BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025694
Article Type:	Research
Date Submitted by the Author:	26-Jul-2018
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Keywords:	Africa, HAEMATOLOGY, Chronic Kidney Disease

HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY

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Word count: 3085

Abstract word count: 294

References: 50

Tables: 3

ABSTRACT

Objectives: The objectives were to characterise the haematological profile of screen-detected chronic kidney disease (CKD) participants and to correlate the complete blood count measures with the commonly advocated kidney function estimators. **Methods:** The current cross-sectional study utilized data, collected between February 2015 and November 2016, of 1564 adults of mixed-ancestry, who participated in the Cape Town Vascular and Metabolic Health study. Kidney function was estimated using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. CKD was defined as eGFR <60ml/min/1.73m^2, and anaemia as haemoglobin level <13.5g/dL (men) and <12g/dL (women). **Results:** Based on the MDRD and CKD-EPI equations, the crude prevalence of CKD was 6% and 3%. Irrespective of the equation used, median red blood cell (RBC) indices were consistently lower in those with CKD compared to those without CKD (all p<0.0001). Despite not showing any significant difference in total white blood cell (WBC) count between the two groups, the number of lymphocytes were lower (p=0.0001 and p<0.0001 for MDRD and CKD-EPI, respectively) and neutrophil count (both p≤0.0297) and the ratio of lymphocytes to neutrophil (both p<0.0001) higher in the CKD group compared to those without CKD; with the remaining WBC indices similar in the two groups. The platelet count was similar in both groups. Of the screen-detected CKD participants, 45.5% (MDRD) and 57.8% (CKD-EPI) were anaemic, with the prevalence increasing with increasing severity of CKD, from 37.2% (stage 3) to 82.4% (stages 4-5). Furthermore, CKD-EPI-estimated kidney function, but not MDRD, was positively associated with RBC indices. **Conclusion:** Though it remains unclear whether common kidney function estimators provide accurate estimates of CKD in Africans, the correlation of their estimates with deteriorating RBC profile, suggests that advocated estimators, to some extent approximate kidney function in African populations.

Key words: chronic kidney disease; haematology; Africa

Strengths and limitations of the study

- The first study to characterize the haematological profile of individuals with reduced kidney function in a population-based setting in Africa, even more specific, individuals of mixed-ancestry
- We studied a community with a high burden of obesity, hypertension and diabetes, reflective of the current burden in Africa.
- This study was conducted in only one geographical area, which may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa.
- Our study was based on a single serum creatinine measure to determine CKD and did not include estimates of albuminuria. Albuminuria, which are required for clinical and aetiological diagnosis of CKD, as this information is important particularly in the interpretation of eGFR greater that 60ml/min/1.73m^2 where inaccuracies of the eGFR equations are greatest

BACKGROUND

Chronic kidney disease (CKD) is a major global public health problem [1], estimated to affect more than 10% of the general adult population and up to 50% of some high-risk subpopulations, such as the elderly [2], those with non-communicable diseases (NCD), including type 2 diabetes mellitus (T2D) and hypertension, and communicable diseases (CD), including human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) [3 4]. Africa is currently experiencing the double burden of NCDs and CDs, which are all driving the increasing burden of CKD on the continent [5]. However, the exact burden of CKD in Africa has yet to be fully elucidated [6-9], in part due to the absence of appropriate estimates for predicting reduced kidney function in individuals from African ancestry [9 10].

CKD encompasses a wide range of physiological processes altered by the progressive decline in glomerular filtration rate (GFR) [11 12]. Haematological parameters, particularly red blood cell (RBC) indices, are most commonly affected [13], giving rise to anaemia. Anaemia is the most common, consistent and severe of the various haematological abnormalities, and has been shown to be a very common condition in black Africans [14]. Although anaemia may be found at any stage of CKD, the severity of anaemia increases with CKD progression [15], resultantly affecting nearly all patients with end-stage renal disease (CKD stage 5) [13]. The predominant cause of anaemia in CKD is failure of the kidneys to produce enough endogenous erythropoietin, which accompanies the fall in GFR [16 17]. Untreated, prolonged anaemia is strongly predictive of all-cause and cardiovascular mortality, as well as reduced quality of life and increased morbidity in patients with CKD [13 18]. Untreated anaemia can also accelerate the decline in renal function by causing renal haemodynamic alterations and tissue hypoxia [15]. Other potentially affected haematological parameters in CKD, of which the association with CKD is not yet fully characterized, include total and differential white blood cell (WBC) counts. Persistent, low-grade inflammation is an essential part of the aetiology of CKD and has been recognized as such since the late 1990s, when it was linked to cardiovascular disease (CVD) and mortality [19]. Recently, the ratio of neutrophil-to-lymphocyte count (N/L) has been proposed as a novel measure of inflammation in distinct populations and has been shown to have prognostic value [20]; particularly for mortality risk in patients with myocardial infarction and heart failure [21 22]. However, studies on the relationship of N/L ratio with reduced eGFR are limited [23]. Thus, despite recent advances

in the aetiology governing the development and progression of CKD, population-based data on the haematological profile of people with CKD in Africa, are scanty.

