) (iii) more than 30% reduction of eGFR in two years, whereby percent change in eGFR was calculated as follow up eGFR – baseline eGFR/ baseline eGFR x 100 (54) (iv) more than 30% change in uPCR in two years, whereby change in uPCR was calculated as follow up uPCR – baseline uPCR / baseline uPCR (55). CKD remission/ CKD regression was determined by less than 30% change in uPCR in two years, whereby change in uPCR was calculated as follow up uPCR – baseline uPCR / baseline uPCR (55). The secondary outcome was development of ESKD (dialysis or transplantation) during the study period.

2.2 Study procedures

2.2.1 Clinical procedures

Demographic and clinical data comprising of gender, age, weight, height, body mass index (BMI), aetiology of CKD, glycaemic status, history of smoking, and medications were retrieved

from the KOPD clinic data and face to face interviews at enrolment that were filled in a questionnaire and vital signs; were measured at recruitment and at follow up. Systolic and diastolic blood pressure were assessed 3 times at a clinic visit and the mean of the second and third readings were used. Patients with blood pressure < 140/90 mm Hg) and haemoglobin A_{1c} concentration < 7% for those with diabetes mellitus were enrolled into the study. Uncontrolled hypertension and uncontrolled diabetes mellitus are known to cause and can lead to rapid CKD progression to ESKD (181, 285-287). Body mass index (BMI) was determined using the National Health Services (NHS-UK) BMI calculator (288).

2.2.2 Laboratory procedures

Patients provided blood and urine samples at enrolment and at follow up (24 months). Patients gave consent to have their laboratory data reviewed prospectively for two (2) years. Routine laboratory investigations were done by the National Health Laboratory Services (NHLS) utilizing the Cobas 6000 analyser (Roche Diagnostics, USA). Measurements of serum creatinine, FBP, serum transferrin levels, urinary protein creatinine ratio (uPCR), electrolytes, RBG/FBG, HbA1C, HDL cholesterol calcium and phosphate done at the time of the first visit and at 24 months of follow up. Anaemia was defined as a Hb concentration of <12.0g/dl in females and <13.0g/dl in males (289). The isotope dilution mass spectrometry (IDMS) traceable enzymatic assay was utilized to calculate serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (without using the African American correction factor) was utilized in estimating the glomerular filtration rate (eGFR) (290).

2.2.3 Measurement of baseline serum and urine TGF isomers

At enrolment (baseline), blood samples were collected into plain gel separator vacutainer tubes for the preparation of serum. The samples were allowed to clot for half an hour and then centrifuged at 4000 rpm for 20 minutes after which serum was aliquoted into 1.5ml cryotubes

and kept at -80°C to be defrosted on the day of performing the assay. At enrolment (baseline) urine samples were collected then centrifuged at 4000 rpm for 20 minutes. Supernatant was aliquoted into 1.5ml cryotubes and kept at -80°C to be defrosted on the day of performing the assay. The Human TGF- β DuoSet ELISA (R&D Systems, Inc., Minneapolis, MN) was utilized to perform the baseline serum and urine TGF-β1, TGF-β2 and TGF-β3 levels and the assays were done in accordance with the manufacturer's instructions. 1N hydrochloric acid was used to activate the serum and urine samples and a 1.2N sodium hydroxide/ 0.5M HEPES solution was use for neutralization hence resulting in a 1 in 2 sample dilution. Serum samples assayed for TGF-β1 were further diluted for a final sample assay dilution of 1 in 100. A microplate reader (Biotek 800TS, Agilent, US) set to 450 nm with wavelength correction set at 650nm was utilized to determine optical densities. The serum and urine transforming growth factor-ß isoforms were measured at enrolment (baseline).

2.3 Statistical Methods

The statistics used are described in the respective chapters.

2.4 Ethical considerations

The Human Research Ethics Committee of the University of Witwatersrand, Johannesburg; provided the ethical approval to conduct this study with ethics clearance certificate No. M190553. Each of the participants gave informed consent before enrolment and starting of data collection. The patients did not suffer any penalty for refusing to be part of the study since participation was voluntary. To ensure proper identification without breaching confidentiality, a study identification number was allocated to every participant.

CHAPTER 3 (PUBLISHED IN THE PLOS ONE JOURNAL ON 19.09.2022, APPENDIX D):

DEMOGRAPHIC AND CLINICAL PROFILE OF BLACK PATIENTS WITH CHRONIC KIDNEY DISEASE ATTENDING A TERTIARY HOSPITAL IN JOHANNESBURG, SOUTH AFRICA

Abstract

Background: The prevalence of chronic kidney disease (CKD) is increasing worldwide; black patients have an increased risk of developing CKD and end stage kidney disease (ESKD) at significantly higher rates than other races.

Methods: A cross sectional study was carried out on black patients with CKD attending the kidney outpatient clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa, from September 2019 to March 2020. Demographic and clinical data were extracted from the ongoing kidney outpatient clinic records and interviews, and were filled in a questionnaire. Patients provided blood and urine for laboratory investigations as standard of care, and data were descriptively and inferentially entered into REDcap and analysed using STATA version 17. Multivariable logistic regression analysis was used to identify demographic and clinical variables associated with advanced CKD.

Results: A total of 312 black patients with CKD were enrolled in the study with a median age of 58 (IQR 46 - 67) years; 58% patients had advanced CKD, 31.5 % of whom had grossly increased proteinuria, 96.7 % had hypertension, 38.7 % had diabetes mellitus and 38.1 % had both hypertension and diabetes mellitus. In patients with advanced CKD, the median age was 61 (IQR 51-69) years, eGFR 33 (30 -39) mL/min/1.73 m^2, serum bicarbonate 22 (IQR 20 – 24), haemoglobin 12.9 (IQR 11.5 – 14.0) g/dl and serum uric acid 0.43 (IQR 0.37 – 0.53). The prevalence of metabolic acidosis was 62.4 %, anaemia 46.4 % and gout 30.9 % among those with advanced CKD, while the prevalence of metabolic acidosis and anaemia was 46.6 % and

25.9 % respectively in those with early CKD. Variables with higher odds for advanced CKD after multivariable logistic regression analysis were hypertension (OR 3.3, 95 % CI 1.2 - 9.2, P = 0.020), diabetes mellitus (OR 1.8, 95 % CI 1.1 - 3.3, P = 0.024), severe proteinuria (OR 3.5, 95 % CI 1.9 - 6.5, P = 0.001), angina (OR 2.5, 95 % CI 1.2 - 5.1, P = 0.008), anaemia (OR 2.9, 95% CI 1.7 - 4.9, P= 0.001), hyperuricemia (OR 2.4, 95 % CI 1.4 - 4.1, P = 0.001), and metabolic acidosis (OR 2.0, 95% CI 1.2 - 3.1, P= 0.005). Other associations with advanced CKD were loss of spouse (widow/widower) (OR 3.2, 95 % CI 1.4 - 7.4, P = 0.006), low transferrin (OR 2.4, 95% CI 1.1 - 5.1, P= 0.028), hyperkalemia (OR 5.4, 95% CI 1.2 - 24.1, P= 0.029), use of allopurinol (OR 2.4, 95 % CI 1.4 - 4.3, P = 0.005) and doxazosin (OR 1.9, 95% CI 1.2 - 3.1, P = 0.006).

Conclusion: Hypertension and diabetes mellitus were strongly associated with advanced CKD, suggesting a need for primary and secondary population-based prevention measures. Metabolic acidosis, anaemia with low transferrin levels, hyperuricemia and hyperkalemia were highly prevalent in our patients, including those with early CKD, and they were strongly associated with advanced CKD, hence requiring clinicians and dietitians to be proactive in supporting the needs of CKD patients in meeting their daily dietary requirements towards preventing and slowing the progression of CKD.

Key words: Demographic characteristics, clinical profile, black patients, chronic kidney disease

3.1 Introduction

Chronic kidney disease (CKD), defined as decreased kidney function identified by glomerular filtration rate (GFR) of less than 60 mL/min per 1·73 m^2, or markers of kidney damage, or both, of at least 3 months duration, regardless of the underlying cause (2); is a major public health issue worldwide and contributes immensely to the overall non-communicable disease (NCD) burden, with NCDs also contributing to the burden of CKD (27, 291). Chronic kidney disease is

usually asymptomatic until the more advanced stages and accurate prevalence data are lacking in most regions including sub-Saharan Africa (14). As the prevalence of CKD is increasing worldwide and consequently the demand for kidney replacement therapy (KRT); the incidence of cardiovascular events and death is also increasing (34, 71).

Recent systematic reviews have reported the prevalence of CKD to be 15.8% in Africa, similar to other continents, thus constituting a true public health need with major cost implications to healthcare systems (2, 292). Diabetes mellitus and hypertension are the leading causes of CKD worldwide; and in sub-Saharan Africa, hypertension is the leading cause of CKD (293, 294). Studies have shown that African-Americans have a 2- to 4-fold greater risk for end stage kidney disease (ESKD) thus requiring more renal replacement therapy than their white counterparts (86, 87) . Individuals of black ethnicity due to their genetics, including the presence of *APOL1* high-risk genotypes, are at higher risk of death due to CKD as a result of social, economic and medical causes (15, 89). African ancestry has been associated with higher serum creatinine levels, lower eGFR estimates and more rapid CKD progression (16, 88).

In addition to the known risk factors for advanced CKD, which are age, male sex, black race, arterial hypertension and proteinuria, other modifiable factors including medications (traditional and herbal), hyperuricemia, hyperlipidemia, elevated phosphate levels, heart failure and anaemia are common in CKD at later stages (42, 43). Metabolic acidosis increases with worsening eGFR, with prevalence of around 40% among patients with CKD stage 4 and it is associated with rapid CKD progression (127, 128). Anaemia is common in CKD and is frequently associated with poor outcomes, including increased cardiovascular risks, hospitalization, decreased quality of life and increased risk of mortality (112, 113). Hyperuricemia greatly contributes to the development of CKD and its progression; it appears that increasing uric acid levels increase the risk for CKD development by causing inflammation, endothelial cell injury

and activation of the renin-angiotensin system (44, 295). Hyperkalemia is also common in advanced CKD; its prevalence increases with decreasing eGFR and it is significantly associated with faster CKD progression (124, 296). Thus, the aim of this study was to determine the demographic and clinical profile of black patients with CKD attending Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa.

3.2 Methods

3.2.1 Study design, population and settings

This was a cross-sectional study to evaluate the demographic and clinical profile of black patients with CKD attending the kidney outpatient department (KOPD) clinic at CMJAH from September 2019 to March 2020. The CMJAH is a public accredited central hospital with 1088 beds serving patients from across the Gauteng province and nearby provinces in South Africa. The CMJAH is also the main teaching hospital for The University of the Witwatersrand, faculty of Health Sciences. Johannesburg is the largest city in South Africa and among the largest 50 urban agglomerations in the world. Johannesburg had an estimated population of around 5.9 million in 2021, the most common ethnic groups include black Africans (76.4%), coloreds (5.6%), Whites (12.3%) and Indian/Asians (4.9%).

Inclusion criteria included patients who were >18 years of age, CKD stages 1 – 4, who had controlled hypertension (blood pressure < 140/90 mm Hg) and diabetes mellitus (HbA1C < 7%), attending the KOPD clinic for at least 6 months and were able to give informed consent. Patients who had active infections, active malignancies, autoimmune diseases and who were not black were excluded. Black patients have an increased risk of developing CKD and end stage kidney disease (ESKD) at significantly higher rates than other races (88, 89).

3.2.2 Data collection and laboratory procedures

Demographic and clinical data including age, gender, weight, height, glycemic status, history of smoking, aetiology of CKD and medications; were extracted from the ongoing continuous KOPD clinic records and face to face interviews, and were filled in a questionnaire. Systolic and diastolic blood pressure was measured 3 times, the average of the second and third measurements was used. Body mass index (BMI) was calculated using the National Health Services (NHS-UK) BMI calculator (288). Measurements of urinary protein creatinine ratio (uPCR), serum creatinine, electrolytes, HbA1C, WBC, haemoglobin level, platelets, calcium, phosphate, transferrin and HDL cholesterol were done as standard of care at the time of recruitment during a clinic visit. Serum creatinine was measured using the isotope dilution mass spectrometry (IDMS) traceable enzymatic assay and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation without using the African American correction factor (290). Patients with eGFR < 45 ml/min/1.72m^2 were considered to have advanced CKD, while those patients with eGFR ≥ 45 ml/min/1.72m^2 early CKD.

3.2.3 Data management and analysis

Study data were collected and entered into REDCap (Research Electronic Data Capture) tools (297, 298) hosted at the University of the Witwatersrand and analyzed using STATA version 17 (College Station, Texas, USA). Descriptive statistics were used to summarize demographic and clinical characteristics; continuous variables have been reported as medians with interquartile ranges and Wilcoxon rank-sum test was used for the non-normally distributed variables. Discrete variables have been reported as frequencies and proportions, Pearson's chi-square test were used to test for association between two variables. Odd ratios were used to estimate the strengths of the association between variables and advanced CKD, univariate and multivariate logistic

regression models have been used to estimate the association of variables and advanced CKD. Variables with p-value less than 0.2 on univariate logistic regression models were then fitted into the multivariate logistic regression models with the addition of age and sex as adjusting variables. Variables with a p-value of less than 0.05 were considered to have significant strength of association.

3.2.4 Ethical issues

Ethical approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand, Johannesburg (ethics clearance certificate No. M190553). Written informed consent was obtained from each of the participants before embarking on data collection.

3.3 Results

3.3.1 Demographic and clinical characteristics of the study population

Of the 476 black patients with CKD stages 1- 4, 164 patients were excluded from the study including 110 patients who had uncontrolled hypertension, 35 patients who had uncontrolled diabetes mellitus, 11 patients had autoimmune diseases, 6 patients had active infections and 2 patients had active malignancies. A total of 312 CKD black patients were enrolled into this study, of whom 162 (51.9 %) were males, 292 (93.6%) were hypertensive, 103 (33.0%) were diabetic and 164 (52.6 %) were married. The median age was 61 (IQR 51-69) years for advanced CKD and 53 (IQR 41 -62) years for early CKD; the median eGFR was 33 (30 -39) mL/min/1.73 m^2 for advanced CKD and 60 (IQR 51 – 75) mL/min/1.73 m^2 for early CKD; the median urine protein creatinine ratio (uPCR) was 0.029 (IQR 0.015 -0.67) g/mmol for advanced CKD and 0.016 (IQR 0.008 – 0.034) g/mmol for early CKD. The median serum bicarbonate was 22 (IQR 20 – 24) mmol/L for advanced CKD and 23 (IQR 21 -25) mmol/L for early CKD; the median haemoglobin (Hb) was 12.9 (IQR 11.5 – 14.0) g/dl for advanced CKD and 13.8 (IQR 12.4 – 15.7) g/dl for early CKD; the median serum transferrin was 2.44 (IQR 2.23 – 2.73) g/L for

advanced CKD and 2.62 (IQR 2.37 – 2.89) g/L for early CKD. The median serum uric acid was 0.43 (IQR 0.37 – 0.53) mmol/L for advanced CKD and 0.36 (IQR 0.30 – 0.46) mmol/L for early CKD (Table 3.1).

Table 3.1: Demographic characteristics and clinical profile of 312 CKD patients by eGFR

Characteristic	eGFR < 45 ml/min/1.72m^2 (n=181) Proportion (%) or Median (IQR)	eGFR ≥ 45 ml/min/1.72m^2 (n= 131) Proportion (%) or Median (IQR)	P-value
Age (years)	61 (51-69)	53 (41 -62)	**0.001**
Sex			
Male	96 (53.0%)	66 (50.4%)	
Female	85(47.0%)	65 (49.6%)	0.643
Marital status			
Single	43(23.8%)	48(36.6%)	
Married	98 (54.1%)	66 (50.4%)	
Widow/Widower	30(16.6%)	10 (7.6%)	
Separated/Divorced	10(5.5%)	7 (5.4%)	**0.026**
Highest level of education			
No formal education	24(13.3%)	14 (10.7%)	
Primary	41(22.7%)	30 (22.9%)	
Secondary	56(30.9%)	43 (32.8%)	
Tertiary	60(33.1%)	44 (33.6%)	0.505
Occupation			
Unemployed	26(14.4%)	19(14.5%)	
Domestic workers	37(20.4%)	25(19.1%)	
Self employed	43(23.8%)	27(20.6%)	
Public / Private servant	57(31.5%)	52(39.7%)	
Retired	18(9.9%)	8(6.1%)	0.523
BMI (kg/m^2)	30.2 (26.0 -34.5)	30.2 (26.6 - 34.7)	0.731
SBP (mmHg)	140 (130 – 140)	140 (128 -140)	0.339
DBP (mmHg)	82 (72 – 90)	83 (74 -90)	0.630
uPCR (g/mmol)	0.029 (0.015 -0.67)	0.016 (0.008 – 0.034)	**0.001**
Creatinine (umol/L)	163 (141-190)	109 (88 -122)	**0.001**
eGFR (ml/min/1.72m^2)	33 (30 -39)	60 (51 – 75)	**0.001**
FBG (mmol/L)	4.5 (4.2 – 5.2)	4.4 (4.2 – 4.9)	0.146
HbA1C (%)	7.0 (6.6 – 7.0)	7.0 (7.0 – 7.0)	0.222
Haemoglobin (g/dl)	12.9 (11.5 – 14.0)	13.8 (12.4 – 15.7)	**0.001**
Transferrin (g/L)	2.44 (2.23 – 2.73)	2.62 (2.37 – 2.89)	**0.001**
WBC (x 10^9 cells/L)	6.4 (5.13 – 7.71)	6.03 (4.81 – 7.73)	0.420
Platelets (x 10^9 cells/L)	256 (213 -320)	271 (217 – 325)	0.378
Uric acid (mmol/L)	0.43 (0.37 – 0.53)	0.36 (0.30 – 0.46)	**0.001**
HDL cholesterol (mmol /L)	1.23 (0.98 -1.48)	1.22 (1.02 – 1.52)	0.528
Calcium (mmol /L)	2.31 (2.23 – 2.40)	2.33 (2.26 – 2.42)	0.054
Phosphate (mmol /L)	1.1 (0.94 – 1.24)	1.02 (0.85 – 1.15)	**0.005**
Sodium (mmol/L)	141 (138 – 143)	141 (139 – 143)	0.965
Potassium (mmol/L)	4.4 (3.9 – 4.8)	4.1 (3.9 – 4.4)	**0.001**
Bicarbonate (mmol/L)	22 (20 – 24)	23 (21 -25)	**0.013**

IQR, interquartile range; uPCR, urine protein creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1C, glycosylated haemoglobin A1C; WBC, white blood cells; HDL, high density lipoprotein; SBP, systolic blood pressure.

3.3.2 Clinical profile of CKD patients

Of the 312 patients who were enrolled into the study, 308 of the patients had reduced eGFR, 202 of the patients had both reduced eGFR and proteinuria, 110 of the patients had only reduced eGFR and 4 of the patients had proteinuria only. More than half (58%) of the patients had advanced CKD (CKD stage 3b or 4), of whom 57 (31.5 %) patients presented with severely increased proteinuria compared to 23 (17.4 %) patients with early CKD. Metabolic acidosis was present in 113 (62.4 %) of those who had advanced CKD and in 61 (46.6 %) patients with early CKD. Anaemia was present in 84 (46.4 %) patients with advanced CKD including 30 (16.6 %) patients who had low transferrin levels, while 34 (25.9 %) patients with early CKD had anaemia. Among patients with advanced CKD, hyperuricemia was found in 56 (30.9 %) patients and 16 (8.8 %) patients had hyperkalemia. Among patients with advanced CKD, majority (96.7 %) of the patients were diagnosed with hypertension, 70 (38.7 %) patients had diabetes mellitus and 69 (38.1%) had both hypertension and diabetes mellitus compared to 117 (89.3 %) patients who had hypertension, 33 (25.2 %) patients who had diabetes mellitus and 32 (24.4%) had both hypertension and diabetes mellitus among those with early CKD.

Angina was reported in 41 (22.7%) patients with advanced CKD and 15 (11.5 %) patients in early CKD. Most (56.9 %) patients with advanced CKD were using more than 5 medications compared to 41.2 % patents with early CKD who were using 3 – 4 medications for their blood pressure control. Majority (84.0 %) of the patients with advanced CKD and 76.3 % patients in early CKD were on calcium channel blockers (CCBs), while 18.8 % patients with advanced CKD and 21.4 % of those with early CKD were using angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptors blockers (ARBs). For patients with advanced CKD, most (57.5

%) were on diuretics followed by 51.4 % on doxazosin; 29.3 % of patients were on insulin and 16 (8.8 %) on oral hypoglycemic agents (Table 3.2).

Table 3.2: Clinical profile of 312 CKD patients by eGFR

Parameter	**eGFR < 45 ml/min/1.72m^2 (n=181) Proportion (%)**	**eGFR ≥ 45ml/min/1.72m^2 (n= 131) Proportion (%)**	**P-value**
eGFR (ml/min/1.72m^2)			
Stage 1 (> 90)	0 (0.0 %)	4 (3.1 %)	
Stage 2 (60 – 89)	0 (0.0 %)	62 (47.3 %)	
Stage 3a (45 -59)	0 (0.0 %)	65 (49.6 %)	
Stage 3b (30-44)	144 (79.6 %)	0 (0.0 %)	
Stage 4 (16- 29)	37 (20.4 %)	0 (0.0 %)	**0.001**
Diagnoses encountered			
Hypertension	175 (96.7 %)	117 (89.3 %)	**0.009**
Diabetes mellitus	70 (38.7 %)	33 (25.2 %)	**0.012**
Hypertension & Diabetes mellitus	69 (38.1%)	32 (24.4%)	**0.011**
Adult polycystic kidney disease	5 (2.8 %)	7 (5.3 %)	0.242
Reflux nephropathy	0 (0.0 %)	5 (3.8 %)	**0.008**
Obstructive uropathy	1 (0.6 %)	0 (0.0 %)	0.394
Unknown	6 (3. 3 %)	5 (3.8 %)	0.812
Current smoking			
Yes	13 (7.2%)	10 (7.6%)	
No	168 (92.8%)	121 (92.4%)	0.880
Current Alcohol			
Yes	14 (7.7%)	18 (13.7%)	
No	169 (92.3%)	113 (86.3%)	0.084
Cardiovascular Diseases			
None	120 (66.3%)	108 (82.4 %)	
Angina	41 (22.7%)	15 (11.5 %)	
Myocardial infarction	8 (4.4%)	4 (3.0 %)	
Heart failure	6 (3.3%)	2 (1.5 %)	
Stroke	6 (3.3%)	1 (0.8 %)	
Transient ischemic attack	0 (0.0%)	1 (0.8 %)	**0.017**
Medications			
None	2 (1.1 %)	8 (6.1 %)	**0.004**
Diuretics	104 (57.5 %)	58 (44.3 %)	**0.021**
ACEIs / ARBs	34 (18.8 %)	28 (21.4 %)	0.572
Aldactone	6 (3.3 %)	5 (3.8 %)	0.812
CCBs	152 (84.0 %)	100 (76.3 %)	0.092
Statins	100 (55.3 %)	60 (45.8 %)	0.099
Oral hypoglycemics	16 (8.8 %)	21(16.0 %)	0.053
Insulin	53 (29.3 %)	19 (14.5 %)	**0.002**
Allopurinol	22 (12.1 %)	13 (9.9 %)	0.538
Junior ASA	90 (49.7 %)	57 (43.5 %)	0.278
Beta blockers	41 (22.7 %)	30 (22.9 %)	0.959

Aldomet	12 (6.6 %)	7 (5.3 %)	0.639
Hydralazine	4 (2.2 %)	3 (2.3 %)	0.962
Doxazosin	93 (51.4 %)	48 (36.6 %)	**0.010**
Others	68 (37.6 %)	38 (29.0 %)	0.115
Number of medications per patient			
0	2 (1.1 %)	8 (6.1 %)	
1 - 2	20 (11.1 %)	20 (15.3 %)	
3 – 4	56 (30.9 %)	55 (41.2 %)	
≥ 5	103 (56.9 %)	48 (36.6 %)	**0.001**
uPCR (g/mmol)			
Normal to mildly increased (<0.015)	47 (26.0 %)	63 (48.2 %)	
Moderately increased (0.015- 0.05)	77 (42.5 %)	45 (34.4 %)	
Severely increased (> 0.050)	57 (31.5 %)	23 (17.4 %)	**0.001**
Haemoglobin (g/dl)			
Normal (> 12.0 or 13.0)	97 (53.6 %)	97 (74.1 %)	
Anaemia (<12.0 or 13.0)	84 (46.4 %)	34 (25.9 %)	**0.001**
Transferrin g/dl)			
Low (< 2.0)	30 (16.6 %)	10 (7.6 %)	
Normal (2.0-3.60)	148 (81.8 %)	117 (89.3 %)	
High (>3.60)	3 (1.7 %)	4 (3.1 %)	0.052
Uric acid (mmol/l)			
Low (< 0.16 or 0.21)	4 (2.2 %)	15 (11.5 %)	
Normal (0.16/0.21 – 0.36/0.43)	121 (66.9 %)	98 (74.8 %)	
High (> 0.36 or 0.43)	56 (30.9 %)	18 (13.7 %)	**0.001**
Potassium (mmol/l)			
Low (< 3.5)	13 (7.2 %)	15 (11.5 %)	
Normal (3.5 – 5.1)	152 (84.0 %)	114 (87.0 %)	
High (> 5.1)	16 (8.8 %)	2 (1.5 %)	**0.013**
Bicarbonate (mmol/l)			
Low (< 23)	113 (62.4 %)	61 (46.6 %)	
Normal (23 – 29)	67 (37.0 %)	68 (51.9 %)	
High (> 29)	1 (0.6 %)	2 (1.5 %)	**0.018**

uPCR, urine protein creatinine ratio; eGFR, estimated glomerular filtration rate; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers.

3.3.3 Factors associated with advanced CKD

We divided the patients into two subgroups according to their CKD stages [early CKD (eGFR ≥ 45 ml/min/1.72m^2) vs. advanced CKD (eGFR < 45 ml/min/1.72m^2)]. A total of 24 potential variables were identified after performing univariate logistic regression analyses. Backward elimination reduced this to 15 parameters; the factors associated with advanced CKD after adjusting for age and sex on multivariate logistic regression analysis included: hypertension (OR 3.3, 95 % CI 1.2 - 9.2, P = 0.020), diabetes mellitus (OR 1.8, 95 % CI 1.1 - 3.3, P = 0.024),

angina (OR 2.5, 95 % CI 1.2 - 5.1, P = 0.008), severe proteinuria (OR 3.5, 95 % CI 1.9 - 6.5, P = 0.001), moderate proteinuria (OR 2.5, 95% CI 1.5 - 4.3, P=0.001), hyperuricemia (OR 2.4, 95 % CI 1.4 - 4.1, P = 0.001), anaemia (OR 2.9, 95% CI 1.7 - 4.9, P= 0.001), metabolic acidosis(OR 2.0, 95% CI 1.2 - 3.1, P= 0.005), allopurinol (OR 2.4, 95 % CI 1.4 - 4.3, P = 0.005) and doxazosin (OR 1.9, 95% CI 1.2 - 3.1, P = 0.006), low transferrin (OR 2.4, 95% CI 1.1 - 5.1, P= 0.028), hyperkalemia (OR 5.4, 95% CI 1.2 - 24.1, P= 0.029) and, widow/widower (OR 3.2, 95 % CI 1.4 - 7.4, P = 0.006) (Tables 3.3).

Table 3.3: Factors associated with advanced CKD

Characteristic	**Number of patients**	**UNIVARIATE**		**MULTIVARIATE**	
		OR (95% CI)	**P. value**	**OR (95% CI)**	**P. value**
Age	312	1.1(1.0-1.2)	0.001		
Marital status					
Single	91	1(Reference)		1(Reference)	
Married	164	1.7 (0.9-2.8)	0.055	1.5 (0.9-3.0)	0.137
Widow/Widower	40	3.3 (1.5-7.7)	0.004	3.2 (1.4-7.4)	**0.006**
Separated/Divorced	17	1.6 (0.6-4.6)	0.038	1.4 (0.5-4.3)	0.466
Hypertension					
No	20	1(Reference)		1(Reference)	
Yes	292	3.5 (1.3 – 9.3)	0.013	3.3 (1.2-9.2)	**0.020**
Diabetes mellitus					
No	209	1(Reference)		1(Reference)	
Yes	103	1.9 (1.1 – 3.1)	0.013	1.8 (1.1-3.0)	**0.024**
Current Alcohol					
No	280	1(Reference)		1(Reference)	
Yes	32	0.5 (0.3-1.1)	0.088	0.5 (0.2-1.0)	0.059
Cardiovascular Disease:					
None	228	1(Reference)		1(Reference)	
Angina	56	2.7 (1.4-5.3)	0.004	2.5 (1.2-5.1)	**0.008**
Myocardial infarction	12	1.8 (0.5-6.1)	0.348	1.4 (0.4-5.0)	0.578
Heart failure	8	2.7 (0.5-13.7)	0.230	2.8 (0.5-14.5)	0.219
Stroke	8	5.4 (0.6-45.6)	0.121	4.6 (0.5-39.2)	0.167
Medications					
No	10	1 (Reference)		1(Reference)	
Yes	302	11.7 (1.4-94.8)	0.021	0.1 (0.0-0.9)	**0.042**
Diuretics					
No	150	1(Reference)		1(Reference)	
Yes	162	1.7 (1.1-2.7)	0.022	1.6 (1.0-2.5)	0.053
CCB					
No	60	1(Reference)		1(Reference)	
Yes	252	1.6 (0.9-2.9)	0.093	1.6 (0.9-2.8)	0.127
Statins					
No	152	1(Reference)		1(Reference)	
Yes	160	1.5 (0.9-2.3)	0.100	1.4 (0.9-2.2)	0.171
Oral hypoglycemics					
No	275	1(Reference)		1(Reference)	

Yes	37	0.5 (0.3-1.0)	0.056	0.5 (0.2-1.0)	**0.048**
Allopurinol					
No	277	1(Reference)		1(Reference)	
Yes	35	2.5 (1.4 – 4.4)	0.003	2.4 (1.3-4.3)	**0.005**
Nitrates					
No	306	1(Reference)		1(Reference)	
Yes	6	1.9 (1.1-3.3)	0.024	1.7 (1.0-3.0)	0.059
Doxazosin					
No	171	1(Reference)		1(Reference)	
Yes	141	1.8 (1.2-2.9)	0.010	1.9 (1.2-3.1)	**0.006**
Others					
No	206	1(Reference)		1(Reference)	
Yes	106	1.5 (0.9-2.4)	0.116	1.5 (0.9-2.5)	0.100
Proteinuria (g/mmol)					
Normal to mild (<0.015)	110	1(Reference)		1(Reference)	
Moderately (0.015- 0.05)	122	2.3 (1.4 – 3.9)	0.002	2.5 (1.5- 4.3)	**0.001**
Severely (> 0.050)	80	3.9 (2.0 – 7.2)	0.000	3.5 (1.9 - 6.5)	**0.001**
Hyperuricemia					
No	216	1(Reference)		1(Reference)	
Yes	96	2.4 (1.4 - 4.0)	0.001	2.4 (1.4 - 4.1)	**0.001**
Anaemia					
No	194	1(Reference)		1(Reference)	
Yes	118	2.5 (1.5 - 4.0)	0.001	2.9 (1.7 - 4.9)	**0.001**
Transferrin g/dl)					
Normal (2.0-3.60)	265	1(Reference)		1(Reference)	
Low (< 2.0)	40	2.4 (1.1 - 5.1)	0.025	2.4 (1.1- 5.1)	**0.028**
High (>3.60)	7	0.6 (0.1 - 2.7)	0.499	0.5 (0.1- 2.7)	0.491
WBC (x 10^9 cells/l)					
Normal (4-11)	276	1(Reference)		1(Reference)	
Low (> 4)	23	0.5 (0.2 - 1.3)	0.051	1.8 (0.7 - 4.2)	0.202
High (>11)	13	1.1 (0.4 - 3.5)	0.859	1.8 (0.4 - 7.5)	0.418
Calcium (mmol /l)					
Normal (2.15 -2.45)	244	1(Reference)		1(Reference)	
Low (< 2.15)	25	2.2 (0.9 - 5.8)	0.098	2.1 (0.8 - 5.8)	0.125
High (>2.45)	43	0.6 (0.3 - 1.1)	0.081	0.6 (0.3 - 1.1)	0.081
Potassium (mmol/l)					
Normal (3.5 – 5.1)	266	1(Reference)		1(Reference)	
Low (< 3.5)	28	0.7 (0.3 - 1.4)	0.280	0.6 (0.3 - 1.4)	0.261
High (> 5.1)	18	6.0 (1.4 - 26.6)	0.018	5.4 (1.2 -24.1)	**0.029**
Bicarbonate (mmol/l)					
Normal (23 – 29)	135	1(Reference)		1(Reference)	
Low (< 23)	174	1.9 (1.2 - 3.0)	0.007	2.0 (1.2 - 3.1)	**0.005**
High (> 29)	3	0.5 (0.1 - 5.7)	0.583	0.4 (0.0 - 5.1)	0.495

P < 0.05 was used to identify potential variables, those with P <0.2 in univariate were included into the multivariate analysis adjusting for age and sex.

3.4 Discussion

This study evaluated the demographic and clinical profile of black patients with CKD attending the CMJAH kidney outpatient clinic in Johannesburg, South Africa. There were 42% with early CKD and 58% with advanced CKD; 93.6% had hypertension and 33.0 % diabetes mellitus, and

32.3% had both hypertension and diabetes. The prevalence of hypertension among patients with CKD is high and it is strongly associated with advanced CKD and CKD progression (299, 300), the majority (96.7 %) of patients with advanced CKD had hypertension, similar to findings in other studies from SSA (294, 301). Peripherally acting α-blockers like doxazosin are commonly used in the management of hypertension in CKD, mainly due to their pharmacokinetic profile that is undisturbed by worsening kidney function and their role in blood sugar control (74, 302). Approximately 51.4 % of the patients with advanced CKD were using doxazosin for treatment of hypertension with a 1.9 times increased association with advanced CKD, as it has also been reported in other studies (303, 304).

Studies have shown that diabetes related CKD is the leading cause of end stage kidney disease among patients with T2DM patients worldwide (305, 306), approximately 38.7 % of the patients with advanced CKD had T2DM; T2DM had 1.8 increased risk for advanced CKD, similar to other studies conducted among black patients in South Africa and Ethiopia (97, 301). For patients with advanced CKD, approximately 29.3 % of the patients were on insulin and 8.8 % were on oral hypoglycemics for treatment of their T2DM. Oral hypoglycemics were associated with 0.5 times higher risk for advanced CKD, similar to other studies (307, 308).

Metabolic acidosis is common in CKD and it can lead to dysfunction of many organs and systems including the kidney hence resulting in CKD progression (129, 309). The prevalence of metabolic acidosis was 62.4 % in advanced CKD and 46.6 % in early CKD. This prevalence is higher than the 33% - 40% among patients with CKD stage 3 – 4 from other continents (309-311). The possible explanation could be firstly, the more rapid CKD progression which has been shown to occur in black patients even early in their CKD stages (88, 128) and secondly, diet; where replacement of traditional diets with contemporary/ western foods which contain mainly animal proteins, less vegetables and low intake of fruits might increase CKD patients' dietary

acid load (146, 312). Patients with low serum bicarbonate levels were 2-fold more likely to have advanced CKD, as reported also in other studies (127, 310).

Anaemia is common in CKD, and it is associated with decreased quality of life, high morbidity and mortality (313, 314). The prevalence of anaemia was 46.4 % in advanced CKD, including 16.6 % who had low transferrin levels, while 25.9 % of the patients with early CKD had anaemia. Advanced CKD was 2.9 times more prevalent if a patient had anaemia and 2.4 times more prevalent if a patient had low transferrin, similar to other studies (112, 113, 315). High serum uric acid levels are associated with high risk for advanced CKD and CKD progression (316, 317). The prevalence of hyperuricemia was 30.9 % in advanced CKD; advanced CKD had a 2.4-fold higher OR if a patient had hyperuricemia and 2.4-fold higher if a patient was on allopurinol. Similar findings have been reported in other studies (44, 318). Hyperkalemia is common in patients with chronic CKD and its prevalence increases as the eGFR declines (123, 319). Approximately 8.8 % of the patients with advanced CKD were found to have hyperkalemia. Hyperkalemia had a 5.4-fold increased association with advanced CKD. Similar findings have been reported in other studies (124, 296).

Studies have shown that the incidence of cardiovascular events increases with worsening kidney function (320, 321); 22.7% patients with advanced CKD reported angina with a 2.5 times increased risk in advanced CKD, similar to other studies (320, 322). Also 31.5 % patients with advanced CKD presented with severely increased proteinuria. Advanced CKD was 3.5 times higher if a patient had severe proteinuria, like it was also reported in other studies (78, 97, 323). Furthermore, few (18.8 %) patients with advanced CKD and 21.4 % of those with early CKD were using ACEIs or ARBs. Similar findings have been reported from other studies on the underutilization of ACEIs/ARBs when they were clinically indicated or discontinuation of RAAS inhibitors by clinicians during the course of CKD possibly due to their associated side

effects (324-326). Angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptors blockers (ARBs) had no significant association with advanced CKD, possibly due to the small numbers on these agents. This contrasts with the findings from other studies that did demonstrate that the use of ACEIs/ARBs had beneficial effects for kidney events and cardiovascular outcomes compared to other antihypertensive medications in patients with CKD (326-328). The possible explanation could be the fact that the majority (84.0 %) of the study patients with advanced CKD were using calcium channel blockers. Studies have demonstrated that calcium channel blockers have similar kidney and cardiovascular protective effects when compared to RAAS blockers in patients with CKD (329, 330).

3.5 Conclusion

Hypertension and diabetes mellitus were strongly associated with advanced CKD, suggesting a need for primary and secondary population-based prevention measures. Metabolic acidosis, anaemia with low transferrin levels, hyperuricemia and hyperkalemia were highly prevalent in our patients, including those with early CKD, and they were strongly associated with advanced CKD. This calls for the proactive role of clinicians and dietitians in supporting the needs of CKD patients in meeting their daily dietary requirements towards preventing and slowing the progression of CKD. Further studies on the role of diet including plant-based proteins, vegetables and fruits in preventing and slowing CKD progression and other metabolic complications of CKD are warranted.

CHAPTER 4 (Published in the PLOS ONE Journal on 13.02.2023, Appendix E)

PROGRESSION OF CHRONIC KIDNEY DISEASE AMONG BLACK PATIENTS ATTENDING A TERTIARY HOSPITAL IN JOHANNESBURG, SOUTH AFRICA

Abstract

Background: Chronic kidney disease (CKD) is a major public health issue worldwide and is an important contributor to the overall non-communicable disease burden. Chronic kidney disease is usually asymptomatic, and insidiously and silently progresses to advanced stages in resource limited settings.

Methodology: A prospective longitudinal study was carried out on black patients with CKD attending the kidney outpatient clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa, from September 2019 to March 2022. Demographic and clinical data were extracted from the ongoing continuous clinic records, as well as measurements of vital signs and interviews at baseline and at follow up. Patients provided urine and blood samples for laboratory investigations as standard of care at study entry (0) and at 24 months, and were followed up prospectively for two (2) years. Data were descriptively and inferentially entered into REDcap and analysed using STATA version 17, and multivariable logistic regression analysis was used to identify predictors of CKD progression.

Results: A total of 312 patients were enrolled into the study, 297 (95.2%) patients completed the study, 10 (3.2%) patients were lost to follow and 5 (1.6%) patients died during the study period. The prevalence of CKD progression was 49.5%, while that of CKD remission was 33% and CKD regression was 17.5%. For patients with CKD progression, the median age at baseline was

58 (46 - 67) years, the median eGFR was 37 (32 -51) mL/min/1.73 m^2, median urine protein creatinine ratio (uPCR) was 0.038 (0.016 -0.82) g/mmol and the median hemoglobin (Hb) was 13.1 (11.7 – 14.4) g/dl; 95.2% had hypertension, 40.1% patients had diabetes mellitus and 39.5% had both hypertension and diabetes mellitus. Almost half (48.3%) of the patients with CKD progression had severely increased proteinuria and 45.6% had anaemia. Variables associated with higher odds for CKD progression after multivariable logistic regression analysis were moderately elevated proteinuria (OR 2.1, 95% CI (1.1 – 3.9), P= 0.019), severely elevated proteinuria (OR 6.1, 95 % CI (3.2 – 11.6), P = 0.001), hyponatremia (OR 4.5, 95% CI 1.8 - 23.6, P= 0.042), hypocalcemia (OR 3.8, 95 % CI 1.0 - 14.8, P = 0.047), anaemia (OR 2.1, 95% CI 1.0 - 4.3, P= 0.048), elevated HbA1c (OR 1.8, 95 % CI 1.2 - 2.8, P = 0.007), diabetes mellitus (OR 1.8, 95 % CI 1.9 - 3.6, P = 0.047), current smoking (OR 2.8, 95 % CI 1.9 - 8.6, P = 0.049).

Conclusion: Our study identified a higher prevalence of CKD progression in a prospective longitudinal study of black patients with CKD compared with literature reports. CKD Progression was associated with proteinuria, diabetes mellitus, elevated HbA1c, anaemia, hypocalcemia, hyponatremia and current smoking in a cohort of black patients with CKD who had controlled hypertension and diabetes mellitus at baseline.

Key words: Prevalence, predictors, CKD progression, black patients

4.1 Introduction

The global prevalence of chronic kidney disease (CKD) has been progressively increasing and is a significant risk factor for cardiovascular disease morbidity and mortality (331, 332). The burden of CKD is more prominent in low- and lower-middle-income countries (LLMICs) than in high-income countries (HICs) with a higher prevalence among those disadvantaged and indigenous communities who have less/ limited access to health care (331, 333). The global

increase in CKD is driven by the increasing prevalence of diabetes mellitus, hypertension and obesity (334, 335).

Studies have shown that African-Americans have a 2- to 4-fold higher risk for end-stage kidney disease (ESKD) than their white counterparts thus requiring renal replacement therapy (86, 87). Individuals of black ethnicity; due to their genes, including the presence of *APOL1* high-risk genotypes, are at higher risk of increased serum creatinine levels, lower eGFR, more rapid CKD progression and death due to social, economic and medical inequities (15, 16). Studies have reported the prevalence of CKD to be 15.8% in Africa, with major cost implications to overburdened healthcare systems (2, 4). In developing countries, patients with ESKD are increasing in numbers and most die due to poor access to kidney replacement therapies, therefore calling for cost effective preventive strategies to reduce CKD progression (334, 336).

Chronic kidney disease in developing countries also tends to be present in younger people who are unaware of their underlying kidney disease, and very few receive optimal care because of resource constraints (14, 34). Regardless of the underlying cause of CKD, chronic kidney diseases progress slowly to ESKD due to irreversible nephron loss thus leading to premature death (337, 338). Slowing CKD progression, promoting remission, or even regression of CKD, have been based on the control of blood pressure, control of blood sugar, reduction of proteinuria and management of other known risk factors for CKD progression (140, 339).

When CKD progressors are identified reliably and earlier in stages 1 to 3, disease progression can be altered with reduced complications (10, 78). There is a need to identify those patients with CKD and those who are likely to progress more rapidly, to reduce the number of patients progressing to end-stage kidney disease (ESKD). The aim of this study was to study the prevalence and predictors of CKD progression in a cohort of black patients with satisfactory

blood pressure and glycaemic control at baseline attending a tertiary hospital in Johannesburg, South Africa.

4.2 Methods

4.2.1 Study design, population and settings

This was a prospective longitudinal study to evaluate the prevalence and predictors of CKD progression among black patients attending the kidney outpatient clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) from September 2019 to March 2022. The CMJAH is a public accredited central hospital with 1088 beds serving patients from across the Gauteng province and nearby provinces in South Africa. CMJAH is also one of the main teaching hospitals for the Faculty of Health Sciences of the University of the Witwatersrand. Johannesburg is the largest city in South Africa and among the largest 50 urban agglomerations in the world. Johannesburg had an estimated population of around 5.9 million in, 2021; the most common ethnic groups included black Africans (76.4%), Whites (12.3%), mixed ethnicity (5.6%) and Indian/Asians (4.9%). The inclusion criteria for the study were patients who were >18 years of age, with CKD stages 1 – 4, who had controlled hypertension (blood pressure < 140/90 mm Hg) and diabetes mellitus (HbA1C < 7%) (287, 340), attending the kidney outpatient clinic for at least 6 months and were able to give informed consent. Patients who had active infections, active malignancies, autoimmune diseases and who were not black were excluded. Black patients have significantly higher rates of more rapid CKD progression and ESKD than other races (88, 89), hence they were the focus of this study.

4.2.2 Data collection and laboratory procedures

Enrolment occurred between September 2019 to March 2020; consecutive sampling was used to meet the required number of study patients and follow up was conducted between September 2021 to March 2022. Demographic and clinical data including age, gender, weight, height,

glycaemic status, history of smoking, aetiology of CKD and medications were extracted from the ongoing continuous clinic records. Face to face interviews at the time of enrolment were recorded in a questionnaire and vital signs measurements were conducted at first enrolment and at follow up. Patients provided blood and urine samples at study entry (0) and at 24 months. Patients agreed to have their routine medical records accessed prospectively for two (2) years. Systolic and diastolic blood pressure was measured 3 times, and the average of the second and third measurements was used (341).

Body mass index (BMI) was calculated using the National Health Services (NHS-UK) BMI calculator (288). Measurements of urinary protein creatinine ratio (uPCR), serum creatinine, electrolytes, HbA1C, white cell count, haemoglobin level, platelets, calcium, phosphate, transferrin and HDL cholesterol were done as standard of care at the time of recruitment and at follow up during a clinic visit. Anaemia was defined as a Hb concentration of < 13.0g/dl in males and < 12.0g/dl in females (289). Serum creatinine was measured using the isotope dilution mass spectrometry (IDMS) traceable enzymatic assay and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation without using the African American correction factor. Race adjustment of eGFR in black patients may affect their clinical care thus contributing to differences in the management of their kidney disease (342, 343).

CKD progression was assessed by a (i) change to a more advanced stage of CKD (ii) greater than 30% reduction of eGFR in two years, whereby percent change in eGFR was calculated as last eGFR – first eGFR/ first eGFR x 100 (54) (iii) sustained decline in eGFR of > 4 ml/min/1.73 m^2/year or more (53, 344). CKD regression was defined by a sustained increase in eGFR of > 4 ml/min/1.73 m^2/year or an improvement in CKD stage at 24 months from

baseline (53, 339). CKD remission was defined by a stable or change in eGFR of < 4 ml/min/1.73 m^2/year from baseline and at 24 months of follow up (339, 344).

4.2.3 Data management and analysis

Study data were collected and entered into *REDCap* (Research Electronic Data Capture) tools (297, 298) hosted at the University of the Witwatersrand and analyzed using STATA version 17 (College Station, Texas, USA). Descriptive statistics were used to summarize demographic and clinical data. Continuous variables have been reported as medians with interquartile ranges and Wilcoxon rank-sum test was used for the non-normally distributed variables. Discrete variables have been reported as frequencies and proportions. Pearson's chi-square test was used to test for association between variables and CKD progression. Odd ratios were used to estimate the strengths of the association between variables and CKD progression; univariate and multivariate logistic regression models were used to estimate the association of the variables and CKD progression. Variables with p-value less than 0.2 on univariate logistic regression models were then fitted into the multivariate logistic regression models with the addition of age and sex as adjusting variables. Variables with a p-value of less than 0.05 were considered to have significant strength of association.

4.2.4 Ethical issues

Ethical approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand, Johannesburg (ethics clearance certificate No. M190553). Written informed consent was obtained from each of the participants before enrolment and embarking on data collection.

4.3 Results

4.3.1 Demographic and clinical characteristics of the study population

A total of 476 black patients with CKD stages 1- 4 attended the CMJAH kidney outpatient clinic during the enrolment period, 164 patients were excluded from the study including 110 patients who had uncontrolled hypertension, 35 patients who had uncontrolled diabetes mellitus, 11 patients who had autoimmune diseases, 6 patients had active infections and 2 patients had active malignancies. Of the 312 patients who were enrolled into the study, 10 (3.2%) patients were lost to follow and 5 (1.6%) patients died, 297 (95.2%) patients completed the study of whom 156 (52.5 %) were males and 154 (51.8 %) were married. Progression of CKD occurred in 147 (49.5%) patients over 2 years of follow up, the median age was 58 (46 - 67) years for CKD progressors and 57 (45 - 66) years for those without CKD progression. Significant variables were the median eGFR at baseline, 37 (32 -51) mL/min/1.73 m^2 for CKD progressors and 44 (34 – 61) mL/min/1.73 m^2 for those without CKD progression (p=0.003); the median urine protein creatinine ratio (uPCR) was 0.038 (0.016 -0.82) g/mmol for CKD progressors and 0.016 (0.008 – 0.032) g/mmol for those without CKD progression (p=0.001); median haemoglobin of 13.1 (11.7 – 14.4) g/dl for CKD progressors and 13.7 (12.2 – 15.3) g/dl for those without CKD progression, p=0.023 (Table 4.1).

Table 4.1: Baseline demographic and clinical characteristics of CKD progressors and non-progressors

Characteristic	CKD progression (n = 147) Proportion (%) or Median (IQR)	No CKD progression (n = 150) Proportion (%) or Median (IQR)	P-value
Age (years)	58 (46 - 67)	57 (45 -66)	0.355
Sex			
Male	82 (55.78%)	74 (49.33 %)	
Female	65 (44.22%)	76 (50.67%)	0.266
Marital status			
Single	39 (26.53%)	50 (33.33%)	
Married	81 (55.10%)	73 (48.67%)	
Widow/Widower	18 (12.24%)	19 (12.67%)	
Separated/Divorced	9 (6.12%)	8 (5.33%)	0.608
Highest level of education			
No formal education	18 (12.24%)	16 (10.67%)	
Primary	31 (21.09%)	36 (24.00%)	
Secondary	52 (35.37%)	43 (28.67%)	
Tertiary	46 (31.29%)	55 (36.67%)	0.549
Occupation			
Unemployed	23 (15.65%)	21 (14.00%)	
Domestic workers	25 (17.01%)	31 (20.67%)	
Self employed	33 (22.45%)	32 (21.33%)	
Public / Private servant	54 (36.73%)	53 (35.33%)	
Retired	12 (8.16%)	13 (8.67%)	0.943
BMI (kg/m^2)	30.6 (26.84 -35.67)	29.39 (25.97 - 33.4)	0.193
SBP (mmHg)	140 (132 – 140)	140 (126 -140)	0.088
DBP (mmHg)	83 (74 – 90)	82 (73 -90)	0.319
uPCR (g/mmol)	0.038 (0.016 -0.82)	0.016 (0.008 – 0.032)	**0.001**
Creatinine (umol/L)	148 (118 -183)	134 (104 -161)	**0.002**
eGFR ($ml/min/1.72m^2$)	37 (32 -51)	44 (34 – 61)	**0.003**
FBG (mmol/L)	4.5 (4.2 – 5.4)	4.4 (4.2 – 4.9)	0.187
HbA1C (%)	7.0 (7.0 – 7.0)	7.0 (6.6 – 7.0)	0.184
Haemoglobin (g/dl)	13.1 (11.7 – 14.4)	13.7 (12.2 – 15.3)	**0.023**
Transferrin (g/L)	2.49 (2.25 – 2.78)	2.59 (2.29 – 2.85)	0.782
WBC (x 10^9 cells/L)	6.1 (5.06 – 7.84)	6.46 (4.81 – 7.74)	0.549
Platelets (x 10^9 cells/L)	251 (211 -320)	269 (218 – 323)	**0.017**
Uric acid (mmol/L)	0.42 (0.35 – 0.51)	0.40 (0.31 – 0.48)	**0.010**
HDL cholesterol (mmol /L)	1.16 (0.99 -1.43)	1.25 (1.01 – 1.56)	0.114
Calcium (mmol /L)	2.30 (2.23 – 2.40)	2.34 (2.25 – 2.42)	0.095
Phosphate (mmol /L)	1.11 (0.93 – 1.26)	1.02 (0.88 – 1.16)	**0.022**
Sodium (mmol/L)	141 (139 – 143)	141 (138 – 143)	0.849
Potassium (mmol/L)	4.3 (4.0 – 4.7)	4.2 (3.8 – 4.5)	0.068
Bicarbonate (mmol/L)	22 (20 – 24)	22 (20 -24)	0.871

IQR, interquartile range; uPCR, urine protein creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood sugar; HbA1C, glycosylated haemoglobin A1C; WBC, white blood cells; HDL, high density lipoprotein; SBP, systolic blood pressure.

4.3.2 Prevalence of CKD progression, CKD remission and CKD regression

During the 2 years of follow up, a total of 147 (49.5%) patients had progression of CKD whereby 35.0% changed to a more advanced stage of CKD, 19.9% had greater than 30% reduction of eGFR in two years and 48.5 % had a sustained decline in eGFR of > 4 ml/min/1.73 m^2/year. A total of 98 (33%) patients were observed to have remission of CKD, while 52 (17.5%) patients had CKD regression (Figures 4.1a & 4.1b).

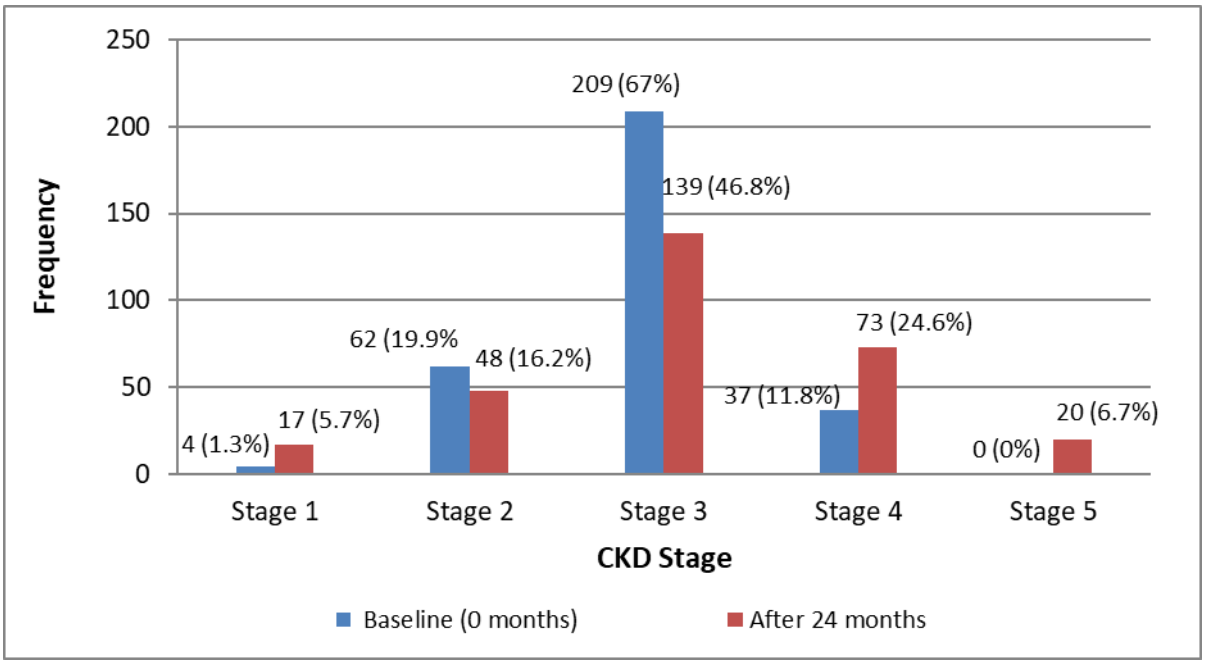

Figure 4.1a: CKD stages at baseline and at 24 months follow up

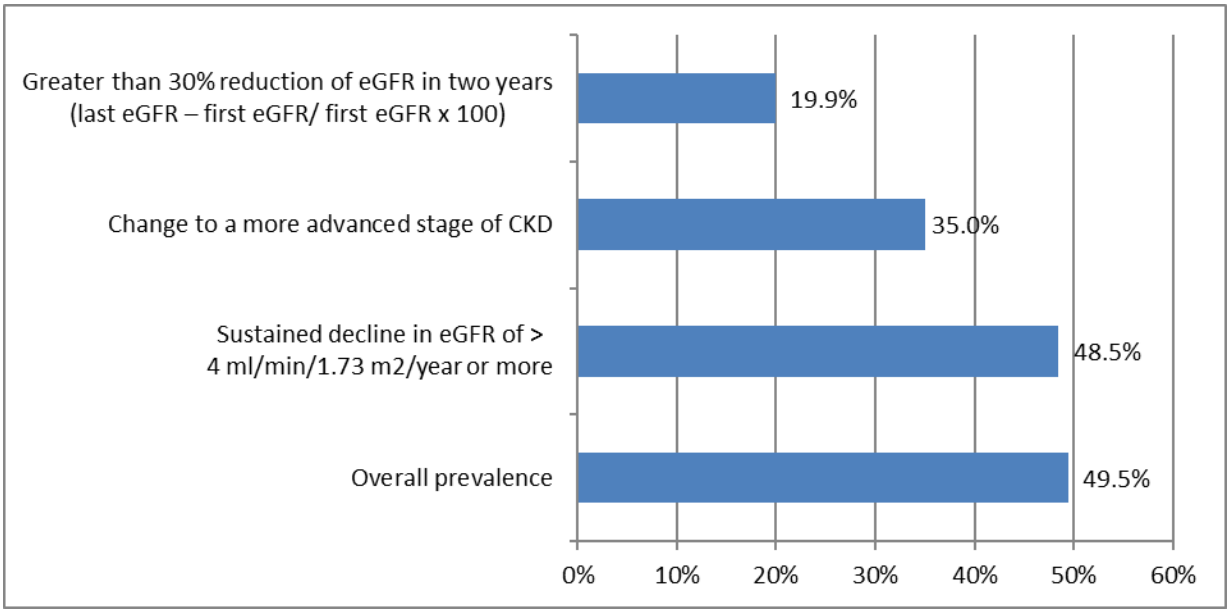

Figure 4.1b: Estimation of CKD progression using different eGFR criteria

4.3.3 Clinical profile of black patients who had CKD progression

Of the 147 black patients who had CKD progression, the majority (95.2%) of the patients had hypertension, 59 (40.1%) patients had diabetes mellitus and 58 (39.5%) had both hypertension and diabetes mellitus. About half (48.3%) of the CKD progressors presented with severely increased proteinuria compared to 30 (20.0%) of those without CKD progression. Anaemia was noted in 67 (45.6%) patients with CKD progression and in 40 (26.7 %) patients in those without CKD progression. Low serum calcium levels were noted in 26 (17.7%) of those with CKD progression and in 10 (6.7%) patients of those without CKD progression, while high serum phosphate levels were noted in 18 (12.3%) patients in those with CKD progression and in 6 (4.0%) patients of those without CKD progression. Low serum sodium levels were noted in 12 (8.2%) of those with CKD progression and in 3 (2.0%) of patients without CKD progression, while high serum potassium levels were noted in 21 (14.3%) patients who had CKD progression and in 7 (4.7%) of patients without CKD progression. Hyperuricaemia was present in 50 (34.0%) patients with CKD progression compared to 24 (16.0%) patients without CKD

progression. Majority (76.9%) of the patients with CKD progression were using more than 4 anti-hypertensives for their blood pressure control compared to 57.3 % in those patients without CKD progression. For patients with CKD progression, the majority (85.7%) of the patients were on calcium channel blockers, 62.6% were on diuretics, 57.1% were on statins, 55.1% were on beta blockers, 31.3% were on insulin, 29.3% and were on aspirin. A total of 35 (23.8 %) CKD progressors were on ACEs/ARBs compared with 25 (16.7 %) non-progressors. There was no significant difference in CKD progression associated with the use of ACEIs/ARBs (Table 4.2).