We therefore aimed to characterise the haematological profile of screen-detected CKD participants in a community-based sample, and to correlate the complete blood count measures with two commonly advocated kidney function estimators of CKD in urban South Africans of mixed-ancestry.

METHODS

Study setting and population

The current study utilized data from the ongoing Cape Town Vascular and Metabolic Health (VMH) study, an extension of the Cape Town Bellville-South study, which has been described in detail previously [24]. Bellville-South, with a population of approximately 29,301, is a township formed in the late 1950s, located in the metropolitan city of Cape Town, South Africa. The population consists predominantly of individuals of mixed-ancestry (coloured) (76%), followed by black Africans (18.5%), with only 1.5% of the population being of Caucasian and Asians ancestry. The data collection for the current analysis took place between February 2015 and November 2016 during a community-based survey involving only mixed-ancestry South Africans. The study was approved by the Research Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003, respectively). The study was conducted in accordance with the Declaration of Helsinki. All participants voluntary signed written informed consent after all the procedures were fully explained in the language of their choice.

Participant involvement

The participants were not involved in the design or recruitment process of this study. However, permission to conduct the study was obtained from relevant authorities including the city and community authorities.

Questionnaires and physical examination

All interviews and physical examinations took place at a research clinic on the campus of Cape Peninsula University of Technology, located within the study suburb. All consenting participants received a standardized interview, explained in great detail elsewhere [25]. Physical examination involved blood pressure (BP) determination, measured according to the World Health Organisation (WHO) guidelines [26], using a semi-automatic digital blood pressure monitor (Omron M6 comfort-preformed cuff BP Monitor), placed on the right arm in sitting position and at rest for at least 10 min. Three measures were taken of which the average of the lowest two was used in all analyses. Body weight (to the nearest 0.1 kg) was measured with the participant in light clothing and without shoes, using an Omron body fat meter HBF-511 digital bathroom scale, which was calibrated and standardized using a weight of known mass. Height (to the nearest centimetre) was measured with a stadiometer, with subjects standing on a flat surface. Body mass index (BMI) was calculated as weight per square meter (kg/m^2). Waist circumference (WC) was measured with a non-elastic tape measure at the level of the narrowest part of the torso, as seen from the anterior view. Anthropometric measurements were performed three times and the average used for analysis.

Biochemical analysis and calculations

All biochemical analyses took place at an ISO 15189 accredited Pathology practice (Path-Care, Reference Laboratory, Cape Town, South Africa). Blood samples were collected from all participants after an overnight fast, and two hours after a 75g oral glucose tolerance test (OGTT) following the WHO recommendations [27]. Plasma glucose levels and haemoglobin A1c (HbA1c) were measured by enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa) and high performance liquid chromatography (Biorad Variant Turbo, BioRad, South Africa), respectively. Insulin was determined by a paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). Triglycerides (TG), total cholesterol (TC), and high-density lipoproteins (HDL-C) were analysed using the Roche Modular auto analyser and enzymatic colorimetric assays, and low-density lipoproteins (LDL-C) were calculated using the Friedewald formula [28]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: HOMA-IR = [fasting insulin concentration (mIU/l) × fasting plasma glucose (mmol/l)/22.5. Serum concentration of high sensitivity C-reactive protein (hsCRP) (Immun Diagnostik AG, Bensheim, Germany) was

analysed using commercially available ELISA kits according to the manufacturer's protocols. Serum creatinine was measured by the modified Jaffe-Kinetic method (Beckman AU, Beckman Coulter, South Africa). Creatinine assays at our Partner pathology service are standardized to the internationally accepted reference method (isotope dilution mass spectrophotometry [IDMS]) since 2009 and eGFR estimators applicable to standardised creatinine values were used. Kidney function was assessed using serum creatinine-based estimators of glomerular filtration rate (eGFR), namely, the 4-variable Modification of Diet in Renal Disease (MDRD) equation [29] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [30]. The African-American ethnicity correction factor was omitted from the eGFR calculation, as the South African Renal Society CKD guidelines promotes the exclusion of the correction factor, except in the case of black Africans. Full blood counts, including total RBC, total WBC, lymphocytes count and percentage, monocyte count and percentage, neutrophil count and percentage, basophil count and percentage, eosinophil count and percentage, haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width, and platelets, were measured on a Coulter LH 750 hematology analyzer (Beckman Coulter, South Africa).