Table 4.2: Baseline clinical and laboratory parameters of CKD progressors and non-progressors

Parameter	**CKD progression (n = 147) Proportion (%)**	**No CKD progression (n = 150) Proportion (%)**	**P-value**
Diagnoses encountered			
Hypertension	140 (95.24 %)	137 (91.33 %)	0.179
Diabetes mellitus	59 (40.14 %)	39 (26.00 %)	**0.010**
Hypertension & Diabetes mellitus	58 (39.46%)	38 (25.3%)	**0.009**
Adult polycystic kidney disease	7 (4.76 %)	4 (2.67 %)	0.339
Reflux nephropathy	2 (1.36 %)	3 (2.00 %)	0.668
Obstructive uropathy	1 (0.68 %)	0 (0.0 %)	0.312
Unknown	2 (1.36 %)	8 (5.33 %)	0.058
Current smoking			
Yes	14 (9.52%)	8 (5.33 %)	0.168
No	133 (90.48%)	142 (96.67%)	
Current Alcohol			
Yes	14 (9.52%)	18 (12.00%)	
No	133 (90.48%)	132 (88.00%)	0.493
Cardiovascular Diseases			
None	99 (67.35%)	121 (80.67 %)	
Angina	28 (19.05%)	22 (14.67 %)	
Myocardial infarction	9 (6.12%)	2 (1.33 %)	
Heart failure	5 (3.40%)	3 (2.00 %)	**0.030**
Stroke	6 (4.08%)	2 (1.33 %)	
Medications			
None	4 (2.72 %)	5 (3.33 %)	0.758
Diuretics	92 (62.59 %)	61 (40.67 %)	**0.001**
ACEIs / ARBs	35 (23.81 %)	25 (16.67 %)	0.125
Aldactone	7 (4.76 %)	4 (2.67 %)	0.339
CCBs	126 (85.71 %)	112 (74.67 %)	**0.017**
Statins	84 (57.14 %)	67 (44.67 %)	**0.032**
Oral hypoglycemics	15 (10.2 %)	21(14.0 0%)	0.316
Insulin	46 (31.29 %)	22 (14.67 %)	**0.001**
Allopurinol	16 (10.88 %)	18 (12.00 %)	0.763
Junior ASA	43 (29.25 %)	26 (17.33 %)	**0.015**
Beta blockers	81 (55.10 %)	61 (40.67 %)	**0.013**

Aldomet	36 (24.49 %)	33 (22.0 %)	0.611
Hydralazine	11 (7.48 %)	8 (5.33 %)	0.449
Nitrates (ISMN/ISDN)	4 (2.72%)	3 (2.00%)	0.682
Doxazosin	68 (46.26 %)	66 (44.00 %)	0.696
Others	47 (31.97 %)	52 (34.67 %)	0.622
No. of antihypertensives per patient			
0	4 (2.72 %)	6 (4.00 %)	
1 - 3	30 (20.40 %)	58 (38.67 %)	
≥ 4	113 (76.88 %)	86 (57.33 %)	**0.002**
uPCR (g/mmol)			
Normal to mildly increased (<0.015)	23 (15.65 %)	59 (39.33 %)	
Moderately increased (0.015- 0.05)	48 (32.65 %)	59 (39.33 %)	
Severely increased (> 0.050)	71 (48.30 %)	30 (20.00 %)	
Nephrotic syndrome (> 0.350)	5 (3.40%)	2 (1.33%)	**0.001**
WBC (x 10^9 cells/l)			
Low (> 4)	5 (3.40 %)	12 (8.00 %)	
Normal (4-11)	139 (94.56 %)	131 (87.33 %)	
High (>11)	3 (2.04 %)	7 (4.67 %)	0.096
Haemoglobin (g/dl)			
Normal (> 12.0 or 13.0)	80 (54.42 %)	110 (73.33 %)	
Anaemia (<12.0 or 13.0)	67 (45.58 %)	40 (26.67 %)	**0.001**
Transferrin g/dl)			
Low (< 2.0)	26 (17.69 %)	13 (8.67 %)	
Normal (2.0-3.60)	117 (79.59 %)	134 (89.33 %)	
High (>3.60)	4 (2.72 %)	3 (2.00 %)	0.061
Platelets (x 10^9 cells/l)			
Low (> 150)	2 (1.36 %)	3 (2.00 %)	
Normal (150 - 450)	142 (96.60 %)	141 (94.00 %)	
High (>450)	3 (2.04 %)	6 (4.00 %)	0.643
Calcium (mmol /l)			
Low (< 2.15)	26 (17.69 %)	10 (6.67 %)	
Normal (2.15 -2.45)	107 (72.79 %)	118 (78.67 %)	
High (>2.45)	14 (9.52 %)	22 (14.67 %)	**0.009**
Phosphate (mmol /l)			
Low (< 0.80)	7 (4.76 %)	16 (10.67 %)	
Normal (0.80 -1.55)	122 (82.99 %)	128 (85.33 %)	
High (>1.55)	18 (12.25 %)	6 (4.00 %)	**0.019**
Uric acid (mmol/l)			
Normal (0.16/0.21 – 0.36/0.43)	97 (65.99 %)	126 (84.00 %)	
High (> 0.36 or 0.43)	50 (34.01 %)	24 (16.00 %)	**0.001**
HDL cholesterol (mmol /l)			
Low (< 1.04)	43 (29.25 %)	35 (23.33 %)	
Normal (1.04 -1.17)	28 (19.05 %)	25 (16.67 %)	
High (>1.17)	76 (51.70 %)	90 (60.00 %)	0.343
Sodium (mmol/l)			
Low (< 136)	12 (8.16 %)	3 (2.00 %)	
Normal (136 -145)	130 (88.44 %)	138 (92.00 %)	
High (>145)	5 (3.40 %)	9 (6.00 %)	**0.034**
Potassium (mmol/l)			
Low (< 3.5)	10 (6.80 %)	11 (7.33 %)	
Normal (3.5 – 5.1)	116 (78.91 %)	132 (88.0 %)	
High (> 5.1)	21 (14.29 %)	7 (4.67 %)	**0.018**
Bicarbonate (mmol/l)			
Low (< 23)	97 (65.99 %)	85 (56.67 %)	

Normal (23 – 29)	46 (31.29 %)	63 (42.00 %)	
High (> 29)	4 (2.72 %)	2 (1.33%)	0.130

uPCR, urine protein creatinine ratio; eGFR, estimated glomerular filtration rate; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers.

4.3.4 Factors associated with CKD progression

We divided the patients into two subgroups according to their CKD progression status after 2 years of follow up: CKD progression vs. no CKD progression. A total of 30 potential variables were identified after performing univariate logistic regression analyses. Backward elimination reduced this to 21 parameters. The factors associated with CKD progression after adjusting for age and sex on multivariate logistic regression analysis included diabetes mellitus (OR 1.8, 95 % CI 1.9 - 3.6, P = 0.047), increasing HbA1C (OR 1.8, 95 % CI 1.2 - 2.8, P = 0.007), moderately elevated proteinuria (OR 2.1, 95% CI (1.1 – 3.9), P= 0.019), severely elevated proteinuria (OR 6.1, 95 % CI (3.2 – 11.6), P = 0.001), hypocalcaemia (OR 3.8, 95 % CI 1.0 - 14.8, P = 0.047), anaemia (OR 2.1, 95% CI 1.0 - 4.3, P= 0.048), hyponatraemia (OR 4.5, 95% CI 1.8 - 23.6, P= 0.042) and current smoking (OR 2.8, 95 % CI 1.9 - 8.6, P = 0.049 (Tables 4.3).

Table 4.3: Factors associated with CKD progression

Characteristic	**No. of patients**	**UNIVARIATE**		**MULTIVARIATE**	
		OR (95% CI)	**P. value**	**OR (95% CI)**	**P. value**
Marital status					
Single	89	1(Reference)		1(Reference)	
Married	154	1.4 (0.8 - 2.4)	0.188	1.7 (0.8 - 3.7)	0.152
Widow/Widower	37	1.2 (0.6 - 2.6)	0.620	0.9 (0.3 - 2.9)	0.969
Separated/Divorced	17	1.4 (0.5 - 4.1)	0.490	1.4 (0.4 - 5.8)	0.605
Hypertension					
No	20	1(Reference)		1(Reference)	
Yes	277	1.8 (0.7 – 4.9)	0.186	0.2 (0.0 - 4.6)	0.343
Diabetes mellitus					
No	199	1(Reference)		1(Reference)	
Yes	98	1.9 (1.2 – 3.1)	0.010	1.8 (1.9 - 3.6)	**0.047**
Current smoking					
No	275	1(Reference)		1(Reference)	
Yes	22	1.8 (0.8 - 4.6)	0.174	2.8 (1.9 - 8.6)	**0.049**
BMI	297	1.0 (0.9 -1.1)	0.189	1.0 (1.0 -1.1)	0.609
SBP (mm hg)	297	1.0 (0.9 -1.0)	0.135	1.0 (0.9 -1.1)	0.700
DBP (mm hg)	297	1.0 (0.9 -1.1)	0.142	1.0 (0.9 -1.1)	0.908

HbA1C (%)	98	1.5 (1.2 - 2.0)	0.002	1.8 (1.2 - 2.8)	**0.007**
Cardiovascular Disease:					
None	220	1(Reference)		1(Reference)	
Angina	50	1.6 (0.8 - 2.8)	0.161	1.5 (0.6 - 3.7)	0.399
Myocardial infarction	11	5.5 (1.2 - 26.0)	0.032	3.8 (0.6 - 23.4)	0.151
Heart failure	8	2.0 (0.5- 8.7)	0.338	1.4 (0.3 - 6.9)	0.710
Stroke	8	7.3 (0.9 - 61.9)	0.067	4.3 (0.4 - 42.3)	0.216
Proteinuria (g/mmol)					
Normal to mildly increased (<0.015)	82	1(Reference)		1(Reference)	
Moderately increased (0.015- 0.05)	107	2.1 (1.1 – 3.9)	0.019	23.3 (2.4 - 230.1)	**0.007**
Severely increased (> 0.050)	101	6.1 (3.2 – 11.6)	0.000	32.3 (2.8 - 368.6)	**0.005**
Nephrotic syndrome (> 0.350)	7	6.4 (1.2 – 35.4)	0.033	3.1 (0.0 - 547.7)	0.665
Anaemia					
No	190	1(Reference)		1(Reference)	
Yes	107	2.3 (1.4 - 3.7)	0.001	2.1 (1.0 - 4.3)	**0.048**
Transferrin g/dl)					
Normal (2.0-3.60)	251	1(Reference)		1(Reference)	
Low (< 2.0)	39	2.3 (1.1 - 4.7)	0.022	1.1 (0.4 - 3.0)	0.858
High (>3.60)	7	1.5 (0.3 - 7.0)	0.584	0.9 (0.1- 5.9)	0.883
WBC (x 10^9 cells/l)					
Normal (4-11)	270	1(Reference)		1(Reference)	
Low (> 4)	17	0.4 (0.1 - 1.2)	0.087	0.6 (0.1 - 2.4)	0.460
High (>11)	10	0.4 (0.1 - 1.6)	0.196	0.3 (0.0 - 2.1)	0.215
Calcium (mmol /l)					
Normal (2.15 -2.45)	225	1(Reference)		1(Reference)	
Low (< 2.15)	36	2.9 (1.3 - 6.2)	0.008	3.8 (1.0 - 14.8)	**0.047**
High (>2.45)	36	0.7 (0.3 - 1.4)	0.335	0.9 (0.4 - 2.1)	0.809
Phosphate (mmol /l)					
Normal (0.80 -1.55)	250	1(Reference)		1(Reference)	
Low (< 0.80)	23	0.5 (0.2 - 1.2)	0.098	0.7 (0.2 - 2.3)	0.567
High (>1.55)	24	3.0 (1.1 - 7.8)	0.027	2.0 (0.5 - 8.1)	0.315
Hyperuricaemia					
No	223	1(Reference)		1(Reference)	
YES	74	2.7 (1.6 - 4.7)	0.001	1.6 (0.8 - 3.5)	0.202
HDL cholesterol (mmol /l)					
Normal (1.04 -1.17)	53	1(Reference)		1(Reference)	
Low (< 1.04)	78	0.8 (0.4 - 1.6)	0.468	0.7 (0.3 - 1.5)	0.333
High (>1.17)	166	0.5 (0.3 - 1.0)	0.051	0.6 (0.3 - 1.2)	0.154
Sodium (mmol/l)					
Normal (136 -145)	268	1(Reference)		1(Reference)	
Low (< 136)	15	4.3 (1.2 - 15.4)	0.028	4.5 (1.8 - 23.6)	**0.042**
High (>145)	14	0.6 (0.2 - 1.8)	0.355	0.5 (1.1 - 2.2)	0.349
Potassium (mmol/l)					
Normal (3.5 – 5.1)	248	1(Reference)		1(Reference)	
Low (< 3.5)	21	1.0 (0.4 - 2.3)	0.941	0.9 (0.3 - 2.5)	0.958
High (> 5.1)	28	3.4 (1.4 - 8.3)	0.007	1.8 (0.7 -5.1)	0.200
Bicarbonate (mmol/l)					
Normal (23 – 29)	109	1(Reference)		1(Reference)	
Low (< 23)	182	1.5 (1.0 - 2.5)	0.068	0.9 (0.4 - 1.8)	0.706
High (> 29)	6	2.7 (0.5 - 15.6)	0.256	7.3 (0.5 - 100.1)	0.135

P < 0.05 was used to identify potential variables, those with P <0.2 in univariate analysis were included in the multivariate analysis while adjusting for age and sex.

4.4 Discussion

Our study identified a higher prevalence of progression of CKD in a prospective longitudinal study of black patients with CKD attending the kidney outpatient clinic at CMJAH in Johannesburg compared to literature reports. We found a high prevalence of CKD progression with 49.5% of the study patients with CKD progression, which is a higher prevalence of progression than the reported prevalence of 46.7% in a UK cohort study and 38 % in a study conducted in northern California in USA (53, 75). The possible explanation could be differences in the study populations; in our study we enrolled only black patients; black patients tend to have more rapid CKD progression even in the early stages of CKD (128, 345). Also, black patients; due to racial/ethnic disparities, are at higher risk of CKD progression and its related complications than other races (84, 85).

This study established that 33% of the patients had CKD remission and 17.5% of the patients had CKD regression, similar to findings from other studies from the UK (75, 346). A sustained decline in eGFR of > 4 ml/min/1.73 m^2/year or more was able to identify 48.5% patients who had CKD progression, including those with rapid CKD progression, similar to other studies (53, 75). Hypertension was prevalent in the study cohort, occurring in 96.7 % of the study cohort at baseline and in 95.2% of the CKD progressors and in 91.3% of those without progression. Of the patients with CKD progression, the majority (95.2%) had hypertension, 40.1% had diabetes mellitus and 39.5% had both hypertension and diabetes mellitus. Studies have reported CKD progression to be associated with CKD aetiology and hypertension (96, 97). Both systolic and diastolic blood pressure were not significantly associated with CKD progression in this study, in contrast with the findings from other studies where higher SBP and DBP levels were associated with CKD and had higher risks of CKD progression (98, 99). This

difference could be due to the high prevalence (above 90%) of the hypertension in both groups and an entry criterion of controlled blood pressure.

Smoking was reported in 9.5 % of our study patients who had CKD progression, with a 2.8-fold increased risk for CKD progression. Studies have reported that smoking is significantly associated with a higher risk of CKD and smoking is strongly associated with CKD progression (100, 101). Obesity and HDL cholesterol were not significantly associated with CKD progression in this study unlike with the findings from other studies where patients who had obesity and/or hyperlipidemia had higher rates of CKD progression (102, 103). This difference could be due to the high prevalence of obesity/overweight in both progressor and non-progressor groups.

Diabetes mellitus had a 1.8-fold increased risk for CKD progression and increased HbA1c levels had a 1.8-fold increased risk for CKD progression similar to the findings from other studies where increased HbA1c levels was associated with CKD progression in patients with T2DM (107, 108). Most (48.3%) patients with CKD progression presented with severely increased proteinuria; CKD progression was 6.1 times higher if a patient had severely increased proteinuria and 2.1 times higher if a patient had moderately increased proteinuria. Studies have reported CKD progression to be increased in patients who present with higher levels of proteinuria (77, 109). ACEIs or ARBs have shown to reduce proteinuria more effectively than other antihypertensives. In this study, few (20.2%) patients were using ACEIs or ARBs, similar to other studies that reported underutilization of ACEIs/ARBs in CKD patients (324, 326). These findings emphasize a critical need for nephrologists and clinicians to optimize safe and effective use of ACEIs/ARBs for RAAS blockade among CKD patients towards reducing proteinuria and slowing CKD progression (110, 111).

Anaemia was reported in 45.6 % of the patients who had CKD progression and was 2.1-fold increased with CKD progression and CKD was more severe if a patient had anaemia. Studies have reported that anaemia in CKD mainly results from impaired production of erythropoietin by the failing kidneys and anaemia is significantly associated with increased odds for CKD, CKD progression and worse clinical outcomes (113, 114). Hyperuricemia was significantly associated with CKD progression on univariate analysis in our study but this significance was lost on multivariate analysis. Studies have reported that high serum uric acid levels commonly occur as a result of the impaired glomerular filtration rate (GFR) that occurs in CKD. Hyperuricemia can also precede the development of CKD, predict incident CKD and is associated with high risk for advanced CKD/ CKD progression (115, 116).

Hypocalcemia was reported in 17.7 % patients who had CKD progression with 3.8-fold association with CKD progression. Low serum calcium levels are commonly caused by increased serum phosphorus and decreased renal production of 1,25(OH)2 vitamin D due to hyperparathyroidism. Hypocalcemia is independently associated with CKD, and it tends to predict rapid CKD progression and severe CKD (117, 118). Hyperphosphatemia was significantly associated with CKD progression on univariate analysis but this significance was lost on multivariate analysis. Studies have reported that high serum phosphate levels predicted higher rates of CKD progression (119, 120).

Hyponatremia was reported in 8.16 % of those patients who had CKD progression; CKD progression was 4.5 times more common if a patient had low serum sodium levels. The kidneys have the ability to modulate sodium and water excretion, and CKD is frequently complicated with hyponatremia probably due to fluid overload or diuretic usage. Hyponatremia in CKD is associated with a higher risk of CKD progression, cardiovascular events, hospitalization and increased mortality (121, 122). Hyperkalemia was significantly associated with CKD progression

on univariate analysis but this significance was lost on multivariate analysis. Studies have reported that high serum potassium levels are common in patients with chronic CKD and it is associated with decreased renal function and CKD progression; its prevalence increases as the eGFR declines (123, 124). Metabolic acidosis was not significantly associated with CKD progression in this study. Studies from other settings reported that patients with low serum bicarbonate levels had higher rates of CKD progression and an accelerated reduction in eGFR with poor outcomes (129, 130). This difference could be due to the high prevalence of metabolic acidosis in both groups.

4.5 Conclusion

Our study identified a higher prevalence of CKD progression in a prospective longitudinal study of black patients with CKD compared with literature reports. CKD Progression was associated with proteinuria, diabetes mellitus, elevated HbA1c, anaemia, hypocalcemia, hyponatremia and current smoking in a cohort of black patients with CKD who had controlled hypertension and diabetes mellitus at baseline. These patients are at relatively high risk for CKD progression thus calling for nephrologists and clinicians to be vigilant in identifying black patients with CKD who are at risk of early CKD progression as this would allow risk stratification to improve their kidney disease outcomes. A sustained decline in eGFR of >4 ml/min/1.73 m^2/year identified the highest number of patients with CKD progression, and this may help nephrologists and clinicians to identify those progressors in addition to capturing those patients with rapid CKD progression who require more frequent monitoring, closer surveillance and management towards halting their CKD progression.

CHAPTER 5

BASELINE MEASUREMENTS OF TRANSFORMING GROWTH FACTOR-ß ISOFORMS WERE NOT ASSOCIATED WITH CHRONIC KIDNEY DISEASE PROGRESSION AMONG BLACK PATIENTS ATTENDING A TERTIARY HOSPITAL IN JOHANNESBURG, SOUTH AFRICA

Abstract

Background: The prevalence of chronic kidney disease (CKD) is rising globally; developing countries are the most affected and are the least equipped to deal with its associated consequences. Chronic kidney disease can rapidly and silently progress to end stage kidney disease (ESKD) in resource constrained settings.

Methods: This was a prospective longitudinal study among black patients with CKD who attended the kidney outpatient clinic between September 2019 and March 2022 at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa. Blood and urine samples were collected for laboratory investigations as standard of care at enrolment and at follow up (24 months). Baseline serum and urine transforming growth factor- (TGF)-β1, TGF-β2 and TGF-β3 levels were measured using the Human TGF-β duoset ELISA. REDcap was used to enter data descriptively and inferentially and data analysis was done by STATA version 17 and multivariable logistic regression analysis was utilized to determine predictors of CKD progression.

Results: A total of 297 (95.2%) patients completed the study. After 2 years of follow up, the prevalence of CKD progression was established as 47.8% (as indicated by a sustained decline in eGFR of >4 ml/min/1.73 m2/year or more) and 51.9% (by a change in uPCR >30 %). Of the patients with CKD progression, 54.9% of patients were men; and at baseline, their median age

was 59 (46 - 67) years; eGFR was 37 (32 -51) mL/min/1.73 m2; urine protein creatinine ratio (uPCR) was increased at 0.039 (0.015-0.085) g/mmol. Majority (85.2 %) of the patients with CKD progression were on calcium channel blockers (amlodipine), 63.4% of the patients were on diuretics and 31.7 % of the patients on insulin. For those patients with CKD progression vs those without CKD progression, the median serum TGF-β1 was 21210 (15915 – 25745) ng/L vs 24200 (17570 – 29560) ng/L, the median urine TGF-β3 was 17.5 (5.4 – 76.2) ng/L vs 2.8 (1.8 – 15.3) ng/L. Variables associated with CKD progression after multivariable logistic regression analysis were baseline serum calcium levels (OR 0.06, 95 % CI 0.01 -0.64, P = 0.019) and medications which were diuretics (OR 2.35, 95 % CI 1.37 – 4.00, P = 0.002), calcium channel blockers (OR 2.07, 95 % CI 1.04 – 4.12, P = 0.038) and insulin (OR 1.96, 95 % CI 1.01 – 3.84, P = 0.048).

Conclusion: Patients with CKD progression had lower baseline concentrations of serum TGF-β1 and increased baseline urinary TGF-β3 concentrations; however, baseline transforming growth factor isoforms did not predict CKD progression. The search for more sensitive biomarkers for predicting CKD progression at baseline is necessary.

Key words: TGF-β, CKD progression, black patients

5.1 Introduction

The global prevalence of chronic kidney disease (CKD) is around 10% and it is more frequently seen in the elderly, women, racial minorities, patients with diabetes mellitus and in patients with hypertension (52, 334). Chronic kidney disease is more prevalent in developing countries, which are most disadvantaged due to inadequate resources and preparedness to provide kidney replacement therapies (KRT) (31, 52). Chronic kidney disease is a common disorder associated with significant health care expenses, thus resulting in cardiovascular events, end stage kidney disease (ESKD) and eventually death (347, 348).

Chronic kidney disease can silently advance to ESKD, thus earlier diagnosis is important for instituting timely measures (136, 349). Age, male sex, proteinuria, arterial hypertension and black race are known risk factors for CKD progression (350). Moreover, there are other modifiable risk factors including medications, anaemia, hyperlipidemia, hyperuricaemia and elevated phosphate levels which tend to influence CKD progression at later stages (42, 43, 351). Albuminuria, serum creatinine and cystatin-C are key and most frequently utilized predictive models of CKD progression. However, these routinely used biomarkers show changes relatively late in the disease process (148, 149).

Chronic kidney disease progression is affected by many factors; thus, it is important to develop biomarkers that effectively predict early CKD progression (352, 353). In this regard, literature has reported a number of biomarkers that occur before alterations in albuminuria, serum creatinine, and cystatin-C (148, 179). Transforming growth factor-β (TGF-β) has been regarded as the master cytokine in kidney inflammation and fibrosis through canonical TGF-β/Smad signalling and non-canonical pathways (221, 354). Renal fibrosis is the common pathway that leads to CKD progression and ESKD (355, 356) and studies have found that TGF-β is an independent risk factor for CKD and CKD progression (243, 357). The TGF-β superfamily comprises 33 members that are further subclassified into several subfamilies, including the TGF-βs (TGF-ß1, -β2, and -β3) (233, 358). Studies have been extensively conducted on TGF-β1 and TGF-β1 is regarded as a profibrotic mediator in most kidney diseases (359, 360). Transforming growth factor-β1 (TGF-β1) through its effects on mesangial cells, podocytes, endothelial and tubular cells is crucial in glomerular and tubulointerstitial disease pathogenesis (235, 361). Transforming growth factor-β1 (TGF-β1) is excessively expressed in almost all kidney diseases that are linked with fibrosis and inflammation (362, 363) and it is responsible for inducing

alterations in the glomerular filtration barrier, glomerulosclerosis and fibrosis, eventually leading to degeneration of tubules thus resulting in permanent renal dysfunction (72, 245).

Laboratory studies have reported that TGF-β1 has some value in diagnosing glomerular diseases and could be used as a biomarker predicting both recovery of glomerular diseases and CKD progression (364, 365). Patients with CKD and HIV expressed higher levels of urine and serum TGF-β1 levels especially in their early CKD stages (366, 367). Studies have reported that TGF-β2 plays a role in the fracture healing process (247, 248) while TGF-β3 has a role in immune-mediated responses (250, 368). The roles of TGF-β2 and TGF-β3 in kidney fibrosis are less clear and there is a paucity of data on TGF-β2 and TGF-β3 as biomarkers in CKD. A recent publication on some of the patients with HIV showed that urinary TGF-β1 and TGF-β2 levels were increased in HIV-positive patients who had CKD (369). The aim of this study was to evaluate whether serum and urinary TGF-β isoforms could predict CKD progression among patients attending a tertiary hospital in Johannesburg, South Africa.

5.2 Methods

5.2.1 Study design, population and settings

To study novel biomarkers that predict CKD progression among black patients attending the kidney outpatient clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a prospective longitudinal study was conducted between September 2019 to March 2022. The enrolment period was from September 2019 to March 2020 and follow up was conducted from September 2021 to March 2023.

The CMJAH is an accredited public hospital with a capacity of 1088 beds serving patients in Gauteng province and close by provinces in South Africa. The CMJAH is also one of the primary teaching and tertiary hospitals for the Faculty of Health Sciences of the University of the Witwatersrand. Johannesburg is the largest city in South Africa and the capital of Gauteng

province. Johannesburg had an estimated population of around 5.9 million in 2021; the ethnic groups included black Africans (76.4%), Whites (12.3%), mixed ethnicity (5.6%) and Indian/Asians (4.9%).

Inclusion criteria for the study were patients who were >18 years of age and were able to give informed consent. Patients in CKD stages 1 – 4, who had controlled hypertension (blood pressure < 140/90 mm Hg) and diabetes mellitus (HbA1c < 7%) and were attending the kidney outpatient clinic for at least 6 months were recruited. Patients with active malignancies, active infections, autoimmune diseases and who were not black were excluded. Literature has reported that black ethnicity is associated with significantly increased rates of faster CKD progression and ESKD, hence comprised the study population (88, 89).

5.2.2 Data collection and laboratory procedures

Kish and Leslie sample size calculation and consecutive sampling were utilized to obtain the required sample size during enrolment which occurred from September 2019 to March 2020; 24 months follow up was conducted from September 2021 to March 2022. Demographic and clinical data comprising of gender, age, weight, height, BMI, aetiology of CKD, glycaemic status, history of smoking, and medications were retrieved from the KOPD clinic data and face to face interviews at enrolment that were filled in a questionnaire and vital signs were measured at recruitment and at follow up.

Systolic and diastolic blood pressure were assessed 3 times at a clinic visit and the mean of the second and third readings were used. Body mass index (BMI) was determined using the National Health Services (NHS-UK) BMI calculator (288). Patients provided blood and urine samples at enrolment and at follow up (24 months); patients gave consent to have their laboratory data reviewed prospectively for two (2) years.

Routine laboratory investigations were done by the National Health Laboratory Services (NHLS) utilizing the Cobas 6000 analyser (Roche Diagnostics, USA). Measurements of serum creatinine, serum transferrin levels, urinary protein creatinine ratio (uPCR), electrolytes, FBP, RBG/FBG, HbA1C, HDL cholesterol calcium and phosphate done at the time of the first visit and at 24 months of follow up as standard of care.

Anaemia was defined as a Hb concentration of <12.0g/dl in females and <13.0g/dl in males (289). The isotope dilution mass spectrometry (IDMS) traceable enzymatic assay was utilized to calculate serum creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (without using the African American correction factor) was utilized in estimating the glomerular filtration rate (eGFR) (290).

Chronic kidney disease progression was determined by: (i) continued decrease in eGFR of > 4 ml/min/1.73 m^2/year or more (53) (ii) change to a more severe stage of CKD (51) (iii) more than 30% reduction of eGFR in two years, whereby percent change in eGFR was calculated as follow up eGFR – baseline eGFR/ baseline eGFR x 100 (54) (iv) more than 30% change in uPCR in two years, whereby change in uPCR was calculated as follow up uPCR – baseline uPCR / baseline uPCR (55).

5.2.3 Measurement of baseline serum and urine TGF-β isomers

At enrolment (baseline) blood samples were collected into plain gel separator vacutainer tubes for the preparation of serum, the samples were allowed to clot for half an hour and then centrifuged at 4000 rpm for 20 minutes after which serum was aliquoted into 1.5ml cryotubes and kept at -80°C to be defrosted on the day of performing the assay. At enrolment (baseline) urine samples were collected then centrifuged at 4000 rpm for 20 minutes.

Supernatant was aliquoted into 1.5ml cryotubes and kept at -80°C to be defrosted on the day of performing the assay. The Human TGF- β DuoSet ELISA (R&D Systems, Inc., Minneapolis,

MN) was utilized to perform the baseline serum and urine TGF-β1, TGF-β2 and TGF-β3 levels and the assays were done in accordance with the manufacturer's instructions. 1N hydrochloric acid was used to activate the serum and urine samples and a 1.2N sodium hydroxide/ 0.5M HEPES solution was used for neutralization thus resulting in a 1 in 2 sample dilution. Serum samples assayed for TGF-β1 were further diluted for a final sample assay dilution of 1 in 100. A microplate reader (Biotek 800TS, Agilent, US) set to 450 nm with wavelength correction set at 650nm was utilized to determine optical densities. The serum and urine transforming growth factor-ß isoforms were measured at enrolment (baseline) only.

5.2.4 Data management and analysis

REDCap (Research Electronic Data Capture) tools hosted at the University of the Witwatersrand was utilized for data entry and processing (297, 298) and data analysis was done utilizing the STATA version 17 (College Station, Texas, USA). To summarize demographic and clinical data, descriptive statistics were utilized. Data were checked for normal distribution by making use of the Shapiro - Wilk test, which showed that all continuous variables did not meet the normality assumption, thus medians with their interquartile ranges were employed as the measure of the centre and dispersion respectively to summarize those continuous variables. Furthermore, the comparison between continuous variables and outcome variables Wilcoxon rank-sum test was employed. Discrete variables were recorded as frequencies and proportions, to test for association between variables and CKD progression Pearson's chi-square test was utilized. To determine the effect of the variables at baseline and changes in the variables on CKD progression at 24 months multivariable logistic regression analysis has been utilized. Variables with p-value less than 0.2 on univariable logistic regression models were then entered into the multivariable logistic regression models with the addition of age as adjusting variable; variables with a p-value of less than 0.05 were thought to have significant power of association. The associations of

urinary and serum TGF-β isomers with CKD progression were assessed by odds ratios. This analysis was repeated with the four definitions of CKD progression as independent variables. A p-value of less than 0.05 was considered significant.

5.2.5 Ethical issues

The Human Research Ethics Committee of the University of Witwatersrand gave the ethical approval to conduct this study with ethics clearance certificate No. M190553. Each of the participants gave written informed consent before enrolment and starting data collection.

5.3 Results

5.3.1 Flow chart of the study participants

During the study period 476 patients visited the clinic for their routine follow ups, 312 patients were enrolled of whom 246 (78.8 %) patients had late CKD (CKD stage 3 &4). The majority (95.2%) of the patients completed the study after the 2 years follow up, of whom 116 (81.7 %) patients were in advanced CKD stages (CKD stage 3 &4) at baseline (Figure 1). Majority (93.6%) of the study patients were diagnosed to have hypertension. Diabetes mellitus was present in 33.0 % of the patients while both hypertension and diabetes mellitus were present in 32.3% patients (Figure 5.1).

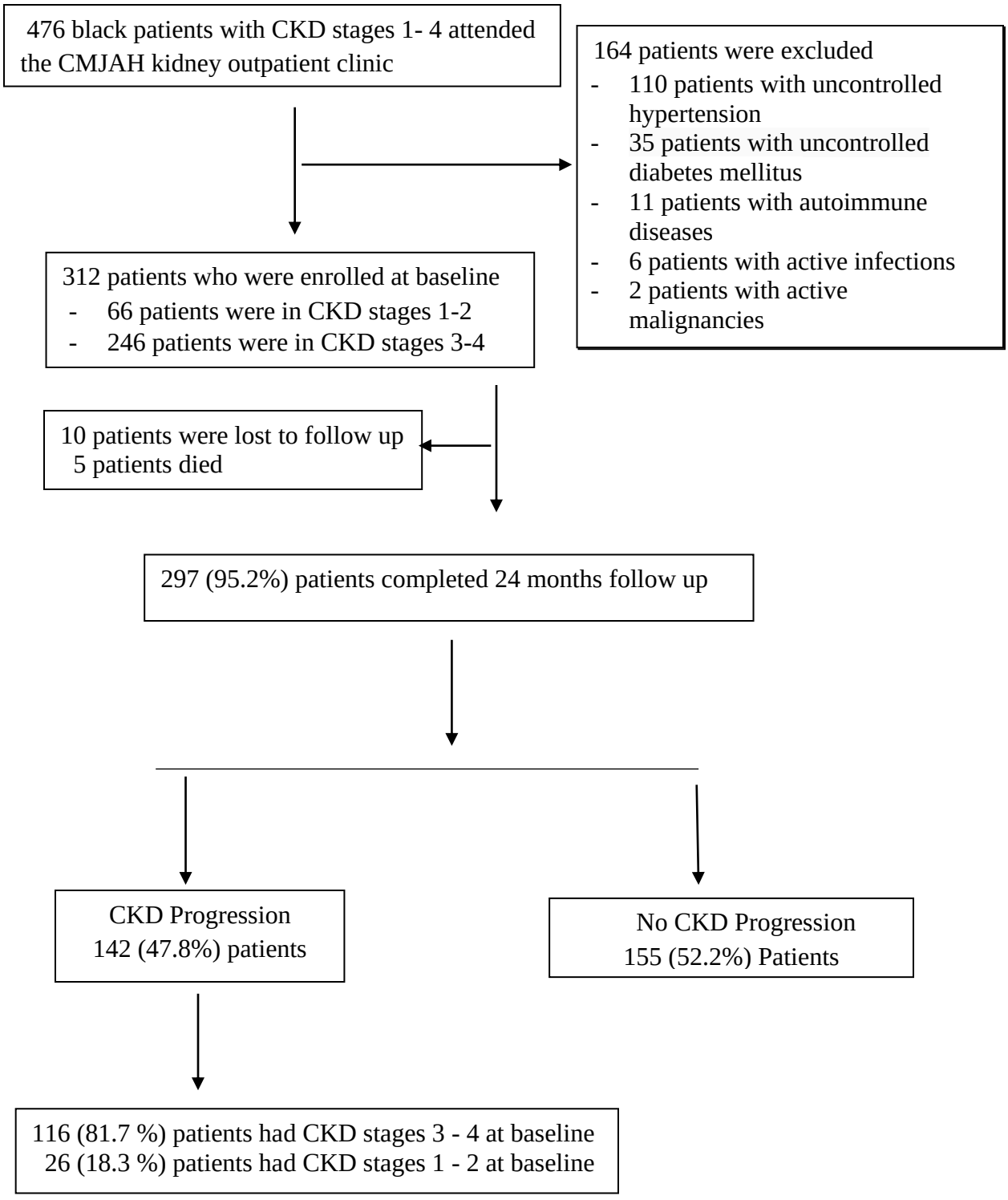

Figure 5.1: Flow chart of the study participants

5.3.2 Prevalence of CKD progression using routine biomarkers

After 24 months of follow up, CKD progression occurred in 142 (47.8%) patients who had a continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more, 104 (35%) patients moved to a more severe stage of CKD, 57 (19.2%) patients had more than 30% reduction of eGFR in two years and 154 (51.9%) patients had a change in uPCR > 30% in two years (Figure 2). Subsequent analysis used the continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more model, unless otherwise stated (Figure 5.2).

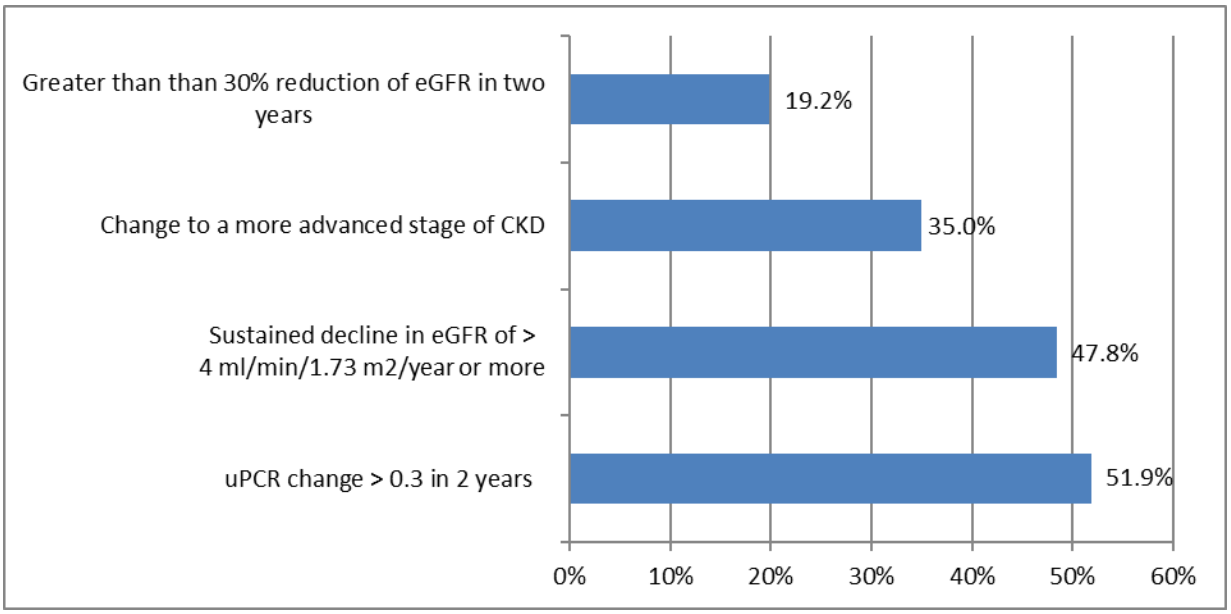

Figure 5.2: CKD progression after 2 years based on using routinely used biomarkers (eGFR and uPCR).

5.3.3 Demographic and clinical characteristics of the study population by CKD progression status

After 24 months follow up, 297 patients completed the study. The prevalence of CKD progression was 47.8% of whom 54.9% were men and the median age was 59 (46 - 67) years in CKD progressors and 56 (45 -66) years in those who had no CKD progression (Table 1). Significant variables were baseline median eGFR and median urine protein creatinine ratio

(uPCR), both of which were significantly different in CKD progressors than in non-progressors. Other factors that were significantly different in progressors vs non progressors were the baseline serum calcium, serum phosphate and haemoglobin. Majority (85.2%) of the patients who were diagnosed with CKD progression were taking calcium channel blockers (amlodipine), 63.4% were using diuretics, 57.0% were on statins, 55.6% were on beta blockers, 31.7% were on insulin, 28.9% were taking aspirin (junior ASA) and 23.2% were on ACEIs/ARBs (Table 5.1).

The baseline median (IQR) serum TGF-β1 was 21210 (15915 – 25745) ng/L for those with CKD progression and 24200 (17570 – 29560) ng/L for those without CKD progression using the sustained eGFR decline model (p = 0.004). Similar results were seen for the models exploring changes in CKD stage and percent reduction in eGFR, but no significant change in TGF-β1 levels was seen using the 30% increase in uPCR model. Baseline median (IQR) urine TGF-β3 was 17.5 (5.4 – 76.2) ng/L for those with CKD progression and 2.8 (1.8 – 15.3) ng/L for those without CKD progression using the sustained eGFR decline model (p = 0.017) but no significant differences were seen for this marker in any of the other models (Table 5.2).

Sensitivity analysis was repeated using those recruited with early stages of CKD (stages 1 & 2) and again with those recruited with more advanced CKD stages (stage 3a, 3b & 4). For those recruited in early CKD stages, 40% (26/65) showed CKD progression with the sustained decline in eGFR model and 62% (40/65) showed progression using any of the four models. In comparison with those recruited with later CKD stages, 50% (116/232) showed CKD progression with the sustained eGFR decline model and 76% (177/232) showed progression with any of the four models. Patients recruited with early CKD stages who had CKD progression had significantly lower platelet counts and significantly higher uric acid and bicarbonate levels than non- progressors (Supplementary Table 1), but showed no significant changes in any of the TGF-β isoforms measured (Supplementary Table 2). Those recruited with later stages of CKD

mimicked the clinical picture seen in the overall cohort with significant differences in creatinine, eGFR, uPCR, haemaglobin, calcium, phosphate seen between CKD progressors and non-progressors (Supplementary Table 3). Of interest however, was the calcium phosphate product which was significantly higher in the progressors than the non-progressors in this sub-cohort. Regarding medication use, the profile in the sub-cohort again mimicked the overall cohort except for calcium channel blockers which did not show significant differences between progressors and non- progressors in those recruited in later CKD stages. The TGF-β profile seen in the later CKD stage cohort reflected that of the overall cohort with significant differences in serum TGF-β1 levels between CKD progressors and non- progressors for the 3 models based on eGFR but no difference in the uPCR model. Urine TGF-β3 also differed significantly in the sustained decline eGFR model (Supplementary 4). The sensitivity analysis and the conclusions are preliminary or hypothesis for further studies, as sample size was not calculated for each of the CKD stages.

Table 5.1: Baseline demographic and clinical characteristics of study patients by CKD progression

Characteristic	CKD progression (n = 142) Proportion (%) or Median (IQR)	No CKD progression (n = 155) Proportion (%) or Median (IQR)	P-value
Demographics:			
Age (years)	59 (46 - 67)	56 (45 -66)	0.249
Sex			
Male	78 (54.9%)	78 (50.3 %)	
Female	64 (45.1%)	77 (49.7 %)	0.427
Marital status			
Single	38 (26.8 %)	51 (32.9 %)	
Married	77 (54.2 %)	77 (49.7 %)	
Widow/Widower	18 (12.7 %)	19 (12.3 %)	
Separated/Divorced	9 (6.3 %)	8 (5.2 %)	0.701
Highest level of education			
No formal education	18 (12.7 %)	16 (10.3 %)	
Primary	30 (21.1 %)	37 (23.9 %)	
Secondary	51 (35.9 %)	44 (28.4 %)	
Tertiary	43 (30.3 %)	58 (37.4 %)	0.387
Occupation			
Unemployed	22 (15.5 %)	22 (14.2 %)	
Domestic workers	25 (17.6 %)	31 (20.0 %)	
Self employed	32 (22.5 %)	33 (21.3 %)	
Public / Private servant	51 (35.9 %)	56 (36.1 %)	
Retired	12 (8.5 %)	13 (8.4 %)	0.985
Clinical Variables:			
BMI (kg/m^2)	30.6 (26.8 -35.7)	29.7 (26.0 – 33.4)	0.268
SBP (mmHg)	140 (132 -140)	140 (125 – 140)	0.070
DBP (mmHg)	83 (74 -90)	82 (73 – 90)	0.334
Creatinine (umol/L)	147.5 (118 -183)	134 (105 -162)	**0.004**
eGFR ($ml/min/1.72m^2$)	37 (32 -51)	44 (34 – 61)	**0.005**
uPCR (g/mmol)	0.039 (0.015-0.085)	0.016 (0.008 – 0.032)	**<0.001**
FBG (mmol/L)	4.5 (4.2 – 5.0)	4.4 (4.2 – 4.9)	0.364
HbA1c (%)	7.0 (6.9 – 7.0)	7.0 (6.6 – 7.0)	0.355
Haemoglobin (g/dl)	13.0 (11.7 – 14.2)	13.7 (12.2 – 15.3)	**0.011**
WBC (x 10^9 cells/L)	6.10 (5.07 – 7.84)	6.47 (4.81 – 7.74)	0.918
Platelets (x 10^9 cells/L)	251 (211 -320)	269 (218 – 323)	0.475
Uric acid (mmol/L)	0.42 (0.35 – 0.51)	0.40 (0.31 – 0.48)	**0.020**
HDL cholesterol (mmol /L)	1.16 (1.00 -1.43)	1.25 (1.01 – 1.56)	0.153
Calcium (mmol /L)	2.30 (2.23 – 2.39)	2.34 (2.25 – 2.42)	**0.004**
Phosphate (mmol /L)	1.12 (0.93 – 1.27)	1.02 (0.88 – 1.16)	**0.007**
Sodium (mmol/L)	141 (139 – 143)	141 (138 – 143)	0.758
Potassium (mmol/L)	4.3 (4.0 – 4.7)	4.2 (3.8 – 4.5)	0.054
Bicarbonate (mmol/L)	22 (20 – 24)	22 (20 - 24)	0.787
Calcium phosphate product ($mmol^2/L^2$)	2.50 (2.12 – 2.89)	2.41 (2.03 – 2.70)	0.092
Medications:			
Diuretics	90 (63.4%)	63 (40.7%)	**0.001**
ACEIs / ARBs	33 (23.2%)	27 (17.4%)	0.212

Aldactone	6 (4.2%)	5 (3.2%)	0.649
Calcium channel blockers	121 (85.2%)	117 (75.5%)	**0.036**
Statins	81 (57.0%)	70 (45.2%)	**0.041**
Oral Hypoglycaemics	14 (9.9%)	22 (14.2%)	0.253
Insulin	45 (31.7%)	23 (14.8%)	**0.001**
Allopurinol	15 (10.6%)	19 (12.3%)	0.647
Junior ASA	41 (28.9%)	28 (18.1%)	**0.028**
Beta blockers	79 (55.6%)	63 (40.7%)	**0.010**
Aldomet	35 (24.7%)	34 (21.9%)	0.580
Hydralazine	11 (7.8%)	8 (5.2%)	0.363
Nitrates (ISMN/ISDN)	4 (2.8%)	3 (1.9%)	0.617
Doxazosin	64 (45.1%)	70 (45.2%)	0.987
Others	45 (31.7%)	54 (34.8%)	0.565

ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers; ASA, acetylsalicylic acid; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood sugar; HbA1c, glycosylated haemoglobin A1C; HDL, high density lipoprotein; IQR, interquartile range; ISMN; isosorbide mononitrate; ISDN, isosorbide dinitrate; uPCR, urine protein creatinine ratio; SBP, systolic blood pressure; WBC, white blood cells

Table 5.2: Baseline serum and urine transforming growth factor-β beta concentrations of study patients by CKD progression using routinely used biomarkers

	eGFR decline > 4 ml/min/1.73 m2/year or more			changed to a more advanced stage of CKD			>30% reduction in eGFR in 2 years			>30% increase in uPCR in 2 years		
Baseline Characteristic	**CKD progression (n = 142) Median (IQR)**	**No CKD progression (n = 155) Median (IQR)**	**p-value**	**CKD progression (n = 104) Median (IQR)**	**No CKD progression (n = 193) Median (IQR)**	**p-value**	**CKD progression (n = 57) Median (IQR)**	**No CKD progression (n = 240) Median (IQR)**	**p-value**	**CKD progression (n = 154) Median (IQR)**	**No CKD progression (n = 143) Median (IQR)**	**p-value**
Serum TGF-β1 (ng/L)	21210 (15915 – 25745)	24200 (17570 – 29560)	**0.004**	20370 (15100 – 24650)	23740 (17470 – 29505)	**0.001**	20425 (15380 – 23630)	23320 (17150 – 29310)	**0.015**	22330 (16780 - 29060)	22080 (17150 - 28270)	0.767
Serum TGF-β2 (ng/L)	66.0 (34.8 – 89.2)	68.2 (36.7 – 100.2)	0.303	60.8 (30.6 – 89.3)	69.1 (38.5 – 94.7)	0.113	55.9 (29.9 – 77.8)	69.3 (37.7 – 94.7)	0.062	73.0 (42.4 - 100.5)	59.5 (30.9 - 88.9)	0.053
Serum TGF-β3 (ng/L)	13.9 (6.3 – 27.2)	14.8 (8.0 - 39.1)	0.506	13.0 (6.3 – 27.8)	14.9 (8.0 – 37.4)	0.512	13.5 (6.3 – 26.3)	14.8 (7.6 – 39.2)	0.421	11.8 (6.6 – 30.4)	16.1 (9.3 - 36.8)	0.286
Urine TGF-β1(ng/L)	5.3 (2.0 – 14.4)	6.9 (2.8 – 29.7)	0.357	4.9 (1.6 – 14.7)	6.9 (2.9 – 25.1)	0.231	5.4 (1.9 – 29.8)	6.8 (2.8 – 21.1)	0.817	11.1 (3.9 – 29.7)	6.2 (1.6 – 14.8)	0.054
Urine TGF-β2 (ng/L)	11.6 (4.6 – 17.3)	7.8 (3.8 – 16.1)	0.585	9.4 (4.1 – 13.9)	8.3 (4.0 – 21.0)	0.545	10.7 (5.0 – 15.4)	8.3 (4.0 – 17.1)	0.979	10.4 (3.3 - 21.6)	6.8 (4.4 - 13.1)	0.255
Urine TGF-β3 (ng/L)	17.5 (5.4 – 76.2)	2.8 (1.8 – 15.3)	**0.017**	7.9 (3.1 – 35.8)	5.9 (1.8 – 42.3)	0.663	10.4 (6.9 – 45.0)	5.9 (1.8 – 34.4)	0.184	4.9 (1.8 - 32.2)	10.4 (2.9 - 42.3)	0.402

TGF β, transforming growth factor beta

[a] Data available for each biomarker as follows: Serum TGF-β1 n=289 (97.3%); Serum TGF-β2 n=245 (82.5%); Serum TGF-β3 n=174 (58.6%); Urine TGF-β1 n=89 (30.0%); Urine TGF-β2 n=52 (17.5%); Urine TGF-β3 n=53 (17.8%). Biomarker levels in remining samples fell below the level of detection for the ELISA.

5.3.4 The association of clinical parameters with CKD progression

We divided the patients into two subgroups according to their CKD progression (by a continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more) status after 2 years of follow up by: CKD progression vs. no CKD progression. Factors associated with CKD progression were included in a multivariable regression analysis model. Significant associations with progression were baseline serum calcium (OR 0.06, 95% CI 0.01 -0.64, P = 0.019) and medications: diuretics (OR 2.35, 95% CI 1.37 – 4.00, P = 0.002), calcium channel blockers (OR 2.07, 95% CI 1.04 – 4.12, P = 0.038) and insulin (OR 1.96, 95% CI 1.01 – 3.84, P = 0.048); (Table 5.3).

Table 5.3: Odds Ratios showing the association between clinical variables and CKD progression

Characteristic	**No. of patients**	**UNIVARIABLE**		**MULTIVARIABLE**	
		OR (95% CI)	**P. value**	**OR (95% CI)**	**P. value**
Age	297	1.01 (0.99 -1.03)	0.223	1.00 (0.98 -1.02)	0.871
Sex					
Male	156	1 (Reference)			
Female	141	0.83 (0.53 – 1.31)	0.427		
SBP (mm hg)	297	1.02 (0.99 -1.04)	0.150	1.00 (0.98 -1.03)	0.833
eGFR (ml/min/1.72m^2)	297	0.98 (0.97 – 1.00)	**0.012**	1.00 (0.98 – 1.02)	0.887
Calcium (mmol /L)	297	0.04 (0.01 -0.28)	**0.001**	0.06 (0.01 -0.64)	**0.019**
Phosphate (mmol /L)	297	3.63 (1.34 -9.84)	**0.011**	1.88 (0.56 -6.28)	0.304
Haemoglobin (g/dl)	297	0.87 (0.78 – 0.97)	**0.012**	0.99 (0.86 - 1.15)	0.915
HDL cholesterol (mmol /L)	297	0.58 (0.32 -1.07)	0.083	0.76 (0.37 -1.54)	0.449
Uric acid (mmol/L)	297	12.07 (1.80 – 81.0)	**0.010**	1.64 (0.16 – 16.72)	0.677
Potassium (mmol/L)	297	1.41 (0.93 – 2.14)	0.105	1.27 (0.76 – 2.11)	0.364
Calcium phosphate product (mmol^2/L^2)	297	1.42 (0.93 – 2.16)	0.107	1.06 (0.65 – 1.74)	0.805
Diuretics	153	2.53 (1.58 – 4.04)	**0.001**	2.35 (1.37 – 4.00)	**0.002**
Calcium Channel Blockers	238	1.87 (1.04 – 3.38)	**0.037**	2.07 (1.04 – 4.12)	**0.038**
Statins	151	1.61 (1.02 – 2.55)	**0.041**	1.29 (0.74 – 2.23)	0.374
Insulin	68	2.66 (1.51 – 4.69)	**0.001**	1.96 (1.01 – 3.84)	**0.048**
Junior ASA	69	1.84 (1.07 – 3.18)	**0.029**	1.13 (0.59 – 2.17)	0.707

Beta blockers	142	1.83 (1.16 – 2.90)	**0.010**	1.37 (0.79 – 2.38)	0.258

P < 0.05 was used to identify potential variables, those with P <0.2 in univariable analysis along with age were included in the multivariable analysis with mutual adjustment.

5.3.5 Association of baseline transforming growth factor -beta (TGF-β) concentrations with CKD progression

Using the four different models for estimating CKD progression previously described, no significant association of TGF-β isoforms with CKD progression were seen on the multivariable regression analysis. The odd ratios for all biomarkers were around 1 indicating no increased or decreased odds of developing CKD progression based on the TGF-β isoform biomarkers (Table 5.4).

Table 5.4: The association of baseline serum and urine TGF-β with CKD progression in different models

		eGFR decline > 4 ml/min/1.73 m2/year or more				**Changed to a more advanced stage of CKD**				**> 30% reduction in eGFR in 2 years**				**> 30% increase in uPCR in 2 years**			
		Unadjusted		**Adjusted***		**Unadjusted**		**Adjusted***		**Unadjusted**		**Adjusted***		**Unadjusted**		**Adjusted***	
Baseline Biomarker	**No. of patients**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Serum TGF-β1 (ng/L)	289	1.00 (1.00 – 1.00)	0.074	1.00 (1.00 – 1.00)	0.194	1.00 (1.00 – 1.00)	**0.042**	1.00 (1.00 – 1.00)	0.158	1.00 (1.00 – 1.00)	**0.046**	1.00 (1.00 – 1.00)	0.223	1.00 (1.00 – 1.00)	0.933	1.00 (1.00 – 1.00)	0.989
Serum TGF-β2 (ng/L)	245	1.00 (0.99 – 1.00)	0.187	1.00 (0.99 – 1.00)	0.165	1.00 (0.99 – 1.00)	0.206	1.00 (0.99 – 1.00)	0.181	0.99 (0.99 – 1.00)	0.130	0.99 (0.99 – 1.00)	0.178	1.01 (1.00 – 1.01)	**0.038**	1.01 (1.00 – 1.01)	0.052
Serum TGF-β3 (ng/L)	174	1.00 (1.00 – 1.00)	0.814	1.00 (1.00 – 1.00)	0.786	1.00 (1.00 – 1.00)	0.699	1.00 (1.00 – 1.00)	0.851	1.00 (1.00 – 1.00)	0.646	1.00 (1.00 – 1.00)	0.538	1.00 (1.00 – 1.00)	0.797	1.00 (1.00 – 1.00)	0.893
Urine TGF-β1(ng/L)	89	0.99 (0.98 – 1.00)	0.174	0.99 (0.98 – 1.00)	0.195	0.99 (0.98 – 1.01)	0.312	0.99 (0.98 – 1.01)	0.368	1.00 (0.99 – 1.01)	0.611	1.00 (0.99 – 1.01)	0.673	1.00 (1.00 – 1.01)	0.412	1.00 (1.00 – 1.01)	0.402
Urine TGF-β2 (ng/L)	52	0.99 (0.97 – 1.02)	0.481	1.00 (0.97 – 1.03)	0.846	0.97 (0.91 – 1.02)	0.231	0.97 (0.92 – 1.03)	0.376	0.98 (0.92 – 1.04)	0.513	1.01 (0.95 – 1.08)	0.744	1.02 (0.98 – 1.07)	0.267	1.02 (0.98 – 1.06)	0.359
Urine TGF-β3 (ng/L)	53	1.01 (1.00 – 1.02)	0.131	1.01 (1.00 – 1.02)	**0.046**	1.00 (0.99 – 1.01)	0.472	1.00 (0.99 – 1.01)	0.662	1.00 (0.99 – 1.01)	0.808	1.01 (0.99 – 1.02)	0.315	1.00 (0.99 – 1.01)	0.683	1.00 (1.00 – 1.01)	0.416

*Adjusted for age, calcium, diuretics, calcium channel blockers and insulin

5.4 Discussion

Early identification of CKD progression is essential for planning and improving the management of CKD; albuminuria, serum creatinine and cystatin-C that form the core predictive models of CKD progression show alterations when the disease is already advanced. Thus, there is a need to identify biomarkers that are capable of predicting progression early in the disease trajectory. TGF- β has been increasingly touted as a candidate biomarker due to its critical role in kidney fibrosis and inflammation. TGF-β1 plays a major profibrotic role in most kidney diseases resulting in fibrosis and inflammation eventually leading to ESKD (359, 360). Studies have reported that serum and urine TGF-β1 levels were increased in most kidney diseases and were independently associated with lower eGFR and increasing proteinuria (370, 371).

In this study, both serum and urine TGF-β1 were measured at baseline only and the majority (97.3%) of our study patients had detectable serum TGF-β1 and 30% patients had detectable urine TGF-β1 at baseline. After 2 years of follow up, lower baseline serum and urine TGF-β1 concentrations were associated with declining eGFR and CKD progression. However, the baseline serum and urine TGF-β1 did not predict CKD progression in the multivariable analysis. These finding are similar to those from previous studies that have shown a reduction in serum TGF-β1 levels with declining eGFR (372, 373). In spite of the unavailability of clinical studies on the utility of TGF-β1 in predicting CKD progression, laboratory studies have found that TGF-β1 was crucial in progression of glomerular diseases and was significantly associated with CKD progression (365, 374). Amongst the reasons for these findings could be that our study was conducted among patients with CKD who were on treatment and presented with controlled diabetes mellitus and controlled hypertension at baseline.

For patients who were diagnosed with CKD progression, majority (85.2%) of the patients were using calcium channel blockers (amlodipine) and a smaller number (23.2%) of the patients were on ACEI/ARBs for management of hypertension at baseline; use of calcium channel blockers (amlodipine) was associated with CKD progression. Unlike previous studies which demonstrated that calcium channel blockers have similar kidney and cardiovascular protective effects when compared to RAAS blockers in patients with advanced CKD (329, 330). Calcium channel blockers or ACEI/ARBs were reported to have beneficial effects for kidney events including reducing TGF-β levels and albuminuria via RAAS blockade eventually slowing CKD progression (330, 375, 376).

At baseline most (63.4%) patients with CKD progression were on diuretics and using diuretics for management of hypertension had an association with CKD progression. Treatment of hypertension with diuretics was effective in reducing the blood pressure and CKD progression (377, 378). In this study about a third (31.7%) of the patients who reported CKD progression were using insulin for management of diabetes mellitus at baseline and CKD progression was linked with using insulin. Treatment of diabetes mellitus with insulin was effective in reducing the hyperglycaemia and CKD progression (379, 380).

Studies on the role of TGF-β2 in predicting CKD progression are uncommon despite the fact that TGF-β2 is highly expressed in most kidney diseases and is associated with kidney fibrosis (235, 359, 363). In this study, both serum and urine TGF-β2 were measured at baseline only and majority (82.5%) of the patients had detectable serum TGF-β2 and 17.5% patients had detectable urine TGF-β2. After 2 years of follow up, declining eGFR and CKD progression were associated with lower baseline serum TGF-β2 concentrations and increased baseline urine TGF-β2 concentrations. However, baseline serum and urine TGF-β2 did not predict CKD progression. Studies have reported that TGFβ2 induces myofibroblast

differentiation eventually leading to kidney fibrosis, loss of renal function and CKD progression (381, 382).

Studies have shown that TGF-β3 induces kidney fibrosis and inflammation which is less severe when compared to that of TGF-β1 (383, 384). In this study, both serum and urine TGF-β3 were measured at baseline only and more than half (58.6%) of the patients had detectable serum and 17.9% patients had detectable urine TGF-β3. After 2 years of follow up, CKD progression was associated with declining eGFR and higher baseline urinary TGF-β3. However, the baseline urinary TGF-β3 did not predict CKD progression. Previous studies reported that TGF-β3 in the kidney might have antifibrotic and kidney protective properties unlike the activity of TGF-β1, and that TGF-β3 expression levels peak with advanced CKD stages (225, 383). Our study adds to the pool of evidence that renal TGF-β3 might have roles that are opposing or counteracting the activity of TGF-β1.

In previous studies conducted with the aim of determining the application of urine and serum biomarkers in predicting CKD among patients with HIV/AIDS, urinary TGF-β1 and TGF-β2 concentrations were found to increase among CKD patients who had HIV (366, 369). Unlike our study findings, other studies have reported urinary biomarkers to be more sensitive and specific in predicting CKD progression when compared to serum biomarkers (34, 69). In this study, very few CKD patients had detectable urine TGF-β at baseline; amongst the reasons for this was that patients were in late stages of CKD at baseline when TGF- β expression had been dampened (366, 385). A more sensitive assay may have been able to elucidate this issue further. Also, lower baseline urinary TGF-β levels in this study could be accounted to the fact that at baseline almost all study patients were hypertensive and were on antihypertensive agents, calcium channel blockers and diuretics (385, 386).

Studies have reported increased risks for adverse outcomes among CKD patients who present with proteinuria; the presence of proteinuria has been associated with accelerated eGFR

decline among CKD patients (387, 388) and increases in uPCR are consistently associated with CKD progression and ESKD (389, 390). Changes in proteinuria and eGFR have been used in the estimation of CKD progression; proteinuria serves as an indicator of early CKD and the degree of proteinuria correlates with CKD progression (391, 392). After 24 months of follow up, CKD progression denoted by a change in uPCR > 30% in two years occurred in 51.9%. Almost similar findings from a study that was conducted in Australia where the prevalence of CKD progression by a change in uPCR > 30% in two years was 59%, while a lower prevalence of 26.1% was reported in a study that was conducted in Japan (76, 77). In this study, few (20.2%) patients were using ARBs or ACEIs while majority (84.0 %) of the patients were using calcium channel blockers. Previous studies did discover low uptake of ARBs/ ACEIs in patients with CKD (324, 326). Highlighting the requirement for clinicians to consider the most effective and practical use of ARBs/ACEIs in blocking RAAS among patients with CKD aiming at decreasing proteinuria and halting CKD progression (110, 111).

After 2 years of follow up, the prevalence of CKD progression by a continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more was 47.8%. Almost similar findings to the prevalence of CKD progression by a continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more of 46.7% was reported in a UK cohort study, while a lower prevalence of 38% was reported in a study in northern California (53, 75). The difference in findings could be accounted for the differences in the study populations; due to their African ancestry and racial disparities, black patients are at increased risk of faster CKD progression than other races (84, 128, 345).

A major limitation of this study was the inability to detect TGF-β isoforms in the majority of urine samples at baseline; also, we were not able to repeat the serum and urine TGF-β measurements after the 2 years follow up mainly due to resource constraints. Like the other routinely used biomarkers of eGFR and uPCR, changes in serum and urine TGF-β levels at

baseline and at follow up may have assisted in predicting CKD progression. In addition, the COVID 19 pandemic and a major fire at CMJAH in April 2021 that resulted in closure of the hospital for 6 months might have had an impact on blood pressure and diabetes mellitus control. With clinic closure, patients were unable to continue with the routine follow at this hospital but instead had to follow up in nearby hospitals. Despite these limitations, the results of our study add to the current body of literature on baseline TGF-β isoforms and CKD progression.

5.5 Conclusion

Patients with CKD progression had lower baseline concentrations of serum TGF-β1 and increased baseline urinary TGF-β3 concentrations compared to patients who did not progress. However, based on both univariate and multivariable analyses, baseline serum or urine TGF-β isoforms were not shown to be predictors of CKD. The roles of the various serum and urine TGF-β isoforms in CKD progression are still unclear and highlight the importance of further studies to determine their isoform specific effects. An increase in uPCR at two years follow up identified the highest prevalence of patients who had CKD progression when compared to other routinely used biomarkers.

Supplementary Table 5.1: Baseline demographic and clinical characteristics of the study patients recruited at early stage (stage 1 and 2) by CKD progression

Characteristic	CKD progression (n = 26)	No CKD progression (n = 39)	P-value
	Proportion (%) or Median (IQR)	Proportion (%) or Median (IQR)	
Demographics:			
Age (years)	52.5 (38 - 60)	43 (37 -59)	0.597
Sex			
Male	13 (50.0%)	15 (38.5 %)	
Female	13 (50.0%)	24 (61.5 %)	0.357
Marital status			
Single	10 (38.5 %)	16 (41.0 %)	
Married	13 (50.0 %)	20 (51.3 %)	
Widow/Widower	1 (3.9 %)	3 (7.7 %)	
Separated/Divorced	2 (7.7 %)	0	0.333
Highest level of education			
No formal education	5 (19.2 %)	0	
Primary	6 (23.1 %)	11 (28.2 %)	
Secondary	5 (19.2 %)	14 (35.9 %)	

Tertiary	10 (38.5 %)	14 (35.9 %)	**0.027**
Occupation			
Unemployed	2 (7.7 %)	8 (20.5 %)	
Domestic workers	7 (26.9 %)	7 (18.0 %)	
Self employed	6 (23.1 %)	9 (23.1 %)	
Public / Private servant	11 (42.3 %)	12 (30.8 %)	
Retired	0	3 (7.7 %)	0.304
Clinical Variables:			
BMI (kg/m^2)	29.7 (26.7 -33.5)	29.4 (23.3 – 33.6)	0.453
SBP (mmHg)	135.5 (126 -140)	140 (122 – 140)	0.949
DBP (mmHg)	82 (78 -90)	83 (73 – 90)	0.924
Creatinine (umol/L)	91.5 (80 -109)	85 (78 -100)	0.263
eGFR ($ml/min/1.72m^2$)	74 (67 -81)	75 (69 – 85)	0.533
uPCR (g/mmol)	0.012 (0.008-0.027)	0.014 (0.007 – 0.019)	0.768
FBG (mmol/L)	4.4 (4.2 – 4.7)	4.4 (4.1 – 4.9)	0.961
HbA1c (%)	7.0 (7.0 – 7.0)	7.0 (6.8 – 7.0)	0.676
Haemoglobin (g/dl)	13.7 (12.5 – 15.9)	14.3 (12.3 – 16.0)	0.525
WBC (x 10^9 cells/L)	5.49 (4.25 – 7.15)	6.01 (4.81 – 7.50)	0.139
Platelets (x 10^9 cells/L)	218.5 (200 -300)	276 (218 – 335)	**0.036**
Uric acid (mmol/L)	0.34 (0.30 – 0.42)	0.31 (0.22 – 0.35)	**0.009**
HDL cholesterol (mmol /L)	1.22 (1.02 -1.52)	1.26 (1.06 – 1.55)	0.784
Calcium (mmol /L)	2.33 (2.28 – 2.37)	2.36 (2.26 – 2.47)	0.469
Phosphate (mmol /L)	0.99 (0.89 – 1.16)	1.04 (0.94 – 1.13)	0.529
Sodium (mmol/L)	142 (139 – 144)	140 (138 – 142)	0.118
Potassium (mmol/L)	4.1 (4.0 – 4.4)	4.2 (4.0 – 4.4)	0.471
Bicarbonate (mmol/L)	24 (21 – 25)	22 (20 - 24)	**0.018**
Calcium phosphate product ($mmol^2/L^2$)	2.30 (2.10 – 2.70)	2.45 (2.18 – 2.77)	0.278
Medications:			
Diuretics	10 (38.5%)	12 (30.8%)	0.521
ACEIs / ARBs	8 (30.8%)	6 (15.4%)	0.139
Aldactone	0	2 (5.1%)	0.241
Calcium channel blockers	20 (76.9%)	24 (61.5%)	0.194
Statins	8 (30.8%)	14 (35.9%)	0.669
Oral Hypoglycemics	2 (7.7%)	6 (15.4%)	0.355
Insulin	4 (15.4%)	6 (15.4%)	1.00
Allopurinol	0	3 (7.7%)	0.148
Junior ASA	3 (11.5%)	5 (12.8%)	0.878
Beta blockers	9 (34.6%)	13 (33.3%)	0.915
Aldomet	5 (19.2%)	8 (20.5%)	0.899
Hydralazine	1 (3.9%)	3 (7.7%)	0.527
Nitrates (ISMN/ISDN)	0	1 (2.6%)	0.411
Doxazosin	10 (38.5%)	11 (28.2%)	0.386
Others	9 (34.6%)	10 (25.6%)	0.436

Supplementary Table 5.2: Baseline demographic and clinical characteristics of the study patients recruited at later stages (stage 3a, 3b and 4) by CKD progression

Characteristic	CKD progression (n = 116) Proportion (%) or Median (IQR)	No CKD progression (n = 116) Proportion (%) or Median (IQR)	P-value
Demographics:			
Age (years)	59.5 (49 – 68.5)	59.5 (49 -68)	0.515
Sex			
Male	65 (56.0%)	63 (54.3 %)	
Female	51 (44.0%)	53 (45.7 %)	0.792
Marital status			
Single	28 (24.1 %)	35 (30.2 %)	
Married	64 (55.2 %)	57 (49.1 %)	
Widow/Widower	17 (14.7 %)	16 (13.8 %)	
Separated/Divorced	7 (6.0 %)	8 (6.9 %)	0.734
Highest level of education			
No formal education	13 (11.2 %)	16 (13.8 %)	
Primary	24 (20.7 %)	26 (22.4 %)	
Secondary	46 (39.7 %)	30 (25.9 %)	
Tertiary	33 (28.5 %)	44 (37.9 %)	0.149
Occupation			
Unemployed	20 (17.2 %)	14 (12.1 %)	
Domestic workers	18 (15.5 %)	24 (20.7 %)	
Self employed	26 (22.4 %)	24 (20.7 %)	
Public / Private servant	40 (34.5 %)	44 (37.9 %)	
Retired	12 (10.3 %)	10 (8.6 %)	0.668
Clinical Variables:			
BMI (kg/m^2)	30.6 (26.8 -35.8)	29.8 (26.5 – 33.2)	0.483
SBP (mmHg)	140 (133.5 -140)	140 (127 – 140)	**0.044**
DBP (mmHg)	83 (74 -90)	82 (72 – 90)	0.287
Creatinine (umol/L)	156 (133 -187.5)	147 (122 -174)	**0.021**
eGFR ($ml/min/1.72m^2$)	35 (31 -43.5)	39.5 (32 – 47.5)	**0.011**
uPCR (g/mmol)	0.051 (0.021-0.095)	0.018 (0.01 – 0.038)	**<0.001**
FBG (mmol/L)	4.5 (4.2 – 5.6)	4.4 (4.2 – 4.9)	0.373
HbA1c (%)	7.0 (6.8 – 7.0)	7.0 (6.6 – 7.0)	0.383
Haemoglobin (g/dl)	12.8 (11.5 – 14.1)	13.6 (12.2 – 14.8)	**0.018**
WBC (x 10^9 cells/L)	6.16 (5.20 – 7.91)	6.48 (4.81 – 7.85)	0.683
Platelets (x 10^9 cells/L)	256.5 (222 -327.5)	263.5 (216 – 319)	0.877
Uric acid (mmol/L)	0.45 (0.38 – 0.53)	0.43 (0.36 – 0.51)	0.357
HDL cholesterol (mmol /L)	1.15 (0.98 -1.43)	1.24 (1.00 – 1.59)	0.163
Calcium (mmol /L)	2.29 (2.20 – 2.39)	2.34 (2.25 – 2.42)	**0.007**
Phosphate (mmol /L)	1.13 (0.94 – 1.30)	1.01 (0.88 – 1.17)	**0.002**
Sodium (mmol/L)	140 (138.5 – 143)	141 (138 – 143)	0.666
Potassium (mmol/L)	4.4 (4.1 – 4.7)	4.2 (3.75 – 4.65)	**0.041**
Bicarbonate (mmol/L)	22 (20 – 24)	22 (20 - 24)	0.506
Calcium phosphate product ($mmol^2/L^2$)	2.56 (2.16 – 2.92)	2.38 (1.98 – 2.70)	**0.024**
Medications:			
Diuretics	80 (69.0%)	51 (44.0%)	**<0.001**
ACEIs / ARBs	25 (21.6%)	21 (18.1%)	0.510

Aldactone	6 (5.2%)	3 (2.6%)	0.308
Calcium channel blockers	101 (87.1%)	93 (80.2%)	0.156
Statins	73 (62.9%)	56 (48.3%)	**0.025**
Oral Hypoglycemics	12 (10.3%)	16 (13.8%)	0.420
Insulin	41 (35.3%)	17 (14.7%)	**<0.001**
Allopurinol	15 (12.9%)	16 (13.8%)	0.847
Junior ASA	38 (32.8%)	23 (19.8%)	**0.025**
Beta blockers	70 (60.3%)	50 (43.1%)	**0.009**
Aldomet	30 (25.9%)	26 (22.4%)	0.539
Hydralazine	10 (8.6%)	5 (4.3%)	0.182
Nitrates (ISMN/ISDN)	4 (3.5%)	2 (1.7%)	0.408
Doxazosin	54 (46.6%)	59 (50.9%)	0.511
Others	36 (31.0%)	44 (37.9%)	0.269

Supplementary Table 5.3: Baseline serum and urine transforming growth factor-beta of the study patients recruited at early stage (stages 1 and 2) by CKD progression using routinely used biomarkers.

	eGFR decline > 4 ml/min/1.73 m2/year or more			Changed to a more advanced stage of CKD			> 30% reduction in eGFR in 2 years			> 30% increase in uPCR in 2 years		
Baseline Characteristics	**CKD progression (n = 26)**	**No CKD progression (n = 39)**	**p-value**	**CKD progression (n = 15)**	**No CKD progression (n = 50)**	**p-value**	**CKD progression (n = 6)**	**No CKD progression (n = 59)**	**p-value**	**CKD progression (n = 28)**	**No CKD progression (n = 37)**	**p-value**
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Serum TGF-β1 (ng/L)	23380 (20370 – 30190)	27620 (21580 – 30660)	0.256	23560 (21290 – 33030)	26090 (21240 – 30570)	0.943	28295 (21760 – 42560)	25960 (21240 – 30370)	0.333	28520 (23225 – 30515)	23970 (19055 – 31180)	0.140
Serum TGF-β2 (ng/L)	66.3 (34.8 – 87.1)	79.1 (44.7 – 119.3)	0.193	41.2 (34.8 – 75.3)	82.8 (47.1 – 106.9)	0.106	58.2 (38.0 – 77.8)	73.7 (34.8 – 103.5)	0.677	86.6 (66.7 – 106.9)	54.5 (27.1 – 92.7)	0.080
Serum TGF-β3 (ng/L)	10.4 (2.3 – 20.6)	16.0 (4.9 – 35.4)	0.260	3.7 (1.2 – 13.0)	14.8 (5.2 – 35.4)	0.066	7.1 (1.2 – 88.4)	12.4 (4.0 – 28.9)	0.616	11.5 (4.0 – 18.0)	14.9 (3.7 – 47.7)	0.301
Urine TGF-β1(ng/L)	11.6 (1.8 – 21.4)	11.5 (2.3 – 122.4)	0.505	8.9 (1.3 – 21.4)	11.5 (2.3 – 71.8)	0.390	6.3 (1.3 – 26.1)	11.6 (2.3 – 58.0)	0.558	17.4 (11.5 – 122.4)	6.8 (0.9 – 26.1)	0.091
Urine TGF-β2 (ng/L)	4.7 (4.6 – 12.6)	10.9 (3.8 – 34.3)	0.517	4.7 (4.6 – 4.7)	12.6 (3.8 – 34.3)	0.352	–[b]	9.2 (3.8 – 34.3)		27.8 (7.1 – 69.3)	5.9 (3.8 - 12.6)	0.160
Urine TGF-β3 (ng/L)	138.0 (0.8 – 275.1)	4.4 (0.8 – 13.6)	0.686	0.8 (0.8 – 0.8)	7.9 (0.8 – 1.3)	0.281	–[b]	4.4 (0.8 – 15.3)		7.9 (0.8 – 15.3)	0.9 (0.8 – 11.8)	0.747

[a] Data available for each biomarker as follows: Serum TGF-β1 n=64 (98.5%); Serum TGF-β2 n=57(87.7%); Serum TGF-β3 n=43 (66.2%); Urine TGF-β1 n=25 (38.5%); Urine TGF-β2 n=19 (29.2%); Urine TGF-β3 n=10 (15.4%). Biomarker levels in remaining samples fell below the level of detection for the ELISA.

[b] No individuals with data in this category

Supplementary Table 5.4: Baseline serum and urine transforming growth factor-beta of the study patients recruited at later stage (stages 3a, 3b and 4) by CKD progression using routinely used biomarkers.

	eGFR decline > 4 ml/min/1.73 m2/year or more			Changed to a more advanced stage of CKD			> 30% reduction in eGFR in 2 years			> 30% increase in uPCR in 2 years		
Baseline Characteristics	**CKD progression (n = 116)**	**No CKD progression (n = 116)**	**p-value**	**CKD progression (n = 89)**	**No CKD progression (n = 143)**	**p-value**	**CKD progression (n = 51)**	**No CKD progression (n = 181)**	**p-value**	**CKD progression (n = 126)**	**No CKD progression (n = 106)**	**p-value**
	Median (IQR)	**Median (IQR)**		**Median (IQR)**	**Median (IQR)**		**Median (IQR)**	**Median (IQR)**		**Median (IQR)**	**Median (IQR)**	
Serum TGF-β1 (ng/L)	20220 (14770 – 24850)	23255 (16360 – 29280)	**0.026**	19035 (14700 – 23700)	22840 (16410 – 29280)	**0.004**	19435 (14700 – 22600)	22080 (15840 – 29130)	**0.023**	20775 (15440 – 26780)	21840 (15840 – 28080)	0.426
Serum TGF-β2 (ng/L)	66.0 (33.7 – 92.6)	67.0 (35.0 – 92.8)	0.656	62.2 (30.4 – 92.1)	67.0 (37.7 – 93.3)	0.364	55.9 (29.9 – 76.3)	68.8 (37.7 – 94.7)	0.070	68.3 (39.9 – 99.1)	60.3 (31.7 – 87.5)	0.230
Serum TGF-β3 (ng/L)	16.1 (8.0 – 28.3)	14.3 (9.0 – 43.0)	0.852	15.5 (8.0 – 31.3)	15.5 (8.7 – 37.4)	0.933	15.0 (7.4 – 26.3)	15.5 (9.0 – 43.3)	0.335	12.4 (7.4 – 43.0)	16.1 (10.5 – 28.3)	0.396
Urine TGF-β1(ng/L)	4.9 (2.3 – 14.2)	6.7 (2.9 – 19.7)	0.496	4.9 (1.6 – 14.4)	6.5 (2.9 – 17.3)	0.425	4.9 (2.3 – 33.5)	5.6 (2.9 – 14.2)	0.831	5.6 (3.8 – 23.1)	5.6 (1.6 – 12.8)	0.160
Urine TGF-β2 (ng/L)	11.8 (6.5 – 17.3)	7.3 (4.0 – 8.9)	0.109	10.7 (3.5 – 14.4)	7.5 (4.2 – 16.1)	0.870	10.7 (5.0 – 15.4)	7.6 (4.2 – 13.5)	0.769	8.9 (3.3 – 16.9)	7.1 (5.9 – 13.5)	0.613
Urine TGF-β3 (ng/L)	17.5 (5.9 – 72.4)	2.9 (1.8 – 16.4)	**0.022**	8.9 (3.4 – 45.0)	5.9 (1.8 – 45.1)	0.499	10.4 (6.9 – 45.0)	5.9 (1.8 – 42.3)	0.232	4.9 (1.8 – 47.9)	15.5 (4.3 – 43.7)	0.192

[a] Data available for each biomarker as follows: Serum TGF-β1 n=225 (97.0%); Serum TGF-β2 n=188 (81.0%); Serum TGF-β3 n=131 (56.5%); Urine TGF-β1 n=64 (27.6%); Urine TGF-β2 n=33 (14.2%); Urine TGF-β3 n=43 (18.5%). Biomarker levels in remining samples fell below the level of detection for the ELISA.

CHAPTER 6

DISCUSSION

The hypothesis tested in this study was that baseline transforming growth factor-beta (TGF-β) isoforms may have a role in predicting CKD progression among black patients attending a tertiary hospital in Johannesburg, South Africa and that urinary transforming growth factor-beta (TGF-β) may be more accurate in predicting CKD progression compared to serum transforming growth factor-beta (TGF-β) among black CKD patients with controlled hypertension and diabetes mellitus in South Africa.

6.1 Summary of the study findings

This study revealed the following;

6.1.1 At baseline, 58% of the study patients presented with advanced CKD; of whom 31.5% were found to have grossly elevated proteinuria. Hypertension was present in 96.7% patients, diabetes mellitus in 38.7% patients and both hypertension and diabetes mellitus were present in 38.1% of the patients. In patients with advanced CKD, most (62.4%) of the patients had metabolic acidosis. Anaemia was detected in 46.4% patients and hyperuricaemia was present in 30.9% patients while the prevalence of metabolic acidosis and anaemia in those patients with early CKD was 46.6% and 25.9% respectively.

6.1.2 CKD progression occurred in 49.5% of the study patients after two years of follow up, 33% of patients had CKD remission and 17.5% patients had CKD regression. Almost half (47.8%) of the patients with CKD progression had a continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more, while the prevalence of CKD progression by alterations in uPCR > 30% was 51.9% after two years.

6.1.3 The majority (95.2%) of the patients with CKD progression had hypertension, 40.1% of the patients had diabetes mellitus and 39.5% of the patients had both hypertension and diabetes

mellitus. Almost half (48.3%) of the patients had severely increased proteinuria, 45.6% patients were found to have anaemia, 34.0% had hyperuricaemia and 17.7% had hypocalcemia at baseline.

6.1.4 Serum and urine TGF-β were measured only at baseline in this study and the majority (97.3%) of our study patients had detectable serum TGF-β1 and 30% patients had detectable urine TGF-β1. After 24 months of follow up, lower baseline serum and urine TGF-β1 concentrations were associated with declining eGFR and CKD progression. The baseline serum and urine TGF-β1 did not predict CKD progression on both univariate and multivariable analyses.

6.1.5 The majority (82.5%) of the patients had detectable serum TGF-β2 and 17.5% patients had detectable urine TGF-β2 at baseline. After 24 months of follow up, declining eGFR and CKD progression were associated with lower baseline serum TGF-β2 concentrations and increased baseline urine TGF-β2 concentrations. The baseline serum and urine TGF-β2 did not predict CKD progression on both univariate and multivariable analyses.

6.1.6 Most (58.6%) of the patients had detectable serum TGF-β3 and 17.9% patients had detectable urine TGF-β3 at baseline. After 2 years of follow up, CKD progression was associated with declining eGFR and higher baseline urinary TGF-β3. The baseline urinary TGF-β3 did not predict CKD progression on both univariate and multivariable analyses.

Table 6.1: Summary of the study findings

	Objectives	Chapter	Findings
1.	To establish the factors associated with CKD among black CKD patients in South Africa.	3	Variables with higher odds for CKD after multivariable logistic regression analysis were: • metabolic acidosis (OR 2.0, 95% CI 1.2 - 3.1, P= 0.005), • anaemia (OR 2.9, 95% CI 1.7 - 4.9, P= 0.001), • severe proteinuria (OR 3.5, 95% CI 1.9 - 6.5, P = 0.001), • hyperuricaemia (OR 2.4, 95% CI 1.4 - 4.1, P = 0.001), • hypertension (OR 3.3, 95% CI 1.2 - 9.2, P = 0.020), • diabetes mellitus (OR 1.8, 95% CI 1.1 - 3.3, P = 0.024) • angina (OR 2.5, 95% CI 1.2 - 5.1, P = 0.008).
2.	To establish the role of routinely used biomarker in predicting CKD progression among black patients in South Africa.	4	The prevalence of CKD progression in two years by changes in: • uPCR > 30% was 51.9% • by continued decrease in eGFR of > 4 ml/min/1.73 m2/year or more was 47.8 %
3.	To establish the factors associated with CKD progression among black patients in South Africa.	4	Variables associated with higher odds for CKD progression after multivariable logistic regression analysis were: • current smoking (OR 2.8, 95% CI 1.9 - 8.6, P = 0.049), • diabetes mellitus (OR 1.8, 95% CI 1.9 - 3.6, P = 0.047), • increased HbA1c (OR 1.8, 95% CI 1.2 - 2.8, P = 0.007) • anaemia (OR 2.1, 95% CI 1.0 - 4.3, P= 0.048) • grossly elevated proteinuria (OR 6.1, 95 % CI (3.2 – 11.6), P = 0.001), • moderately elevated proteinuria (OR 2.1, 95% CI (1.1 –

			3.9), P= 0.019), • hyponatraemia (OR 4.5, 95% CI 1.8 - 23.6, P= 0.042) • hypocalcaemia (OR 3.8, 95% CI 1.0 - 14.8, P = 0.047).
4.	To establish the role of TGF-β isomers in predicting CKD progression among black patients in South Africa.	5	Transforming growth factor-ß isomers were not associated with CKD progression among black patients attending a tertiary hospital in Johannesburg, South Africa
5.	To compare baseline urinary and serum TGF-ß isoforms in predicting CKD progression among black patients in South Africa.	5	Very few patients had detectable urine TGF-ß isoforms at baseline; baseline urine TGF-ß levels had no association with CKD progression.

6.2 Factors associated with CKD

In this study, at baseline 58% of the study patients presented with advanced CKD. Hypertension is common in CKD and it has a strong association with CKD progression (299, 300). Among the patients with advanced CKD, the majority (96.7%) of patients were found to be hypertensive, similar to findings from previous studies from SSA (294, 301). Hypertension had 3.3 times increased odds for advanced CKD, similar to results reported from previous studies (303, 304). Globally diabetic kidney disease is a major cause of ESKD and death among patients with T2DM (305, 306). More than one third (38.7%) of the patients who were diagnosed with advanced CKD

presented with T2DM; T2DM had 1.8 higher odds for advanced CKD, similar to previous studies conducted in Ethiopia and South Africa (97, 301).

Metabolic acidosis is commonly frequent in CKD and it can eventually lead to impairment of multiple systems and organs including the kidney thus causing CKD progression that culminates into ESKD (129, 309). Most (62.4%) patients with advanced CKD were discovered to have metabolic acidosis and 46.6% with early CKD were also found to have metabolic acidosis. These findings are higher than that reported from US states and Puerto Rico where 33% - 40% of the patients with CKD stage 3 – 4 were found to have metabolic acidosis (309-311). These differences could be attributed to: firstly, the greater and more rapid CKD progression which has been reported among black patients including even those presenting with early CKD stages (88, 128). Secondly, diet whereby changing of local diets to western foods which comprise of principally proteins from animals, very few vegetables and reduced eating of fruits might accelerate CKD patients' dietary acid burden (146, 312). Patients with metabolic acidosis showed a 2-fold higher burden for advanced CKD, similar to the findings from other studies (127, 310).

Anaemia is usually associated with CKD, and it frequently leads to decreased quality of life and poor outcomes thus culminating to death (313, 314). Almost half (46.4%) of the patients with advanced CKD had anaemia, comprising 16.6% who presented with reduced transferrin levels, and among those patients with early CKD, 25.9% had anaemia. Patients with anaemia had 2.9 times increased risk for advanced CKD and those patients with reduced serum transferrin levels were 2.4 times more at higher risk; similar findings have been previously reported from Turkey and South Africa (112, 113, 315).

Advanced CKD and CKD progression is frequently present among patients with elevated serum uric acid levels (316, 317). In this study, among the patients with advanced CKD, 30.9% of the patients had hyperuricaemia. Patients with hyperuricemia had 2.4 higher odds for advanced CKD; similar results were noted in previous studies from China (44, 318). Hyperkalaemia is common in

CKD and its magnitude rises with decreasing eGFR (123, 319). In this study, very few (8.8%) patients with advanced CKD were found to have hyperkalaemia; hyperkalaemia occurred 5.4-fold more frequently with advanced CKD, similar to reports from previous literature (124, 296).

The magnitude of cardiovascular events rises with declining kidney function (320, 321). In this study, 22.7% of the patients among those with advanced CKD in this study were found to have angina with 2.5 fold higher odds for advanced CKD. These findings are similar to previous studies (320, 322). Almost one third (31.5%) of the patients who had advanced CKD had grossly elevated proteinuria; markedly elevated proteinuria had a 3.5 fold increased odds for advanced CKD, similar to previous studies (78, 97, 323). ACEIs or ARBs were used in very few (18.8%) patients for treatment of hypertension with advanced CKD and in only 21.4% of patients with early CKD. Underutilization of angiotensin ACEIs/ARBs when they were clinically indicated or terminating RAAS inhibitors in the course of CKD treatment has been reported previously (324-326). Due to the fact that very few patients were using ARBs or ACEIs, presumably these medications were not utilised due to adverse effects or fear of adverse effects in advanced CKD. Previous studies demonstrated that using ACEIs/ARBs had additional benefits for kidney and cardiovascular incidents among patients with CKD (326-328). This could be attributed to the fact that the majority (84.0%) of the patients presenting with advanced CKD were using calcium channel blockers and it has been demonstrated that calcium channel blockers and RAAS blockers possess identical protective functions on the kidney and cardiovascular events in patients with CKD (329, 330).

6.3 Role of routinely used biomarkers in predicting CKD progression

After 24 months of follow up, almost half (49.5%) of the patients had CKD progression by eGFR estimation. This prevalence is almost similar to the 46.7% that was reported in a UK cohort study and it is higher than the 38% in a study from the USA (53, 75). The differences could be attributed to the fact that our study enrolled exclusively black patients; studies have demonstrated that black

patients are prone to more rapid and increased CKD progression including those patients with early stages of CKD (128, 345).

Additionally, Africans; as a consequence of ethnic/racial disparities; are at increased odds for CKD and CKD progression (84, 85). In this study, we found that after the 24 months follow up, CKD remission occurred in 33% patients and CKD regression was recorded in 17.5% patients, similar discoveries have been previously reported from UK (75, 346). After 24 months of follow up, almost half (47.8%) of the study patients were discovered to have CKD progression by continued decrease in eGFR of > 4 ml/min/1.73 m^2/year or longer. Similar findings were reported where the prevalence of CKD progression by a continued decrease in eGFR of > 4 ml/min/1.73 m^2/year or longer was 46.7% in a cohort study conducted in UK, while a prevalence of 38% was reported from a study that was conducted in USA (53, 75).

Studies have reported increased risks for adverse outcomes among CKD patients who present with proteinuria; the presence of proteinuria has been associated with accelerated eGFR decline among CKD patients (387, 388) and increases in uPCR are consistently associated with CKD progression and ESKD (389, 390). After 24 months of follow up, CKD progression denoted by a change in uPCR > 30% in two years occurred in 51.9% of the patients. Identical discoveries have been previously reported where changes in proteinuria and eGFR have been used in the estimation of CKD progression. Proteinuria serves as an indicator of early CKD and the degree of proteinuria correlates with CKD progression (391, 392). Almost similar findings were noted in a study that was conducted in Australia where the prevalence of CKD progression by a change in uPCR > 30% in two years which was 59%, while a lower prevalence of 26.1% was reported in a study conducted in Japan (76, 77).

6.4 Factors associated with CKD progression

In this study, the majority (96.7%) of the study patients were diagnosed with hypertension at baseline. Among those patients diagnosed with CKD progression, 95.2% were found to have hypertension and in patients without CKD progression, 91.3% were hypertensive. Like previous studies hypertension was strongly associated with CKD progression (96, 97). In this study, both diastolic and systolic blood pressure had no significant association with CKD progression, unlike previous studies which reported both elevated DBP and SBP levels to be correlated with CKD and had increased odds for CKD progression (98, 99). This disparity could be attributed to the fact that large numbers of both the patients diagnosed with CKD progression and those without CKD progression had hypertension at baseline.

Very few (9.5%) patients among those with CKD progression reported smoking. Smoking was found to have 2.8 times higher odds for CKD progression. Smoking has been previously reported to be positively correlated with CKD and CKD progression (100, 101). In this study, obesity and/or elevated cholesterol levels had no significant association with CKD progression contrary to other studies where it was reported that obesity and/or hyperlipidemia were found to be positively correlated with CKD progression (102, 103). This disparity could be attributed to the fact that the greater numbers of the patients diagnosed with CKD progression and those without CKD progression were obese/overweight at baseline.

Among patients with CKD progression, 40.1% had diabetes mellitus; diabetes mellitus and elevated HbA1c were each associated with 1.8 times higher odds for CKD progression. Studies have reported elevated HbA1c to be positively correlated with diabetic nephropathy and CKD progression among patients with T2DM (107, 108). Almost half (48.3%) of the patients with CKD progression were discovered with grossly elevated proteinuria. Massively elevated proteinuria was associated with 6.1 times higher odds for CKD progression while moderately elevated proteinuria

was associated with 2.1 times higher odds for CKD progression. Previous studies demonstrated CKD progression to be highly prevalent in patients with markedly elevated proteinuria (77, 109). Previous studies reported that using ARBs or ACEIs was most effective in decreasing proteinuria, in contrast to other antihypertensive drugs. In this study, few (20.2%) patients were using ARBs or ACEIs, probably accounting for the apparent lack of effect of these drugs on proteinuria and CKD progression. Previous studies reported low uptake of ARBs/ ACEIs in patients with late CKD stages (324, 326), highlighting the need for clinicians to consider the use of ARBs/ACEIs in blocking RAAS in patients with late stages of CKD aimed at decreasing proteinuria and halting CKD progression (110, 111). The majority (84.0 %) of the patients were using calcium channel blockers. Studies have also demonstrated that calcium channel blockers have similar kidney and cardiovascular protective effects when compared to RAAS blockers in patients with CKD (329, 330).

Among the patients who had CKD progression, 45.6% of the patients had anaemia. Anaemia conferred a 2.1times higher risk for CKD progression. Anaemia in CKD is highly prevalent as a result of decreased production of erythropoietin by the diseased kidneys(113). Anaemia has been found to be positively correlated with CKD, CKD progression and worse clinical outcomes (114). In this study, there was no significant association of hyperuricaemia and CKD progression unlike previous studies which reported that elevated serum uric acid levels were frequently observed with reduced eGFR. Hyperuricaemia usually occurs before CKD, predicts CKD and is positively correlated with CKD progression (115, 116). Among patients who had CKD progression, few (17.7%) had hypocalcemia and hypocalcemia had 3.8-fold increased odds for CKD progression. Reduced serum calcium levels frequently occur as a result of higher serum phosphate and reduced production of 1,25(OH)2 vitamin D by the kidney due to hyperparathyroidism (118). Hypocalcaemia is positively correlated with CKD, and it culminates in more rapid CKD progression (117). Hyperphosphatemia was not significantly associated with CKD progression in

our study; it had been reported previously that elevated serum phosphate levels predicted CKD progression (119, 120).

Very few (8.16%) patients among those with CKD progression reported hyponatremia and reduced serum sodium levels had 4.5-fold increased odds for CKD progression. The kidney plays a crucial role in regulating sodium and water homeostasis. Hyponatremia as a result of excess water or use of diuretics is common in CKD and it culminates in increased odds of CKD progression, hospitalisation, cardiovascular events and higher mortality (121, 122). In this study, hyperkalaemia was found not to have any significant association with CKD progression. Hyperkalemia is commonly frequent in advanced stages of CKD and it is positively associated with CKD progression; (123, 124). Patients with metabolic acidosis were at increased odds for speedy decline in eGFR, CKD progression and worse consequences (129, 130). In this study, metabolic acidosis was highly prevalent in our study patients but it was found not to have any significant association with CKD progression. This dissimilarity could be attributed to the fact that greater part of the patients diagnosed with CKD progression and those without CKD progression presented with metabolic acidosis at baseline.

6.5 Baseline transforming growth factor-ß isoforms were not associated with chronic kidney disease progression among black patients attending a tertiary hospital in Johannesburg, South Africa

The objective of this study was to evaluate the role of baseline TGF-β isoforms in predicting CKD progression among black patients. Early identification of CKD progression is essential for optimizing the treatment and management of CKD. Albuminuria, serum creatinine and cystatin-C that form the core predictive models of CKD progression show changes relatively late in the disease pathway. Thus, there was a need to early identify biomarkers that are capable of predicting CKD progression.

TGF-β has been increasingly touted as a candidate biomarker due to its critical role in kidney fibrosis and inflammation. TGF-β1 plays a major profibrotic role in most kidney diseases hence resulting in fibrosis and inflammation which eventually leads to ESKD (359, 360). Studies have reported that serum and urine TGF-β1 had high concentrations in most kidney diseases and were independently associated with lower eGFR and increasing proteinuria (370, 371).

In this study both serum and urine TGF-β1 were only measured at baseline and the majority (97.3%) of our study patients had detectable serum TGF-β1 and 30% patients had detectable urine TGF-β1. After 2 years of follow up, lower baseline serum and urine TGF-β1 concentrations were associated with declining eGFR and CKD progression. However, the baseline serum and urine TGF-β1 did not predict CKD progression on both univariate and multivariable analyses. These finding are similar to those from previous studies that have shown a reduction in serum TGF-β1 levels with declining eGFR (372, 373). In spite of the lack of clinical studies on the utility of TGF-β1 in predicting CKD progression, laboratory studies have demonstrated that TGF-β1 has a crucial role in the progression of glomerular diseases and was positively correlated with CKD progression (365, 374). This disparity could be attributed to the fact that our study was conducted in CKD patients who were on treatment and had controlled diabetes mellitus and hypertension at baseline at enrolment. Also, the majority (85.2%) of the patients with CKD progression at baseline were using calcium channel blockers (amlodipine) and a smaller number (23.2%) of the patients were on ACEI/ARBs for the management of hypertension. Studies have shown calcium channel blockers or ACEI/ARBs to have beneficial effects for kidney events including reducing TGF-β levels and albuminuria, thus slowing CKD progression (330, 375, 376). Also, at baseline, most (63.4%) patients with CKD progression were on diuretics. It has been demonstrated previously that the treatment of the hypertension with diuretics was effective in blood pressure control, reduced TGF-β levels and lower CKD progression (377, 378). Few (31.7%) patients of those who were diagnosed with CKD progression were on insulin at baseline for the management of diabetes mellitus. It has

been demonstrated previously from other studies that the treatment of the diabetes mellitus with insulin was effective in controlling the hyperglycaemia, reduced TGF-β levels and was associated with slowing CKD progression (379, 380).

Studies on the role of TGF-β2 in predicting CKD progression are uncommon despite the fact that TGF-β2 is mostly evident in most kidney diseases and it is positively correlated with kidney fibrosis (235, 359, 363). In this study, both serum and urine TGF-β2 were only measured at baseline and the majority (82.5%) of the patients had detectable serum TGF-β2 and 17.5% of the patients had detectable urine TGF-β2 at baseline. After 48 months follow up, declining eGFR and CKD progression was associated with lower baseline serum TGF-β2 concentrations and increased baseline urine TGF-β2 concentrations. The baseline serum and urine TGF-β2 did not predict CKD progression on both univariate and multivariable analyses. Studies have reported that TGFβ2 induces myofibroblast differentiation eventually leading to kidney fibrosis, loss of renal function and CKD progression (381, 382).

Studies have shown that TGF-β3 induces kidney fibrosis and inflammation which is less severe when compared to that of TGF-β1 (383, 384). In this study, both serum and urine TGF-β3 were only measured at baseline and more than half (58.6%) of the patients had detectable serum and 17.9% of the patients had detectable urine TGF-β3 at baseline. After 48 months follow up, CKD progression was associated with declining eGFR and higher baseline urinary TGF-β3. However, the baseline urinary TGF-β3 did not predict CKD progression on both univariate and multivariable analyses. Previous studies reported that TGF-β3 in the kidney might have antifibrotic and kidney protective properties unlike the activity of TGF-β1, and that TGF-β3 expression levels peak with advanced CKD stages (225, 383). Our study adds to the pool of knowledge that renal TGF-β3 might have roles that are opposing or counteracting the activity of TGF-β1 (249, 250).

In previous studies conducted with the aim of determining the use of urinary and serum biomarkers in studying CKD progression among patients with HIV/AIDS, it was reported that urinary TGF-β1

and TGF-β2 concentrations were elevated among HIV positive CKD patients (366, 369). Unlike our study findings, other studies have reported that urinary biomarkers do better in predicting CKD progression when compared to biomarkers from blood samples (34, 69). In this study, very few CKD patients had detectable urine TGF-β at baseline; amongst the reasons for this was that patients were in late stages of CKD when TGF- β expression had been dampened (366, 385). A more sensitive assay may have been able to elucidate this issue further. Also, lower urinary TGF-β levels in this study could be accounted for by the fact that almost all of the patients, who had CKD progression were hypertensive and were on antihypertensives (385, 386).

In recent years, various prediction models using machine algorithms and Artificial Intelligence (AI) for predicting CKD progression are being established in nephrology (393, 394). Studies are reporting that machine algorithms and AI prediction models have high accuracy in predicting CKD progression (395, 396). Hopefully in the near future we can use machine algorithms and AI technologies for predicting and managing CKD progression in routine clinical practice.

6.6 Future research and recommendations

1. The roles of the various serum and urine TGF-β isoforms in CKD progression are still not clear and this highlights the importance of multicentre studies to determine the isoform specific effects of TGF-β isomers in CKD progression in patients with African ancestry. There is need to estimate changes in serum and urine TGF-β isoforms at baseline and at follow up to define the roles of urine and serum TGF-β as biomarkers for predicting CKD progression.
2. Studies on the interaction between miRNAs and TGF-β isoforms in CKD and their role in CKD progression among black patients in South Africa and other SSA countries are warranted as the search for novel biomarkers continues.

3. Studies on the role played by the interaction of genetic susceptibility including the *APOL1* gene and TGF-β isoforms in CKD and their role in predicting CKD progression among black patients in South Africa and other SSA countries are warranted.
4. There is a need to identify more sensitive biomarkers and venture into prediction tools for CKD progression including using machine algorithms and AI technologies.

6.7 The study's strengths

This study advances knowledge of the role of novel biomarkers in predicting CKD progression especially as there is not much literature on chronic kidney disease (CKD) in black patients in Africa. The positive aspect of this study is that it was a prospective longitudinal study investigating the role of baseline TGF-β isoforms in predicting CKD progression among black patients with a large sample size. The study reports a cohort of South African patients with CKD and a relatively high rate of progression. The study has taken relevant factors that are associated with CKD progression among black patients and factors that are associated with advanced CKD and the differentiation of association and cause.

6.8 Study limitations

There are limitations of this study: it is an observational study conducted in a single tertiary hospital in Johannesburg, South Africa and may not be generalisable. A major limitation of this study was the inability to detect TGF-β isoforms in the majority of urine samples. Also, we were not able to repeat the serum and urine TGF-β measurements after the 2 years follow up mainly due to resource constraints. Also, the COVID-19 pandemic and a major hospital fire at CMJAH in April 2021 that resulted in closure of hospital for 6 months might have had an impact on blood pressure and diabetes mellitus control. Patients were unable to continue with the routine follow at this hospital but instead had to follow up in nearby hospitals. Blood pressure measurement was recorded at enrolment and at 24 months follow up; serial routine clinic BP measurements were not utilized to

monitor blood pressure control throughout the study period. Furthermore, ambulatory blood pressure measurements were not available, hence the control of blood pressure may be open to question. Poor uptake of RAAS blockade due to their feared side effects in late CKD stages could account for the high prevalence of CKD progression in this study. Recent guidelines recommend urine albumin-to-creatinine ratio (ACR) to predict risk of ESKD, which was not routinely available at our centre; urine protein-to-creatinine ratio (uPCR) and eGFR estimations were the only routine standard of care methods available in our hospital to asses CKD progression. This study was conducted in a single institution in Johannesburg, Gauteng province and therefore may not be representative of all black CKD patients in South Africa. Despite these limitations, the results of our study add to the current body of literature on TGF-β isoforms and CKD progression.

6.9 Conclusions

Our study has demonstrated that patients with CKD progression had lower baseline concentrations of serum TGF-β1 and increased baseline urinary TGF-β3 concentrations compared to patients who did not progress. However, based on both univariate and multivariable analyses, none of the baseline serum or urine TGF-β isoforms were shown to be predictors of CKD. The roles of the various serum and urine TGF-β isoforms in CKD progression at baseline are still unclear and hence highlight the importance of further studies to determine their isoform specific effects. This study has demonstrated a higher prevalence of CKD progression in a prospective longitudinal study among black patients with CKD than previous studies. An increase in uPCR > 30% at two years identified most patients with CKD progression. Therefore, clinicians and nephrologists should utilize changes in uPCR > 30% at various time points to identify those patients with CKD who are likely to progress more rapidly, who therefore will require closer surveillance and monitoring with emphasis on slowing or stopping progression of CKD.

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CC: Supervisor: Professors S Naicker and R Duarte
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Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

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REF: *R14/49*

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DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine - Nephrology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Role of novel biomakers in prediction of disease progression and its outcomes among Black chronic kidney disease patients in South Africa
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Appendix C: Participant information sheet

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Section A

STUDY TITLE: **Role of novel biomarkers in prediction of kidney disease progression among black CKD patients in South Africa**

INVESTIGATOR: Dr. Alfred Jackson Meremo

SITE: Charlotte Maxeke Johannesburg Academic Hospital

TELEPHONE: Cell: 0649545495, Land line (Department): 011-488-3672

Section B

Introduction

Good day, my name is Alfred Jackson Meremo. I am a PhD student doing research at the University of the Witwatersrand. This research is carried out in order to establish facts and find new conclusions for evidence-based patient centred care. I am conducting a study on **"Role of novel biomarkers in prediction of kidney disease progression among black CKD patients in South Africa**".

The information that I will collect from this research project will be kept confidential. You are hereby invited to participate in this voluntary research.

It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this hospital will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

If you have any questions, you may ask them now or later, even after the study has started.

It is important that you read and fully understand the information on this leaflet as it will help you in making the decision.

Purpose of the study

I am conducting a study to look at people with chronic kidney disease and how novel (new) biomarkers (TGF-β1 and MiRNAs) both in urine and blood can predict chronic kidney disease progression earlier compared to routine biomarkers (serum creatinine and proteinuria).

Studies on novel (new) biomarker (TGF-β1 and MiRNAs) discovery are essential to allow more accurate prediction of chronic kidney disease progression in its earlier stages and correlate better with the decline of their kidney function and take appropriate decisions towards interrupting or reversing the process of progressive kidney injury compared to routinely used biomarkers

Procedures of the study

If you agree to participate in the study, your medical records will be reviewed. You will be examined by a medical doctor and also interviewed to collect information regarding the progression of chronic kidney disease. Blood and urine samples will be collected to measure the biomarkers now (0) months and at 24 months.

A total of 10mls of blood (2 teaspoons of blood) will be drawn. the drawn blood will be spun very quickly and the serum separated out. Your coded (*i.e*. anonymized) blood samples will be sent to the Department of Internal Medicine research laboratory for the above-mentioned tests. At this point, the blood and urine samples can still be linked back to you. This is necessary to ensure that you get the appropriate treatment from your doctor. With your prior consent, any left-over blood will be frozen and stored in a designated freezer for an unlimited period of time for future use in research related to diseases. Any researcher proposing to work on a frozen blood sample at a future date would be required to get the approval of the Human Ethics Research Committee (Medical) before proceeding. You can read about the role of this Committee on the next page. Anonymized

blood samples like this can no longer be linked to an individual, so if you do consent to the long-term storage of your blood, this consent cannot subsequently be withdrawn.

Benefits of the study

The benefits of participating in this study are that we will be able to identify the most predictive novel biomarker for chronic kidney disease progression. Predicting kidney disease progression using new biomarkers, will be beneficial to you as it will help in administering targeted optimal care which eventually would lead to improved outcomes by delaying progression to end stage kidney disease and its complications compared to routinely used biomarkers.

Possible risks

Possible side effects from drawing the blood sample include mild pain, bleeding, and bruising at the site of the needle insertion. Qualified personnel will collect the blood samples to prevent such complications.

Financial arrangements

You will not be paid for participating in the study. However, there will be no costs to you for any related study visits and procedures, as any costs incurred will be compensated by research funds.

Confidentiality

All information obtained during the course of this study will be kept strictly confidential. Your records will be given unique identification numbers and the initial identification details won't be used. All physical records will be kept in a locked locker with access limited only to the research team. Electronic data will be password protected.

Source of information

If you have any questions, queries and clarifications, *please contact:*

Principal Investigator

Dr. Alfred Jackson Meremo*,* who will be reachable 24 hours every day on the number, **0649545495.**

Supervisor

Professor Saraladevi Naicker on telephone no. 011 7172709, or by e-mail on Saraladevi.Naicker@wits.ac.za

This study has been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Dr Clement Penny, who may be contacted on telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za.

The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

Thank you for reading this Study Information Sheet

August 2019

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Informed Consent Form: General (patient)

STUDY TITLE: **Role of novel biomarkers in prediction of kidney disease progression among black CKD patients in South Africa**.

I confirm that I have been informed about the study by Dr. Alfred Jackson Meremo. I understand that my personal details will be kept strictly confidential and that I may at any stage up to the long-term storage of my blood withdraw my consent and participation in the study and continue to receive the appropriate treatment. I have also received, read and understood the study as explained in the participant information sheet and hence consent to taking part in this research study.

I consent my blood and urine samples to be used for the purposes of the present study (please initial the appropriate box)

Yes ☐

No ☐

I consent to the indefinite storage of my blood for future research (please initial the appropriate box). Note that once given, this consent cannot be rescinded.

Yes ☐

No ☐

PARTICIPANT (printed name) …….

Signature or thumb print …… Date ……...

Witness (printed name) ……

Signature……...Date…….

I, Dr. Alfred Jackson Meremo confirm that the participant has been fully informed about the nature of the above study.

STUDY INVESTIGATOR

Printed Name ……..……...…. Signature……...……Date……

PLOS ONE

RESEARCH ARTICLE

Demographic and clinical profile of black patients with chronic kidney disease attending a tertiary hospital in Johannesburg, South Africa

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Data Availability Statement: Data cannot be shared publicly because of ethics policy at University of Witwatersrand, the participants signed a consent form, which states that data is exclusively available for professional research staff. Data are available to qualifying organizations and/or individuals from the chairperson of the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee") who is Dr. Clement Penny, who may be contacted by e-mail on Clement.

Abstract

Background

The prevalence of chronic kidney disease (CKD) is increasing worldwide; black patients have an increased risk of developing CKD and end stage kidney disease (ESKD) at significantly higher rates than other races.

Methods

A cross sectional study was carried out on black patients with CKD attending the kidney outpatient clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa, *between September 2019 to March 2020*. Demographic and clinical data were extracted from the ongoing kidney outpatient clinic records and interviews, and were filled in a questionnaire. Patients provided blood and urine for laboratory investigations as standard of care, and data were descriptively and inferentially entered into REDcap and analysed using STATA version 17. Multivariable logistic regression analysis was used to identify demographic and clinical variables associated with advanced CKD.

Results

A total of 312 black patients with CKD were enrolled in the study with a median age of 58 (IQR 46–67) years; 58% patients had advanced CKD, 31.5% of whom had grossly increased proteinuria, 96.7% had hypertension, 38.7% had diabetes mellitus and 38.1% had both hypertension and diabetes mellitus. In patients with advanced CKD, the median age was 61 (IQR 51–69) years, eGFR 33 (30–39) mL/min/1.73 m^2, serum bicarbonate 22 (IQR 20–24), haemoglobin 12.9 (IQR 11.5–14.0) g/dl and serum uric acid 0.43 (IQR 0.37–0.53). The prevalence of metabolic acidosis was 62.4%, anemia 46.4% and gout 30.9% among those with advanced CKD, while the prevalence of metabolic acidosis and anaemia

Penny@wits.ac.za) for researchers who meet the relevant ethics criteria for access to these data.

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Abbreviations: CKD, chronic kidney diseases; CMJAH, Charlotte Maxeke Johannesburg Academic Hospital; CKD-EPI, chronic kidney disease epidemiology collaboration; ESKD, end stage kidney disease; T2DM, Type 2 Diabetes Mellitus; eGFR, estimated glomerular filtration rate; IDMS, isotope dilution mass spectroscopy; uPCR, urine protein creatinine ratio; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers; KDIGO, kidney disease improving global outcomes; NCDs, non-communicable diseases; SSA, sub-Saharan Africa.

was 46.6% and 25.9% respectively in those with early CKD. Variables with higher odds for advanced CKD after multivariable logistic regression analysis were hypertension (OR 3.3, 95% CI 1.2–9.2, P = 0.020), diabetes mellitus (OR 1.8, 95% CI 1.1–3.3, P = 0.024), severe proteinuria (OR 3.5, 95% CI 1.9–6.5, P = 0.001), angina (OR 2.5, 95% CI 1.2–5.1, P = 0.008), anaemia (OR 2.9, 95% CI 1.7–4.9, P = 0.001), hyperuricemia (OR 2.4, 95% CI 1.4–4.1, P = 0.001), and metabolic acidosis (OR 2.0, 95% CI 1.2–3.1, P = 0.005). Other associations with advanced CKD were loss of spouse (widow/widower) (OR 3.2, 95% CI 1.4–7.4, P = 0.006), low transferrin (OR 2.4, 95% CI 1.1–5.1, P = 0.028), hyperkalemia (OR 5.4, 95% CI 1.2–24.1, P = 0.029), use of allopurinol (OR 2.4, 95% CI 1.4–4.3, P = 0.005) and doxazosin (OR 1.9, 95% CI 1.2–3.1, P = 0.006).

Conclusion

Hypertension and diabetes mellitus were strongly associated with advanced CKD, suggesting a need for primary and secondary population-based prevention measures. Metabolic acidosis, anemia with low transferrin levels, hyperuricemia and hyperkalemia were highly prevalent in our patients, including those with early CKD, and they were strongly associated with advanced CKD, requiring clinicians and dietitians to be proactive in supporting the needs of CKD patients in meeting their daily dietary requirements towards preventing and slowing the progression of CKD.

Introduction

Chronic kidney disease (CKD), defined as decreased kidney function identified by glomerular filtration rate (GFR) of less than 60 mL/min per 1·73 m^2, or markers of kidney damage, or both, of at least 3 months duration, regardless of the underlying cause [1], is a major public health issue worldwide and contributes immensely to the overall non-communicable disease (NCD) burden, with NCDs also contributing to the burden of CKD [2, 3]. Chronic kidney disease is usually asymptomatic until the more advanced stages and accurate prevalence data are lacking in most regions including sub-Saharan Africa [4]. As the prevalence of CKD is increasing worldwide and consequently the demand for kidney replacement therapy (KRT), the incidence of cardiovascular events and death is also increasing [5, 6]. Recent systematic reviews have reported the prevalence of CKD to be 15.8% in Africa, similar to other continents, constituting a true public health need with major cost implications to healthcare systems [1, 7]. Diabetes mellitus and hypertension are the leading causes of CKD worldwide; and in sub-Saharan Africa, hypertension is the leading cause of CKD [8, 9]. Studies have shown that African-Americans have a 2- to 4-fold greater risk for end stage kidney disease (ESKD) requiring renal replacement therapy than their white counterparts [10, 11]. Individuals of black ethnicity due to their genetics, including the presence of APOL1 high-risk genotypes, are at higher risk of death due to CKD as a result of social, economic and medical causes [12, 13]. African ancestry has been associated with higher serum creatinine levels, lower eGFR estimates and more rapid CKD progression [14, 15]. In addition to the known risk factors for advanced CKD, which are age, male sex, black race, arterial hypertension and proteinuria, other modifiable factors including medications (traditional and herbal), hyperuricemia, hyperlipidemia, elevated phosphate levels, heart failure and anemia are common in CKD at later stages [16, 17]. Metabolic acidosis increases with worsening eGFR, with prevalence of around 40% among patients with CKD stage 4 and it is associated with rapid CKD progression [18, 19]. Anaemia is common in

CKD and is frequently associated with poor outcomes, including increased cardiovascular risks, hospitalization, decreased quality of life and increased risk of mortality [20, 21]. Hyperuricemia greatly contributes to the development of CKD and its progression, it appears that increasing uric acid levels increase the risk for CKD development by causing inflammation, endothelial cell injury and activation of the renin-angiotensin system [22, 23]. Hyperkalemia is also common in advanced CKD; its prevalence increases with decreasing eGFR and it is significantly associated with faster CKD progression [24, 25]. Thus, the aim of this study was to determine the demographic and clinical profile of black patients with CKD attending Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa.

Methods

Study design, population and settings

This was a cross-sectional study to evaluate the demographic and clinical profile of black patients with CKD attending the kidney outpatient department (KOPD) clinic at CMJAH between September 2019 to March 2020. The CMJAH is a public accredited central hospital with 1088 beds serving patients from across the Gauteng province and nearby provinces in South Africa, CMJAH is also the main teaching hospital for The University of the Witwatersrand, faculty of Health Sciences. Johannesburg is the largest city in South Africa and among the largest 50 urban agglomerations in the world. Johannesburg had an estimated population of around 5.9 million in 2021, the most common racial groups include; black African (76.4%), colored (5.6%), White (12.3%) and Indian/Asian (4.9%).

Inclusion criteria included patients who were >18 years of age, CKD stages 1–4, who had controlled hypertension (blood pressure < 140/90 mm Hg) and diabetes mellitus (HbA1C < 7%), attending the KOPD clinic for at least 6 months and were able to provide informed consent. Patients who had active infections, active malignancies, autoimmune diseases and who were not black were excluded, black patients have an increased risk of developing CKD and end stage kidney disease (ESKD) at significantly higher rates than other races [13, 15].

Data collection and laboratory procedures

Demographic and clinical data including age, gender, weight, height, glycemic status, history of smoking, etiology of CKD and medications were extracted from the ongoing continuous KOPD clinic records and face to face interviews, and were filled in a questionnaire. Systolic and diastolic blood pressure was measured 3 times, the average of the second and third measurements was used. Body mass index (BMI) was calculated using the National Health Services (NHS-UK) BMI calculator [26]. Measurements of urinary protein creatinine ratio (uPCR), serum creatinine, electrolytes, HbA1C, WBC, haemoglobin level, platelets, calcium, phosphate, transferrin and HDL cholesterol were done as standard of care at the time of recruitment during a clinic visit. Serum creatinine was measured using the isotope dilution mass spectrometry (IDMS) traceable enzymatic assay and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation without using the African American correction factor [27]. Patients with eGFR < 45 ml/min/1.72m^2 were considered to have advanced CKD, while those patients with eGFR ≥ 45 ml/min/1.72m^2 early CKD.

Data management and analysis

Study data were collected and entered into REDCap (Research Electronic Data Capture) tools [28, 29] hosted at the University of the Witwatersrand and analyzed using STATA version 17

(College Station, Texas, USA). Descriptive statistics were used to summarize demographic and clinical characteristics; continuous variables have been reported as medians with interquartile ranges and Wilcoxon rank-sum test was used for the non-normally distributed variables. Discrete variables have been reported as frequencies and proportions, Pearson's chi-square test were used to test for association between two variables. Odd ratios were used to estimate the strength of association between variables and advanced CKD, univariate and multivariate logistic regression models have been used to estimate the association of variables and advanced CKD. Variables with p-value less than 0.2 on univariate logistic regression models were then fitted into the multivariate logistic regression models with the addition of age and sex as adjusting variables; variables with a p-value of less than 0.05 were considered to have significant strength of association.

Ethical issues

Ethical approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand, Johannesburg (ethics clearance certificate No. M190553). Written informed consent was obtained from each of the participants before embarking on data collection.

Results

Demographic and clinical characteristics of the study population

Of the 476 black patients with CKD stages 1–4, 164 patients were excluded from the study including 110 patients who had uncontrolled hypertension, 35 patients who had uncontrolled diabetes mellitus, 11 patients had autoimmune diseases, 6 patients had active infections and 2 patients had active malignancies. A total of 312 CKD black patients were enrolled into this study, of whom 162 (51.9%) were male, 292 (93.6%) were hypertensive, 103 (33.0%) were diabetic and 164 (52.6%) were married. The median age was 61 (IQR 51–69) years for advanced CKD and 53 (IQR 41–62) years for early CKD; the median eGFR was 33 (30–39) mL/min/1.73 m^2 for advanced CKD and 60 (IQR 51–75) mL/min/1.73 m^2 for early CKD; the median urine protein creatinine ratio (uPCR) was 0.029 (IQR 0.015–0.67) g/mmol for advanced CKD and 0.016 (IQR 0.008–0.034) g/mmol for early CKD. The median serum bicarbonate was 22 (IQR 20–24) mmol/L for advanced CKD and 23 (IQR 21–25) mmol/L for early CKD; the median haemoglobin (Hb) was 12.9 (IQR 11.5–14.0) g/dl for advanced CKD and 13.8 (IQR 12.4–15.7) g/dl for early CKD; the median serum transferrin was 2.44 (IQR 2.23–2.73) g/L for advanced CKD and 2.62 (IQR 2.37–2.89) g/L for early CKD. The median serum uric acid was 0.43 (IQR 0.37–0.53) mmol/L for advanced CKD and 0.36 (IQR 0.30–0.46) mmol/L for early CKD (Table 1).

Clinical profile of CKD patients

Of the 312 CKD black patients, 58% patients had advanced CKD (CKD stage 3b or 4), of whom 57 (31.5%) patients presented with severely increased proteinuria as compared to 23 (17.4%) patients with early CKD. Metabolic acidosis was present in 113 (62.4%) of those who had advanced CKD and in 61 (46.6%) patients with early CKD. Anaemia was present in 84 (46.4%) patients with advanced CKD including 30 (16.6%) patients who had low transferrin levels, while 34 (25.9%) patients with early CKD had anemia. Among patients with advanced CKD, hyperuricemia was found in 56 (30.9%) patients and 16 (8.8%) patients had hyperkalemia. Among patients with advanced CKD, majority (96.7%) patients were diagnosed with hypertension, 70 (38.7%) patients had diabetes mellitus and 69 (38.1%) had both hypertension and diabetes mellitus as compared to 117 (89.3%) patients who had hypertension, 33 (25.2%)

Table 1. Demographic characteristics and clinical profile of 312 CKD patients by eGFR.

Characteristic	eGFR < 45 ml/min/1.72m^2 (n = 181)	eGFR ≥ 45 ml/min/1.72m^2 (n = 131)	P-value
	Proportion (%) or Median (IQR)	Proportion (%) or Median (IQR)	
Age (years)	61 (51–69)	53 (41–62)	**0.001**
Sex			
Male	96 (53.0%)	66 (50.4%)	
Female	85(47.0%)	65 (49.6%)	0.643
Marital status			
Single	43(23.8%)	48(36.6%)	
Married	98 (54.1%)	66 (50.4%)	
Widow/Widower	30(16.6%)	10 (7.6%)	
Separated/Divorced	10(5.5%)	7 (5.4%)	**0.026**
Highest level of education			
No formal education	24(13.3%)	14 (10.7%)	
Primary	41(22.7%)	30 (22.9%)	
Secondary	56(30.9%)	43 (32.8%)	
Tertiary	60(33.1%)	44 (33.6%)	0.505
Occupation			
Unemployed	26(14.4%)	19(14.5%)	
Domestic workers	37(20.4%)	25(19.1%)	
Self employed	43(23.8%)	27(20.6%)	
Public / Private servant	57(31.5%)	52(39.7%)	
Retired	18(9.9%)	8(6.1%)	0.523
BMI (kg/m^2)	30.2 (26.0–34.5)	30.2 (26.6–34.7)	0.731
SBP (mmHg)	140 (130–140)	140 (128–140)	0.339
DBP (mmHg)	82 (72–90)	83 (74–90)	0.630
uPCR (g/mmol)	0.029 (0.015–0.67)	0.016 (0.008–0.034)	**0.001**
Creatinine (umol/L)	163 (141–190)	109 (88–122)	**0.001**
eGFR (ml/min/1.72m^2)	33 (30–39)	60 (51–75)	**0.001**
FBG (mmol/L)	4.5 (4.2–5.2)	4.4 (4.2–4.9)	0.146
HbA1C (%)	7.0 (6.6–7.0)	7.0 (7.0–7.0)	0.222
Haemoglobin (g/dl)	12.9 (11.5–14.0)	13.8 (12.4–15.7)	**0.001**
Transferrin (g/L)	2.44 (2.23–2.73)	2.62 (2.37–2.89)	**0.001**
WBC (x 10^9 cells/L)	6.4 (5.13–7.71)	6.03 (4.81–7.73)	0.420
Platelets (x 10^9 cells/L)	256 (213–320)	271 (217–325)	0.378
Uric acid (mmol/L)	0.43 (0.37–0.53)	0.36 (0.30–0.46)	**0.001**
HDL cholesterol (mmol /L)	1.23 (0.98–1.48)	1.22 (1.02–1.52)	0.528
Calcium (mmol /L)	2.31 (2.23–2.40)	2.33 (2.26–2.42)	0.054
Phosphate (mmol /L)	1.1 (0.94–1.24)	1.02 (0.85–1.15)	**0.005**
Sodium (mmol/L)	141 (138–143)	141 (139–143)	0.965
Potassium (mmol/L)	4.4 (3.9–4.8)	4.1 (3.9–4.4)	**0.001**
Bicarbonate (mmol/L)	22 (20–24)	23 (21–25)	**0.013**

IQR, interquartile range; uPCR, urine protein creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1C, glycosylated hemoglobin A1C; WBC, white blood cells; HDL, high density lipoprotein; SBP, systolic blood pressure.

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patients who had diabetes mellitus and 32 (24.4%) had both hypertension and diabetes mellitus among those with early CKD. Angina was reported in 41 (22.7%) patients with advanced CKD and 15 (11.5%) patients in early CKD. Most (56.9%) patients with advanced CKD were using more than 5 medications as compared to 41.2% patents with early CKD who were using 3–4

medications for their blood pressure control. Majority (84.0%) of the patients with advanced CKD and 76.3% patients in early CKD were on calcium channel blockers (CCBs), while 18.8% patients with advanced CKD and 21.4% of those with early CKD were using angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptors blockers (ARBs). For patients with advanced CKD, most (57.5%) were on diuretics followed by 51.4% on doxazosin; 29.3% of patients were on insulin and 16 (8.8%) on oral hypoglycemic agents (Table 2).

Factors associated with advanced CKD

We divided the patients into two subgroups according to their CKD stages [early CKD (eGFR $\geq$ 45 ml/min/1.72m^2) vs. advanced CKD (eGFR $<$ 45 ml/min/1.72m^2)]. A total of 24 potential variables were identified after performing univariate logistic regression analyses. Backward elimination reduced this to 15 parameters; the factors associated with advanced CKD after adjusting for age and sex on multivariate logistic regression analysis included: hypertension (OR 3.3, 95% CI 1.2–9.2, P = 0.020), diabetes mellitus (OR 1.8, 95% CI 1.1–3.3, P = 0.024), angina (OR 2.5, 95% CI 1.2–5.1, P = 0.008), severe proteinuria (OR 3.5, 95% CI 1.9–6.5, P = 0.001), moderate proteinuria (OR 2.5, 95% CI 1.5–4.3, P = 0.001), hyperuricemia (OR 2.4, 95% CI 1.4–4.1, P = 0.001), anaemia (OR 2.9, 95% CI 1.7–4.9, P = 0.001), metabolic acidosis(OR 2.0, 95% CI 1.2–3.1, P = 0.005), allopurinol (OR 2.4, 95% CI 1.4–4.3, P = 0.005) and doxazosin (OR 1.9, 95% CI 1.2–3.1, P = 0.006), low transferrin (OR 2.4, 95% CI 1.1–5.1, P = 0.028), hyperkalemia (OR 5.4, 95% CI 1.2–24.1, P = 0.029) and, widow/widower (OR 3.2, 95% CI 1.4–7.4, P = 0.006) (Table 3).

Discussion

This study evaluated the demographic and clinical profile of black patients with CKD attending the CMJAH kidney outpatient clinic in Johannesburg, South Africa. There were 42% with early CKD and 58% with advanced CKD; 93.6% had hypertension and 33.0% diabetes mellitus, and 32.3% had both hypertension and diabetes. The prevalence of hypertension among patients with CKD is high and it is strongly associated with advanced CKD and CKD progression [30, 31], the majority (96.7%) of patients with advanced CKD had hypertension, similar to findings in other studies from SSA [9, 32]. Peripherally acting α-blockers like doxazosin are commonly used in the management of hypertension in CKD, mainly due to their pharmacokinetic profile that is undisturbed by worsening kidney function and their role in blood sugar control [33, 34], approximately 51.4% of patients with advanced CKD were using doxazosin for treatment of hypertension with a 1.9 times increased association with advanced CKD, as also has been reported in other studies [35, 36]. Studies have shown that diabetes related CKD is the leading cause of end stage kidney disease among patients with T2DM patients worldwide [37, 38], approximately 38.7% patients with advanced CKD had T2DM; T2DM had 1.8 increased risk for advanced CKD, similar to other studies conducted among black patients in South Africa and Ethiopia [32, 39]. In patients with advanced CKD, approximately 29.3% of the patients were on insulin and 8.8% were on oral hypoglycemics for treatment of their T2DM. Oral hypoglycemics were associated with 0.5 times higher risk for advanced CKD, similar to other studies [40, 41].

Metabolic acidosis is common in CKD and it can lead to dysfunction of many organs and systems including the kidney resulting in CKD progression [42, 43], the prevalence of metabolic acidosis was 62.4% in advanced CKD and 46.6% in early CKD; this prevalence is higher than the 33% - 40% among patients with CKD stage 3–4 from other continents [43–45]. The possible explanation could be firstly, the more rapid CKD progression which has been shown to occur in black patients even early in their CKD stages [15, 19] and secondly, diet where

Table 2. Clinical profile of 312 CKD patients by eGFR.

Parameter	eGFR < 45 ml/min/1.72m^2 (n = 181) Proportion (%)	eGFR ≥ 45 ml/min/1.72m^2 (n = 131) Proportion (%)	P-value
eGFR (ml/min/1.72m^2)			
Stage 1 (> 90)	0 (0.0%)	4 (3.1%)	
Stage 2 (60–89)	0 (0.0%)	62 (47.3%)	
Stage 3a (45–59)	0 (0.0%)	65 (49.6%)	
Stage 3b (30–44)	144 (79.6%)	0 (0.0%)	
Stage 4 (16–29)	37 (20.4%)	0 (0.0%)	**0.001**
Diagnoses encountered			
Hypertension	175 (96.7%)	117 (89.3%)	**0.009**
Diabetes mellitus	70 (38.7%)	33 (25.2%)	**0.012**
Hypertension & Diabetes mellitus	69 (38.1%)	32 (24.4%)	**0.011**
Adult polycystic kidney disease	5 (2.8%)	7 (5.3%)	0.242
Reflux nephropathy	0 (0.0%)	5 (3.8%)	**0.008**
Obstructive uropathy	1 (0.6%)	0 (0.0%)	0.394
Unknown	6 (3. 3%)	5 (3.8%)	0.812
Current smoking			
Yes	13 (7.2%)	10 (7.6%)	
No	168 (92.8%)	121 (92.4%)	0.880
Current Alcohol			
Yes	14 (7.7%)	18 (13.7%)	
No	169 (92.3%)	113 (86.3%)	0.084
Cardiovascular Diseases			
None	120 (66.3%)	108 (82.4%)	
Angina	41 (22.7%)	15 (11.5%)	
Myocardial infarction	8 (4.4%)	4 (3.0%)	
Heart failure	6 (3.3%)	2 (1.5%)	
Stroke	6 (3.3%)	1 (0.8%)	
Transient ischemic attack	0 (0.0%)	1 (0.8%)	**0.017**
Medications			
None	2 (1.1%)	8 (6.1%)	**0.004**
Diuretics	104 (57.5%)	58 (44.3%)	**0.021**
ACEIs / ARBs	34 (18.8%)	28 (21.4%)	0.572
Aldactone	6 (3.3%)	5 (3.8%)	0.812
CCBs	152 (84.0%)	100 (76.3%)	0.092
Statins	100 (55.3%)	60 (45.8%)	0.099
Oral hypoglycemics	16 (8.8%)	21(16.0%)	0.053
Insulin	53 (29.3%)	19 (14.5%)	**0.002**
Allopurinol	22 (12.1%)	13 (9.9%)	0.538
Junior ASA	90 (49.7%)	57 (43.5%)	0.278
Beta blockers	41 (22.7%)	30 (22.9%)	0.959
Aldomet	12 (6.6%)	7 (5.3%)	0.639
Hydralazine	4 (2.2%)	3 (2.3%)	0.962
Doxazosin	93 (51.4%)	48 (36.6%)	**0.010**
Others	68 (37.6%)	38 (29.0%)	0.115
Number of medications per patient			
0	2 (1.1%)	8 (6.1%)	
1–2	20 (11.1%)	20 (15.3%)	
3–4	56 (30.9%)	55 (41.2%)	

(*Continued*)

Table 2. (Continued)

Parameter	eGFR < 45 ml/min/1.72m^2 (n = 181) Proportion (%)	eGFR ≥ 45 ml/min/1.72m^2 (n = 131) Proportion (%)	P-value
≥ 5	103 (56.9%)	48 (36.6%)	**0.001**
uPCR (g/mmol)			
Normal to mildly increased (<0.015)	47 (26.0%)	63 (48.2%)	
Moderately increased (0.015–0.05)	77 (42.5%)	45 (34.4%)	
Severely increased (> 0.050)	57 (31.5%)	23 (17.4%)	**0.001**
Haemoglobin (g/dl)			
Normal (> 12.0 or 13.0)	97 (53.6%)	97 (74.1%)	
Anaemia (<12.0 or 13.0)	84 (46.4%)	34 (25.9%)	**0.001**
Transferrin g/dl)			
Low (< 2.0)	30 (16.6%)	10 (7.6%)	
Normal (2.0–3.60)	148 (81.8%)	117 (89.3%)	
High (>3.60)	3 (1.7%)	4 (3.1%)	0.052
Uric acid (mmol/l)			
Low (< 0.16 or 0.21)	4 (2.2%)	15 (11.5%)	
Normal (0.16/0.21–0.36/0.43)	121 (66.9%)	98 (74.8%)	
High (> 0.36 or 0.43)	56 (30.9%)	18 (13.7%)	**0.001**
Potassium (mmol/l)			
Low (< 3.5)	13 (7.2%)	15 (11.5%)	
Normal (3.5–5.1)	152 (84.0%)	114 (87.0%)	
High (> 5.1)	16 (8.8%)	2 (1.5%)	**0.013**
Bicarbonate (mmol/l)			
Low (< 23)	113 (62.4%)	61 (46.6%)	
Normal (23–29)	67 (37.0%)	68 (51.9%)	
High (> 29)	1 (0.6%)	2 (1.5%)	**0.018**

uPCR, urine protein creatinine ratio; eGFR, estimated glomerular filtration rate; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers.

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replacement of traditional diets with contemporary/ western foods which contain mainly animal proteins, less vegetables and low intake of fruits might increase CKD patients' dietary acid load [46, 47]. Patients with low serum bicarbonate levels were 2-fold more likely to have advanced CKD, as reported also in other studies [18, 44]. Anaemia is common in CKD, and it is associated with decreased quality of life, high morbidity and mortality [48, 49]. The prevalence of anaemia was 46.4% in advanced CKD, including 16.6% who had low transferrin levels, while 25.9% of the patients with early CKD had anaemia, advanced CKD was 2.9 times more prevalent if a patient had anemia and 2.4 times more prevalent if a patient had low transferrin, similar to other studies [20, 21, 50]. High serum uric acid levels are associated with high risk for advanced CKD and CKD progression [51, 52], the prevalence of hyperuricemia was 30.9% in advanced CKD; advanced CKD had a 2.4-fold higher OR if a patient had hyperuricemia and 2.4-fold higher if a patient was on allopurinol; similar findings have been reported in other studies [23, 53]. Hyperkalemia is common in patients with chronic CKD and its prevalence increases as the eGFR declines [54, 55], approximately 8.8% patients with advanced CKD were found to have hyperkalemia; hyperkalemia had a 5.4-fold increased association with advanced CKD; similar findings have been reported in other studies [24, 25].

Studies have shown that the incidence of cardiovascular events increases with worsening kidney function [56, 57]; 22.7% patients with advanced CKD reported angina with a 2.5 times increased risk in advanced CKD, similar to other studies [56, 58]. Also 31.5% patients with

Table 3. Factors associated with advanced CKD.

Characteristic	Number of patients	UNIVARIATE		MULTIVARIATE	
		OR (95% CI)	P. value	OR (95% CI)	P. value
Age	312	1.1(1.0–1.2)	0.001		
Marital status					
Single	91	1(Reference)		1(Reference)	
Married	164	1.7 (0.9–2.8)	0.055	1.5 (0.9–3.0)	0.137
Widow/Widower	40	3.3 (1.5–7.7)	0.004	3.2 (1.4–7.4)	**0.006**
Separated/Divorced	17	1.6 (0.6–4.6)	0.038	1.4 (0.5–4.3)	0.466
Hypertension					
No	20	1(Reference)		1(Reference)	
Yes	292	3.5 (1.3–9.3)	0.013	3.3 (1.2–9.2)	**0.020**
Diabetes mellitus					
No	209	1(Reference)		1(Reference)	
Yes	103	1.9 (1.1–3.1)	0.013	1.8 (1.1–3.0)	**0.024**
Current Alcohol					
No	280	1(Reference)		1(Reference)	
Yes	32	0.5 (0.3–1.1)	0.088	0.5 (0.2–1.0)	0.059
Cardiovascular Disease:					
None	228	1(Reference)		1(Reference)	
Angina	56	2.7 (1.4–5.3)	0.004	2.5 (1.2–5.1)	**0.008**
Myocardial infarction	12	1.8 (0.5–6.1)	0.348	1.4 (0.4–5.0)	0.578
Heart failure	8	2.7 (0.5–13.7)	0.230	2.8 (0.5–14.5)	0.219
Stroke	8	5.4 (0.6–45.6)	0.121	4.6 (0.5–39.2)	0.167
Medications					
No	10	1 (Reference)		1(Reference)	
Yes	302	11.7 (1.4–94.8)	0.021	0.1 (0.0–0.9)	**0.042**
Diuretics					
No	150	1(Reference)		1(Reference)	
Yes	162	1.7 (1.1–2.7)	0.022	1.6 (1.0–2.5)	0.053
CCB					
No	60	1(Reference)		1(Reference)	
Yes	252	1.6 (0.9–2.9)	0.093	1.6 (0.9–2.8)	0.127
Statins					
No	152	1(Reference)		1(Reference)	
Yes	160	1.5 (0.9–2.3)	0.100	1.4 (0.9–2.2)	0.171
Oral hypoglycemics					
No	275	1(Reference)		1(Reference)	
Yes	37	0.5 (0.3–1.0)	0.056	0.5 (0.2–1.0)	**0.048**
Allopurinol					
No	277	1(Reference)		1(Reference)	
Yes	35	2.5 (1.4–4.4)	0.003	2.4 (1.3–4.3)	**0.005**
Nitrates					
No	306	1(Reference)		1(Reference)	
Yes	6	1.9 (1.1–3.3)	0.024	1.7 (1.0–3.0)	0.059
Doxazosin					
No	171	1(Reference)		1(Reference)	
Yes	141	1.8 (1.2–2.9)	0.010	1.9 (1.2–3.1)	**0.006**
Others					

(*Continued*)

Table 3. (Continued)

Characteristic	Number of patients	UNIVARIATE		MULTIVARIATE	
		OR (95% CI)	P. value	OR (95% CI)	P. value
No	206	1(Reference)		1(Reference)	
Yes	106	1.5 (0.9–2.4)	0.116	1.5 (0.9–2.5)	0.100
Proteinuria (g/mmol)					
Normal to mild (<0.015)	110	1(Reference)		1(Reference)	
Moderately (0.015–0.05)	122	2.3 (1.4–3.9)	0.002	2.5 (1.5–4.3)	**0.001**
Severely (> 0.050)	80	3.9 (2.0–7.2)	0.000	3.5 (1.9–6.5)	**0.001**
Hyperuricemia					
No	216	1(Reference)		1(Reference)	
Yes	96	2.4 (1.4–4.0)	0.001	2.4 (1.4–4.1)	**0.001**
Anaemia					
No	194	1(Reference)		1(Reference)	
Yes	118	2.5 (1.5–4.0)	0.001	2.9 (1.7–4.9)	**0.001**
Transferrin g/dl)					
Normal (2.0–3.60)	265	1(Reference)		1(Reference)	
Low (< 2.0)	40	2.4 (1.1–5.1)	0.025	2.4 (1.1–5.1)	**0.028**
High (>3.60)	7	0.6 (0.1–2.7)	0.499	0.5 (0.1–2.7)	0.491
WBC (x 10^9 cells/l)					
Normal (4–11)	276	1(Reference)		1(Reference)	
Low (> 4)	23	0.5 (0.2–1.3)	0.051	1.8 (0.7–4.2)	0.202
High (>11)	13	1.1 (0.4–3.5)	0.859	1.8 (0.4–7.5)	0.418
Calcium (mmol /l)					
Normal (2.15–2.45)	244	1(Reference)		1(Reference)	
Low (< 2.15)	25	2.2 (0.9–5.8)	0.098	2.1 (0.8–5.8)	0.125
High (>2.45)	43	0.6 (0.3–1.1)	0.081	0.6 (0.3–1.1)	0.081
Potassium (mmol/l)					
Normal (3.5–5.1)	266	1(Reference)		1(Reference)	
Low (< 3.5)	28	0.7 (0.3–1.4)	0.280	0.6 (0.3–1.4)	0.261
High (> 5.1)	18	6.0 (1.4–26.6)	0.018	5.4 (1.2–24.1)	**0.029**
Bicarbonate (mmol/l)					
Normal (23–29)	135	1(Reference)		1(Reference)	
Low (< 23)	174	1.9 (1.2–3.0)	0.007	2.0 (1.2–3.1)	**0.005**
High (> 29)	3	0.5 (0.1–5.7)	0.583	0.4 (0.0–5.1)	0.495

P < 0.05 was used to identify potential variables, those with P <0.2 in univariate were included into the multivariate analysis adjusting for age and sex.

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advanced CKD presented with severely increased proteinuria; advanced CKD was 3.5 times higher if a patient had severe proteinuria, as also reported in other studies [39, 59, 60]. Furthermore, few (18.8%) patients with advanced CKD and 21.4% of those with early CKD were using ACEIs or ARBs; similar findings have been reported from other studies on the underutilization of ACEIs/ARBs when they were clinically indicated or discontinuation of RAAS inhibitors by clinicians during the course of CKD possibly due to their associated side effects [61–63]. Angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptors blockers (ARBs) had no significant association with advanced CKD, possibly due to the small numbers on these agents, unlike findings from other studies that did demonstrate that the use of ACEIs/ARBs had beneficial effects for kidney events and cardiovascular outcomes compared to other anti-hypertensive medications in patients with CKD [63–65]. The possible explanation could be the

fact that the majority (84.0%) of the study patients with advanced CKD were using calcium channel blockers. Studies have demonstrated that calcium channel blockers have similar kidney and cardiovascular protective effects when compared to RAAS blockers in patients with CKD [66, 67].

Conclusion

Hypertension and diabetes mellitus were strongly associated with advanced CKD, suggesting a need for primary and secondary population-based prevention measures. Metabolic acidosis, anaemia with low transferrin levels, hyperuricemia and hyperkalemia were highly prevalent in our patients, including those with early CKD, and they were strongly associated with advanced CKD. This calls for the proactive role of clinicians and dietitians in supporting the needs of CKD patients in meeting their daily dietary requirements towards preventing and slowing the progression of CKD. Further studies on the role of diet including plant-based proteins, vegetables and fruits in preventing and slowing CKD progression and other metabolic complications of CKD are warranted.

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PLOS ONE

RESEARCH ARTICLE

Progression of chronic kidney disease among black patients attending a tertiary hospital in Johannesburg, South Africa

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Abstract

Background

Chronic kidney disease (CKD) is a major public health issue worldwide and is an important contributor to the overall non-communicable disease burden. Chronic kidney disease is usually asymptomatic, and insidiously and silently progresses to advanced stages in resource limited settings.

Methodology

A prospective longitudinal study was carried out on black patients with CKD attending the kidney outpatient clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa, between September 2019 to March 2022. Demographic and clinical data were extracted from the ongoing continuous clinic records, as well as measurements of vital signs and interviews at baseline and at follow up. Patients provided urine and blood samples for laboratory investigations as standard of care at study entry (0) and at 24 months, and were followed up prospectively for two (2) years. Data were descriptively and inferentially entered into REDcap and analysed using STATA version 17, and multivariable logistic regression analysis was used to identify predictors of CKD progression.

Results

A total of 312 patients were enrolled into the study, 297 (95.2%) patients completed the study, 10 (3.2%) patients were lost to follow and 5 (1.6%) patients died during the study period. The prevalence of CKD progression was 49.5%, while that of CKD remission was 33% and CKD regression was 17.5%. For patients with CKD progression the median age at baseline was 58 (46–67) years, the median eGFR was 37 (32–51) mL/min/1.73 m^2, median urine protein creatinine ratio (uPCR) was 0.038 (0.016–0.82) g/mmol and the median haemoglobin (Hb) was 13.1 (11.7–14.4) g/dl; 95.2% had hypertension, 40.1% patients had diabetes mellitus and 39.5% had both hypertension and diabetes mellitus. Almost half (48.3%) of patients with CKD progression had severely increased proteinuria and 45.6% had

za) for researchers who meet the relevant ethics criteria for access to these data.

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Abbreviations: CKD, chronic kidney diseases; CMJAH, Charlotte Maxeke Johannesburg Academic Hospital; CKD-EPI, chronic kidney disease epidemiology collaboration; ESKD, end stage kidney disease; T2DM, Type 2 Diabetes Mellitus; eGFR, estimated glomerular filtration rate; IDMS, isotope dilution mass spectroscopy; uPCR, urine protein creatinine ratio; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers; KDIGO, kidney disease improving global outcomes; NCDs, non-communicable diseases; SSA, sub Saharan Africa; UK, United Kingdom; USA, United States of America.

anaemia. Variables associated with higher odds for CKD progression after multivariable logistic regression analysis were severely increased proteinuria (OR 32.3, 95% CI 2.8–368.6, P = 0.005), moderately increased proteinuria (OR 23.3, 95% CI 2.6–230.1, P = 0.007), hypocalcaemia (OR 3.8, 95% CI 1.0–14.8, P = 0.047), hyponatraemia (OR 4.5, 95% CI 0.8–23.6, P = 0.042), anaemia (OR 2.1, 95% CI 1.0–4.3, P = 0.048), diabetes mellitus (OR 1.8, 95% CI 0.9–3.6, P = 0.047), elevated HbA1c (OR 1.8, 95% CI 1.2–2.8, P = 0.007) and current smoking (OR 2.8, 95% CI 0.9–8.6, P = 0.049).

Conclusion

Our study identified a higher prevalence of CKD progression in a prospective longitudinal study of black patients with CKD compared with literature reports. CKD Progression was associated with proteinuria, diabetes mellitus, elevated HbA1c, anaemia, hypocalcaemia, hyponatraemia and current smoking in a cohort of black patients with CKD who had controlled hypertension and diabetes mellitus at baseline.

Introduction

The global prevalence of chronic kidney disease (CKD) has been progressively increasing and is a significant risk factor for cardiovascular disease morbidity and mortality [1, 2]. The burden of CKD is more prominent in low- and lower-middle-income countries (LLMICs) than in high-income countries (HICs) with a higher prevalence among those disadvantaged and indigenous communities who have less/ limited access to health care [1, 3]. The global increase in CKD is driven by the increasing prevalence of diabetes mellitus, hypertension and obesity [4, 5]. Studies have shown that African-Americans have a 2- to 4-fold higher risk for end-stage kidney disease (ESKD) than their white counterparts requiring renal replacement therapy [6, 7]. Individuals of black ethnicity due to their genes, including the presence of *APOL*1 high-risk genotypes, are at higher risk of increased serum creatinine levels, lower eGFR, more rapid CKD progression and death due to social, economic and medical inequities [8, 9]. Studies have reported the prevalence of CKD to be 15.8% in Africa, with major cost implications to overburdened healthcare systems [10, 11]. In developing countries, patients with ESKD are increasing in numbers and most die due to poor access to kidney replacement therapies, calling for cost effective preventive strategies to reduce CKD progression [4, 12]. Chronic kidney disease in developing countries also tends to be present in younger people who are unaware of their underlying kidney disease, and very few receive optimal care because of resource constraints [13, 14]. Regardless of the underlying cause of CKD, chronic kidney diseases progress slowly to ESKD due to irreversible nephron loss leading to premature death [15, 16]. Slowing CKD progression, promoting remission, or even regression of CKD, have been based on the control of blood pressure, control of blood sugar, reduction of proteinuria and management of other known risk factors for CKD progression [17, 18]. When CKD progressors are identified reliably and earlier in stages 1 to 3, disease progression can be altered with reduced complications [19, 20]. There is a need to identify those patients with CKD and those who are likely to progress more rapidly, to reduce the number of patients progressing to end-stage kidney disease (ESKD). The aim of this study was to study the prevalence and predictors of CKD progression in a cohort of black patients with satisfactory blood pressure and glycaemic control at baseline attending a tertiary hospital in Johannesburg, South Africa.

Methods

Study design, population and settings

This was a prospective longitudinal study to evaluate the prevalence and predictors of CKD progression among black patients attending the kidney outpatient clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between September 2019 to March 2022. The CMJAH is a public accredited central hospital with 1088 beds serving patients from across the Gauteng province and nearby provinces in South Africa. CMJAH is also one of the main teaching hospitals for the Faculty of Health Sciences of the University of the Witwatersrand. Johannesburg is the largest city in South Africa and among the largest 50 urban agglomerations in the world. Johannesburg had an estimated population of around 5.9 million in 2021, the most common racial groups include black African (76.4%), White (12.3%), mixed ethnicity (5.6%) and Indian/Asian (4.9%). Inclusion criteria for the study were patients who were >18 years of age, with CKD stages 1–4, who had controlled hypertension (blood pressure < 140/90 mm Hg) and diabetes mellitus (HbA1C < 7%) [21, 22], attending the kidney outpatient clinic for at least 6 months and were able to provide informed consent. Patients who had active infections, active malignancies, autoimmune diseases and who were not black were excluded. Black patients have significantly higher rates of more rapid CKD progression and ESKD than other races [23, 24], hence they were the focus of this study.

Data collection and laboratory procedures

Enrollment occurred between September 2019 to March 2020; consecutive sampling was used to meet the required number of study patients and follow up was conducted between September 2021 to March 2022. Demographic and clinical data including age, gender, weight, height, glycaemic status, history of smoking, aetiology of CKD and medications were extracted from the ongoing continuous clinic records. Face to face interviews at the time of enrolment were recorded in a questionnaire and vital signs measurements were conducted at first enrolment and at follow up. Patients provided blood and urine samples at study entry (0) and at 24 months; patients agreed to have their routine medical records accessed prospectively for two (2) years. Systolic and diastolic blood pressure was measured 3 times, and the average of the second and third measurements was used [25]. Body mass index (BMI) was calculated using the National Health Services (NHS-UK) BMI calculator [26]. Measurements of urinary protein creatinine ratio (uPCR), serum creatinine, electrolytes, HbA1C, white cell count, haemoglobin level, platelets, calcium, phosphate, transferrin and HDL cholesterol were done as standard of care at the time of recruitment and at follow up during a clinic visit. Anaemia was defined as a Hb concentration of < 13.0g/dl in males and < 12.0g/dl in females [27]. Serum creatinine was measured using the isotope dilution mass spectrometry (IDMS) traceable enzymatic assay and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation without using the African American correction factor, race adjustment of eGFR in black patients may affect their clinical care contributing to differences in the management of their kidney disease [28, 29]. CKD progression was assessed by a (i) change to a more advanced stage of CKD (ii) greater than 30% reduction of eGFR in two years, whereby percent change in eGFR was calculated as last eGFR–first eGFR/first eGFR x 100 [30] (iii) sustained decline in eGFR of > 4 ml/min/1.73 m^2/year or more [31, 32]. CKD regression was defined by a sustained increase in eGFR of > 4 ml/min/1.73 m^2/year or an improvement in CKD stage at 24 months from baseline [18, 31]. CKD remission was defined by a stable or change in eGFR of < 4 ml/min/1.73 m^2/year from baseline and at 24 months of follow up [18, 32].

Data management and analysis

Study data were collected and entered into *REDCap* (Research Electronic Data Capture) tools [33, 34] hosted at the University of the Witwatersrand and analyzed using STATA version 17 (College Station, Texas, USA). Descriptive statistics were used to summarize demographic and clinical data; continuous variables have been reported as medians with interquartile ranges and Wilcoxon rank-sum test was used for the non-normally distributed variables. Discrete variables have been reported as frequencies and proportions, Pearson's chi-square test was used to test for association between variables and CKD progression. Odd ratios were used to estimate the strengths of association between variables and CKD progression; univariate and multivariate logistic regression models were used to estimate the association of variables and CKD progression. Variables with p-value less than 0.2 on univariate logistic regression models were then fitted into the multivariate logistic regression models with the addition of age and sex as adjusting variables; variables with a p-value of less than 0.05 were considered to have significant strength of association.

Ethical issues

Ethical approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand, Johannesburg (ethics clearance certificate No. M190553). Written informed consent was obtained from each of the participants before enrolment and embarking on data collection.

Results

Demographic and clinical characteristics of the study population

A total of 476 black patients with CKD stages 1–4 attended the CMJAH kidney outpatient clinic during the enrolment period, 164 patients were excluded from the study including 110 patients who had uncontrolled hypertension, 35 patients who had uncontrolled diabetes mellitus, 11 patients who had autoimmune diseases, 6 patients had active infections and 2 patients had active malignancies. Of the 312 patients who were enrolled into the study, 10 (3.2%) patients were lost to follow and 5 (1.6%) patients died, 297 (95.2%) patients completed the study of whom 156 (52.5%) were male and 154 (51.8%) were married. Progression of CKD occurred in 147 (49.5%) patients over 2 years of follow up. The median age was 58 (46–67) years for CKD progressors and 57 (45–66) years for those without CKD progression. Significant variables were the median eGFR at baseline, 37 (32–51) mL/min/1.73 m^2 for CKD progressors and 44 (34–61) mL/min/1.73 m^2 for those without CKD progression (p = 0.003); the median urine protein creatinine ratio (uPCR) was 0.038 (0.016–0.82) g/mmol for CKD progressors and 0.016 (0.008–0.032) g/mmol for those without CKD progression (p = 0.001); median haemoglobin of 13.1 (11.7–14.4) g/dl for CKD progressors and 13.7 (12.2–15.3) g/dl for those without CKD progression, p = 0.023 (Table 1).

Prevalence of CKD progression, CKD remission and CKD regression. During the 2 years of follow up, a total of 147 (49.5%) patients had progression of CKD whereby 35.0% changed to a more advanced stage of CKD, 19.9% had greater than 30% reduction of eGFR in two years and 48.5% had a sustained decline in eGFR of $>$ 4 ml/min/1.73 m^2/year. A total of 98 (33%) patients were observed to have remission of CKD, while 52 (17.5%) patients had CKD regression (Fig 1A and 1B).

Clinical profile of black patients who had CKD progression. Of the 147 black patients who had CKD progression, the majority (95.2%) of patients had hypertension, 59 (40.1%) patients had diabetes mellitus and 58 (39.5%) had both hypertension and diabetes mellitus.

Table 1. Baseline demographic and clinical characteristics of CKD progressors and non-progressors.

Characteristic	CKD progression (n = 147)	No CKD progression (n = 150)	P-value
	Proportion (%) or Median (IQR)	Proportion (%) or Median (IQR)	
Age (years)	58 (46–67)	57 (45–66)	0.355
Sex			
Male	82 (55.78%)	74 (49.33%)	
Female	65 (44.22%)	76 (50.67%)	0.266
Marital status			
Single	39 (26.53%)	50 (33.33%)	
Married	81 (55.10%)	73 (48.67%)	
Widow/Widower	18 (12.24%)	19 (12.67%)	
Separated/Divorced	9 (6.12%)	8 (5.33%)	0.608
Highest level of education			
No formal education	18 (12.24%)	16 (10.67%)	
Primary	31 (21.09%)	36 (24.00%)	
Secondary	52 (35.37%)	43 (28.67%)	
Tertiary	46 (31.29%)	55 (36.67%)	0.549
Occupation			
Unemployed	23 (15.65%)	21 (14.00%)	
Domestic workers	25 (17.01%)	31 (20.67%)	
Self employed	33 (22.45%)	32 (21.33%)	
Public / Private servant	54 (36.73%)	53 (35.33%)	
Retired	12 (8.16%)	13 (8.67%)	0.943
BMI (kg/m^2)	30.6 (26.84–35.67)	29.39 (25.97–33.4)	0.193
SBP (mmHg)	140 (132–140)	140 (126–140)	0.088
DBP (mmHg)	83 (74–90)	82 (73–90)	0.319
uPCR (g/mmol)	0.038 (0.016–0.82)	0.016 (0.008–0.032)	**0.001**
Creatinine (umol/L)	148 (118–183)	134 (104–161)	**0.002**
eGFR ($ml/min/1.72m^2$)	37 (32–51)	44 (34–61)	**0.003**
FBG (mmol/L)	4.5 (4.2–5.4)	4.4 (4.2–4.9)	0.187
HbA1C (%)	7.0 (7.0–7.0)	7.0 (6.6–7.0)	0.184
Haemoglobin (g/dl)	13.1 (11.7–14.4)	13.7 (12.2–15.3)	**0.023**
Transferrin (g/L)	2.49 (2.25–2.78)	2.59 (2.29–2.85)	0.782
WBC (x 10^9 cells/L)	6.1 (5.06–7.84)	6.46 (4.81–7.74)	0.549
Platelets (x 10^9 cells/L)	251 (211–320)	269 (218–323)	**0.017**
Uric acid (mmol/L)	0.42 (0.35–0.51)	0.40 (0.31–0.48)	**0.010**
HDL cholesterol (mmol /L)	1.16 (0.99–1.43)	1.25 (1.01–1.56)	0.114
Calcium (mmol /L)	2.30 (2.23–2.40)	2.34 (2.25–2.42)	0.095
Phosphate (mmol /L)	1.11 (0.93–1.26)	1.02 (0.88–1.16)	**0.022**
Sodium (mmol/L)	141 (139–143)	141 (138–143)	0.849
Potassium (mmol/L)	4.3 (4.0–4.7)	4.2 (3.8–4.5)	0.068
Bicarbonate (mol/L)	22 (20–24)	22 (20–24)	0.871

IQR, interquartile range; uPCR, urine protein creatinine ratio; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood sugar; HbA1C, glycosylated hemoglobin A1C; WBC, white blood cells; HDL, high density lipoprotein; SBP, systolic blood pressure.

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About half (48.3%) of the CKD progressors presented with severely increased proteinuria as compared to 30 (20.0%) of those without CKD progression. Anaemia was noted in 67 (45.6%) patients with CKD progression and in 40 (26.7%) patients in those without CKD progression. Low serum calcium levels were noted in 26 (17.7%) of those with CKD progression and in 10

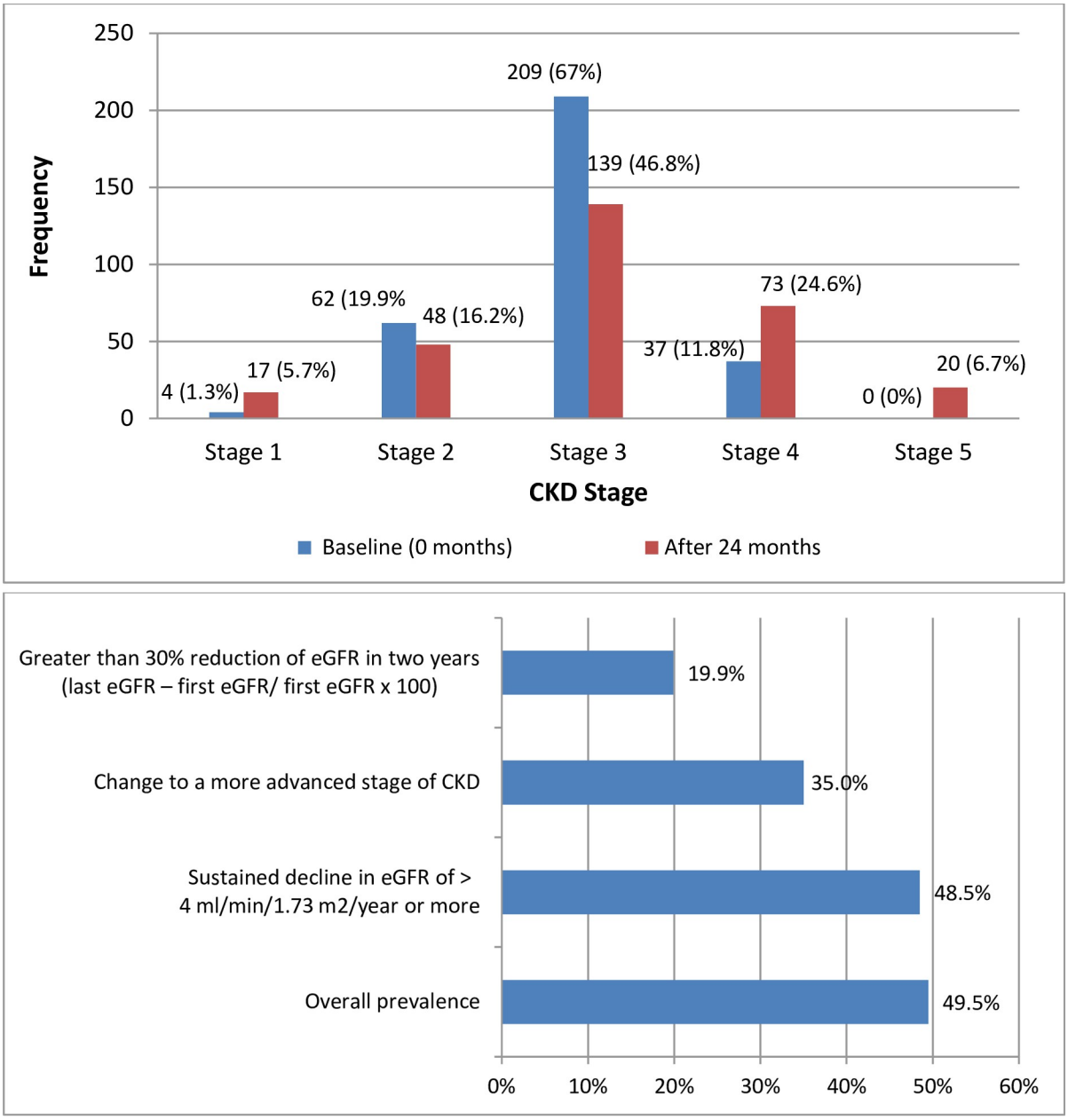

Fig 1. a: CKD stages at baseline and at 24 months follow up; b: Estimation of CKD progression using different eGFR criteria.

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(6.7%) patients of those without CKD progression, while high serum phosphate levels were noted in 18 (12.3%) patients in those with CKD progression and in 6 (4.0%) patients of those without CKD progression. Low serum sodium levels were noted in 12 (8.2%) of those with CKD progression and in 3 (2.0%) of patients without CKD progression, while high serum potassium levels were noted in 21 (14.3%) patients who had CKD progression and in 7 (4.7%) of patients without CKD progression. Hyperuricemia was present in 50 (34.0%) patients with CKD progression as compared to 24 (16.0%) patients without CKD progression. Majority (76.9%) patients with CKD progression were using more than 4 anti-hypertensives for their blood pressure control as compared to 57.3% in those patients without CKD progression. For

patients with CKD progression, the majority (85.7%) of patients were on calcium channel blockers, 62.6% were on diuretics, 57.1% were on statins, 55.1% were on beta blockers, 31.3% were on insulin, 29.3% and were on aspirin. A total of 35 (23.8%) CKD progressors were on ACEs/ARBs compared with 25 (16.7%) non-progressors; there was no significant difference in CKD progression associated with the use of ACEIs/ARBs (Table 2).

Predictors of CKD progression. We divided the patients into two subgroups according to their CKD progression status after 2 years of follow up: CKD progression vs. no CKD progression. A total of 30 potential variables were identified after performing univariate logistic regression analyses. Backward elimination reduced this to 21 parameters; the factors associated with CKD progression after adjusting for age and sex on multivariate logistic regression analysis included diabetes mellitus (OR 1.8, 95% CI 0.9–3.6, P = 0.047), increasing HbA1C (OR 1.8, 95% CI 1.2–2.8, P = 0.007), severely increased proteinuria (OR 32.3, 95% CI 2.8–368.6, P = 0.005), moderately increased proteinuria (OR 23.3, 95% CI 2.6–230.1, P = 0.007), hypocalcaemia (OR 3.8, 95% CI 1.0–14.8, P = 0.047), anaemia (OR 2.1, 95% CI 1.0–4.3, P = 0.048), hyponatraemia (OR 4.5, 95% CI 0.8–23.6, P = 0.042) and current smoking (OR 2.8, 95% CI 0.9–8.6, P = 0.049 (Table 3).

Discussion

Our study identified a higher prevalence of progression of CKD in a prospective longitudinal study of black patients with CKD attending the kidney outpatient clinic at CMJAH in Johannesburg compared to literature reports. We found a high prevalence of CKD progression with 49.5% of the study patients with CKD progression, which is a higher prevalence of progression than the reported prevalence of 46.7% in a UK cohort study and 38% in a study conducted in northern California in USA [31, 35]. The possible explanation could be differences in the study populations; in our study we enrolled only black patients; black patients tend to have more rapid CKD progression even in the early stages of CKD [36, 37]. Also, black patients due to racial/ethnic disparities are at higher risk of CKD progression and its related complications than other races [38, 39]. This study established that 33% patients had CKD remission and 17.5% patients had CKD regression, similar to findings from other studies from the UK [35, 40]. A sustained decline in eGFR of $>$ 4 ml/min/1.73 m^2/year or more was able to identify 48.5% patients who had CKD progression, including those with rapid CKD progression, similar to other studies [31, 35].

Hypertension was prevalent in the study cohort, occurring in 96.7% of the study cohort at baseline and in 95.2% of CKD progressors and in 91.3% of those without progression. Of the patients with CKD progression, the majority (95.2%) had hypertension, 40.1% had diabetes mellitus and 39.5% had both hypertension and diabetes mellitus. Studies have reported CKD progression to be associated with CKD etiology and hypertension [41, 42]. Both systolic and diastolic blood pressure were not significantly associated with CKD progression in this study, unlike findings from other studies where higher SBP and DBP levels were associated with CKD and had higher risks of CKD progression [43, 44]. This difference could be due to the high prevalence (above 90%) of hypertension in both groups and an entry criterion of controlled blood pressure. Smoking was reported in 9.5% of our study patients who had CKD progression, with a 2.8-fold increased risk for CKD progression; studies have reported that smoking is significantly associated with a higher risk of CKD and smoking is strongly associated with CKD progression [45, 46]. Obesity and HDL cholesterol were not significantly associated with CKD progression in this study unlike findings from other studies where patients who had obesity and/or hyperlipidaemia had higher rates of CKD progression [47, 48]. This difference could be due to the high prevalence of obesity/overweight in both progressor and non-progressor groups.

Table 2. Baseline clinical and laboratory parameters of CKD progressors and non-progressors.

Parameter	CKD progression (n = 147) Proportion (%)	No CKD progression (n = 150) Proportion (%)	P-value
Diagnoses encountered			
Hypertension	140 (95.24%)	137 (91.33%)	0.179
Diabetes mellitus	59 (40.14%)	39 (26.00%)	**0.010**
Hypertension & Diabetes mellitus	58 (39.46%)	38 (25.3%)	**0.009**
Adult polycystic kidney disease	7 (4.76%)	4 (2.67%)	0.339
Reflux nephropathy	2 (1.36%)	3 (2.00%)	0.668
Obstructive uropathy	1 (0.68%)	0 (0.0%)	0.312
Unknown	2 (1.36%)	8 (5.33%)	0.058
Current smoking			
Yes	14 (9.52%)	8 (5.33%)	
No	133 (90.48%)	142 (96.67%)	0.168
Current Alcohol			
Yes	14 (9.52%)	18 (12.00%)	
No	133 (90.48%)	132 (88.00%)	0.493
Cardiovascular Diseases			
None	99 (67.35%)	121 (80.67%)	
Angina	28 (19.05%)	22 (14.67%)	
Myocardial infarction	9 (6.12%)	2 (1.33%)	
Heart failure	5 (3.40%)	3 (2.00%)	
Stroke	6 (4.08%)	2 (1.33%)	**0.030**
Medications			
None	4 (2.72%)	5 (3.33%)	0.758
Diuretics	92 (62.59%)	61 (40.67%)	**0.001**
ACEIs / ARBs	35 (23.81%)	25 (16.67%)	0.125
Aldactone	7 (4.76%)	4 (2.67%)	0.339
CCBs	126 (85.71%)	112 (74.67%)	**0.017**
Statins	84 (57.14%)	67 (44.67%)	**0.032**
Oral hypoglycemics	15 (10.2%)	21(14.0 0%)	0.316
Insulin	46 (31.29%)	22 (14.67%)	**0.001**
Allopurinol	16 (10.88%)	18 (12.00%)	0.763
Junior ASA	43 (29.25%)	26 (17.33%)	**0.015**
Beta blockers	81 (55.10%)	61 (40.67%)	**0.013**
Aldomet	36 (24.49%)	33 (22.0%)	0.611
Hydralazine	11 (7.48%)	8 (5.33%)	0.449
Nitrates (ISMN/ISDN)	4 (2.72%)	3 (2.00%)	0.682
Doxazosin	68 (46.26%)	66 (44.00%)	0.696
Others	47 (31.97%)	52 (34.67%)	0.622
No. of antihypertensives per patient			
0	4 (2.72%)	6 (4.00%)	
1–3	30 (20.40%)	58 (38.67%)	
≥ 4	113 (76.88%)	86 (57.33%)	**0.002**
uPCR (g/mmol)			
Normal to mildly increased (<0.015)	23 (15.65%)	59 (39.33%)	
Moderately increased (0.015–0.05)	48 (32.65%)	59 (39.33%)	
Severely increased (> 0.050)	71 (48.30%)	30 (20.00%)	
Nephrotic syndrome (> 0.350)	5 (3.40%)	2 (1.33%)	**0.001**
WBC (x 10^9 cells/l)			

(*Continued*)

Table 2. (Continued)

Parameter	CKD progression (n = 147) Proportion (%)	No CKD progression (n = 150) Proportion (%)	P-value
Low (> 4)	5 (3.40%)	12 (8.00%)	
Normal (4–11)	139 (94.56%)	131 (87.33%)	
High (>11)	3 (2.04%)	7 (4.67%)	0.096
Haemoglobin (g/dl)			
Normal (> 12.0 or 13.0)	80 (54.42%)	110 (73.33%)	
Anemia (<12.0 or 13.0)	67 (45.58%)	40 (26.67%)	**0.001**
Transferrin (g/dl)			
Low (< 2.0)	26 (17.69%)	13 (8.67%)	
Normal (2.0–3.60)	117 (79.59%)	134 (89.33%)	
High (>3.60)	4 (2.72%)	3 (2.00%)	0.061
Platelets (x 10^9 cells/l)			
Low (> 150)	2 (1.36%)	3 (2.00%)	
Normal (150–450)	142 (96.60%)	141 (94.00%)	
High (>450)	3 (2.04%)	6 (4.00%)	0.643
Calcium (mmol /l)			
Low (< 2.15)	26 (17.69%)	10 (6.67%)	
Normal (2.15–2.45)	107 (72.79%)	118 (78.67%)	
High (>2.45)	14 (9.52%)	22 (14.67%)	**0.009**
Phosphate (mmol /l)			
Low (< 0.80)	7 (4.76%)	16 (10.67%)	
Normal (0.80–1.55)	122 (82.99%)	128 (85.33%)	
High (>1.55)	18 (12.25%)	6 (4.00%)	**0.019**
Uric acid (mmol/l)			
Normal (0.16/0.21–0.36/0.43)	97 (65.99%)	126 (84.00%)	
High (> 0.36 or 0.43)	50 (34.01%)	24 (16.00%)	**0.001**
HDL cholesterol (mmol /l)			
Low (< 1.04)	43 (29.25%)	35 (23.33%)	
Normal (1.04–1.17)	28 (19.05%)	25 (16.67%)	
High (>1.17)	76 (51.70%)	90 (60.00%)	0.343
Sodium (mmol/l)			
Low (< 136)	12 (8.16%)	3 (2.00%)	
Normal (136–145)	130 (88.44%)	138 (92.00%)	
High (>145)	5 (3.40%)	9 (6.00%)	**0.034**
Potassium (mmol/l)			
Low (< 3.5)	10 (6.80%)	11 (7.33%)	
Normal (3.5–5.1)	116 (78.91%)	132 (88.0%)	
High (> 5.1)	21 (14.29%)	7 (4.67%)	**0.018**
Bicarbonate (mmol/l)			
Low (< 23)	97 (65.99%)	85 (56.67%)	
Normal (23–29)	46 (31.29%)	63 (42.00%)	
High (> 29)	4 (2.72%)	2 (1.33%)	0.130

uPCR, urine protein creatinine ratio; eGFR, estimated glomerular filtration rate; CCBs, calcium channel blockers; ACEIs, angiotensin converting enzyme inhibitors; ARBs, Aldosterone receptors blockers.

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Table 3. Predictors of CKD progression.

Characteristic	No. of patients	UNIVARIATE		MULTIVARIATE	
		OR (95% CI)	P. value	OR (95% CI)	P. value
Marital status					
Single	89	1(Reference)		1(Reference)	
Married	154	1.4 (0.8–2.4)	0.188	1.7 (0.8–3.7)	0.152
Widow/Widower	37	1.2 (0.6–2.6)	0.620	0.9 (0.3–2.9)	0.969
Separated/Divorced	17	1.4 (0.5–4.1)	0.490	1.4 (0.4–5.8)	0.605
Hypertension					
No	20	1(Reference)		1(Reference)	
Yes	277	1.8 (0.7–4.9)	0.186	0.2 (0.0–4.6)	0.343
Diabetes mellitus					
No	199	1(Reference)		1(Reference)	
Yes	98	1.9 (1.2–3.1)	0.010	1.8 (0.9–3.6)	**0.047**
Current smoking					
No	275	1(Reference)		1(Reference)	
Yes	22	1.8 (0.8–4.6)	0.174	2.8 (0.9–8.6)	**0.049**
BMI	297	1.0 (0.9–1.1)	0.189	1.0 (1.0–1.1)	0.609
SBP (mm hg)	297	1.0 (0.9–1.0)	0.135	1.0 (0.9–1.1)	0.700
DBP (mm hg)	297	1.0 (0.9–1.1)	0.142	1.0 (0.9–1.1)	0.908
HbA1C (%)	98	1.5 (1.2–2.0)	0.002	1.8 (1.2–2.8)	**0.007**
Cardiovascular Disease:					
None	220	1(Reference)		1(Reference)	
Angina	50	1.6 (0.8–2.8)	0.161	1.5 (0.6–3.7)	0.399
Myocardial infarction	11	5.5 (1.2–26.0)	0.032	3.8 (0.6–23.4)	0.151
Heart failure	8	2.0 (0.5–8.7)	0.338	1.4 (0.3–6.9)	0.710
Stroke	8	7.3 (0.9–61.9)	0.067	4.3 (0.4–42.3)	0.216
Proteinuria (g/mmol)					
Normal to mildly increased (<0.015)	82	1(Reference)		1(Reference)	
Moderately increased (0.015–0.05)	107	2.1 (1.1–3.9)	0.019	23.3 (2.4–230.1)	**0.007**
Severely increased (> 0.050)	101	6.1 (3.2–11.6)	0.000	32.3 (2.8–368.6)	**0.005**
Nephrotic syndrome (> 0.350)	7	6.4 (1.2–35.4)	0.033	3.1 (0.0–547.7)	0.665
Anaemia					
No	190	1(Reference)		1(Reference)	
Yes	107	2.3 (1.4–3.7)	0.001	2.1 (1.0–4.3)	**0.048**
Transferrin g/dl)					
Normal (2.0–3.60)	251	1(Reference)		1(Reference)	
Low (< 2.0)	39	2.3 (1.1–4.7)	0.022	1.1 (0.4–3.0)	0.858
High (>3.60)	7	1.5 (0.3–7.0)	0.584	0.9 (0.1–5.9)	0.883
WBC (x 10^9 cells/l)					
Normal (4–11)	270	1(Reference)		1(Reference)	
Low (> 4)	17	0.4 (0.1–1.2)	0.087	0.6 (0.1–2.4)	0.460
High (>11)	10	0.4 (0.1–1.6)	0.196	0.3 (0.0–2.1)	0.215
Calcium (mmol /l)					
Normal (2.15–2.45)	225	1(Reference)		1(Reference)	
Low (< 2.15)	36	2.9 (1.3–6.2)	0.008	3.8 (1.0–14.8)	**0.047**
High (>2.45)	36	0.7 (0.3–1.4)	0.335	0.9 (0.4–2.1)	0.809
Phosphate (mmol /l)					
Normal (0.80–1.55)	250	1(Reference)		1(Reference)	

(*Continued*)

Table 3. (Continued)

Characteristic	No. of patients	UNIVARIATE		MULTIVARIATE	
		OR (95% CI)	P. value	OR (95% CI)	P. value
Low (< 0.80)	23	0.5 (0.2–1.2)	0.098	0.7 (0.2–2.3)	0.567
High (>1.55)	24	3.0 (1.1–7.8)	0.027	2.0 (0.5–8.1)	0.315
Hyperuricaemia					
No	223	1(Reference)		1(Reference)	
YES	74	2.7 (1.6–4.7)	0.001	1.6 (0.8–3.5)	0.202
HDL cholesterol (mmol /l)					
Normal (1.04–1.17)	53	1(Reference)		1(Reference)	
Low (< 1.04)	78	0.8 (0.4–1.6)	0.468	0.7 (0.3–1.5)	0.333
High (>1.17)	166	0.5 (0.3–1.0)	0.051	0.6 (0.3–1.2)	0.154
Sodium (mmol/l)					
Normal (136–145)	268	1(Reference)		1(Reference)	
Low (< 136)	15	4.3 (1.2–15.4)	0.028	4.5 (0.8–23.6)	**0.042**
High (>145)	14	0.6 (0.2–1.8)	0.355	0.5 (1.1–2.2)	0.349
Potassium (mmol/l)					
Normal (3.5–5.1)	248	1(Reference)		1(Reference)	
Low (< 3.5)	21	1.0 (0.4–2.3)	0.941	0.9 (0.3–2.5)	0.958
High (> 5.1)	28	3.4 (1.4–8.3)	0.007	1.8 (0.7–5.1)	0.200
Bicarbonate (mmol/l)					
Normal (23–29)	109	1(Reference)		1(Reference)	
Low (< 23)	182	1.5 (1.0–2.5)	0.068	0.9 (0.4–1.8)	0.706
High (> 29)	6	2.7 (0.5–15.6)	0.256	7.3 (0.5–100.1)	0.135

P < 0.05 was used to identify potential variables, those with P <0.2 in univariate analysis were included in the multivariate analysis while adjusting for age and sex.

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Diabetes mellitus had a 1.8-fold increased risk for CKD progression and increased HbA1c levels had a 1.8-fold increased risk for CKD progression similar to findings from other studies where increased HbA1c levels was associated with CKD progression in patients with T2DM [49, 50]. Most (48.3%) patients with CKD progression presented with severely increased proteinuria; CKD progression was 32.3 times higher if a patient had severely increased proteinuria and 23.3 times higher if a patient had moderately increased proteinuria; studies have reported CKD progression to be increased in patients who present with higher levels of proteinuria [51, 52]. ACEIs or ARBs have shown to reduce proteinuria more effectively than other antihypertensives; in this study, few (20.2%) patients were using ACEIs or ARBs, similar to other studies reporting underutilization of ACEIs/ARBs in CKD patients [53, 54]. Emphasizing the critical need for nephrologists and clinicians to optimize safe and effective use of ACEIs/ARBs for RAAS blockade among CKD patients towards reducing proteinuria and slowing CKD progression [55, 56].

Anaemia was reported in 45.6% of patients who had CKD progression and was 2.1-fold increased with CKD progression and CKD was more severe if a patient had anaemia. Studies have reported that anaemia in CKD mainly results from impaired production of erythropoietin by the failing kidneys and anaemia is significantly associated with increased odds for CKD, CKD progression and worse clinical outcomes [57, 58]. Hyperuricaemia was significantly associated with CKD progression on univariate analysis in our study but this significance was lost on multivariate analysis; studies have reported that high serum uric acid levels commonly occur as a result of the impaired glomerular filtration rate (GFR) that occurs in CKD. Hyperuricaemia can also precede the development of CKD, predict incident CKD and is associated

with high risk for advanced CKD/ CKD progression [59, 60]. Hypocalcaemia was reported in 17.7% patients who had CKD progression with 3.8-fold association with CKD progression; low serum calcium levels are commonly caused by increased serum phosphorus and decreased renal production of 1,25(OH)2 vitamin D due to hyperparathyroidism. Hypocalcaemia is independently associated with CKD, and it tends to predict rapid CKD progression and severe CKD [61, 62]. Hyperphosphataemia was significantly associated with CKD progression on univariate analysis but this significance was lost on multivariate analysis; studies have reported that high serum phosphate levels predicted higher rates of CKD progression [63, 64].

Hyponatraemia was reported in 8.16% of those patients who had CKD progression; CKD progression was 4.5 times more common if a patient had low serum sodium levels. The kidneys have the ability to modulate sodium and water excretion, and CKD is frequently complicated with hyponatraemia probably due to fluid overload or diuretic usage. Hyponatraemia in CKD is associated with a higher risk of CKD progression, cardiovascular events, hospitalization and increased mortality [65, 66]. Hyperkalaemia was significantly associated with CKD progression on univariate analysis but this significance was lost on multivariate analysis. Studies have reported that high serum potassium levels are common in patients with chronic CKD and it is associated with decreased renal function and CKD progression; its prevalence increases as the eGFR declines [67, 68]. Metabolic acidosis was not significantly associated with CKD progression in this study; studies from other settings reported that patients with low serum bicarbonate levels had higher rates of CKD progression and an accelerated reduction in eGFR with poor outcomes [69, 70]. This difference could be due to the high prevalence of metabolic acidosis in both groups.

Conclusion

Our study identified a higher prevalence of CKD progression in a prospective longitudinal study of black patients with CKD compared with literature reports. CKD Progression was associated with proteinuria, diabetes mellitus, elevated HbA1c, anaemia, hypocalcaemia, hyponatraemia and current smoking in a cohort of black patients with CKD who had controlled hypertension and diabetes mellitus at baseline. These patients are at relatively high risk for CKD progression calling for nephrologists and clinicians to be vigilant in identifying black patients with CKD who are at risk of early CKD progression as this would allow risk stratification to improve their kidney disease outcomes. A sustained decline in eGFR of >4 ml/min/ 1.73 m^2/year identified the highest number of patients with CKD progression, and this may help nephrologists and clinicians to identify those progressors in addition to capturing those patients with rapid CKD progression who require more frequent monitoring, closer surveillance and management towards halting their CKD progression.

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