Classification of renal function and co-morbidities

Staging of kidney function was based on the National Kidney Foundation Disease Outcomes Quality Initiative (NKF-KDOQI) classification [31]. An eGFR<60 ml/min/1.73 m^2 was used to define CKD (or CKD stage 3–5). Anaemia was defined using the National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines (haemoglobin level <13.5g/dL for men and <12g/dL for women) [32] and further classified into micro-, normo- and macrocytic based on the MCV. Microcytic anaemia was defined as an MCV of <80fL, normocytic as 100-80 fL, and macrocytic as >100fL [33]. Hypertension was based on either a history of diagnosed hypertension (receiving medications for hypertension) or screen-detected hypertension. The latter being classified if they had a SBP≥140mmHg and/or DBP≥90 mmHg [34]. Diabetes status was based on a history of diagnosed diabetes or screen-detected diabetes. OGTT glucose values were used to classify the glucose tolerance status of participants as recommended by WHO [35] as: (1) normal glucose tolerance (fasting plasma glucose (FPG) <6.1 mmol/l and 2-h glucose <7.8 mmol/l); (2) pre-diabetes including impaired fasting glycaemia (IGT, 6.1≤FPG< 7.0 mmol/l),

impaired glucose tolerance (IGT, 7.8<2-h glucose< 11.1 mmol/l) and the combination of both; and (3) diabetes (FPG≥7.0 mmol/l and/or 2-h glucose≥11.1 mmol/l). A BMI≥25kg/m^2 and BMI≥30kg/m^2 was classified as overweight obese, respectively.

Statistical analysis

All statistical analyses were performed using STATA version 13 (Statcorp, College Station, TX) and statistical significance was based on a p-value <0.05. General characteristics of the participants are summarized as count and percentage for qualitative variables and median and 25th-75th percentiles for quantitative variables. Group comparisons used chi-squared test for qualitative variables, and Wilcoxon rank-sum test for quantitative variables, respectively. Multiple linear regression models were used to assess the independent association between eGFR and haematological indices, while adjusting for age and gender.

RESULTS

Participant characteristics

The initial study sample comprised 1,647 participants. Of those, 83 were excluded due to missing data on serum creatinine or any of the variables required to estimate kidney function, including age and gender. The general characteristics and the haematological profile of the study population are summarised in Tables 1 and 2, respectively. The final sample included 1,564 participants, of which 24.9% were male, with a group median age of 50 years. The crude prevalence of CKD was 6% and 3%, based on the MDRD and CKD-EPI equations respectively. Of those participants with MDRD-diagnosed CKD, 80.7%, 14.8% and 4.5% where in stages 3, 4 and 5, respectively. Similarly, of those diagnosed by means of the CKD-EPI equation, 68.9%, 24.4% and 6.7% where in stages 3, 4 and 5, respectively. MDRD-diagnosed CKD participants had higher creatinine levels (111.5 vs. 59 μmol/l; p<0.0001) and lower eGFR (48.2 vs. 104 ml/min/1.73m^2; p<0.0001), were on average older (68 vs. 49 years; p<0.0001), with a higher WC (97.7 vs. 91.2 cm; p=0.0001), BMI (30.3 vs. 28.3 kg/m^2; p=0.0096), and SBP (142 vs. 125 mmHg; p<0.0001), compared to participants with normal kidney function. Furthermore, MDRD-diagnosed CKD participants had higher fasting and 2-hour blood glucose (5.3 vs. 5.0 mmol/l; p<0.0001 and 7.2 vs. 6.0 mmol/l; p<0.0001, respectively), HbA1c levels (6.2 vs. 5.7%; p<0.0001), fasting and 2-hour insulin levels (8.4 vs. 6.7 IU/l; p=0.0089 and 62.0 vs. 37.5 IU/l;

p=0.0002, respectively), higher HOMA-IR index (2.1 vs. 1.6; p=0.0004), hsCRP (4.7 vs. 4.0 µg/ml; p=0.0492), TG (1.6 vs. 1.2 mmol/l; p<0.0001) and TC (5.4 vs. 5.1 mmol/l; p=0.024); with similar LDL-C (3.2 vs. 3.1 mmol/l; p=0.0668) and HDL-C levels (1.3 vs. 1.3 mmol/l; p=0.7106) compared to those without CKD. When sub-dividing the groups based on CKD diagnosed by the CKD-EPI equation, similar differences were observed, with the exception of BMI, hsCRP and TC, which showed no difference between the groups (28.3 vs. 28.4 kg/m^2; p=0.384, 4.8 vs. 4.0 µg/ml; p=0.4268, 5.3 vs. 5.1 mmol/l; p=0.2226, respectively). Participants with reduced kidney function, both MDRD and CKD-EPI-diagnosed, had a similar prevalence of overweight and obesity, however had a higher prevalence of hypertension and T2D, despite similar prevalence of pre-diabetes (IFG and IGT) between the two groups.

The red blood cell indices, including RBC count, haematocrit and haemoglobin levels were consistently lower in CKD participants compared to the group with normal kidney function (all p<0.0001), irrespective of the eGFR equation used. Conversely, the morphology of the RBC's were not different, as similar values for MCV, MCH, MCHC and RDW were observed between CKD participants and the participants with normal kidney function. Despite not showing any significant difference in total WBC count between the two groups, the number of lymphocytes were lower and neutrophil count and the ratio of lymphocytes to neutrophil higher in the CKD group compared to those individuals with normal kidney function; with the remaining WBC indices similar in the two groups. The platelet count was similar in both groups. Furthermore, based on the K/DOQI guidelines, 45.5% (MDRD) and 57.8% (CKD-EPI) of the CKD participants had anaemia, with the majority of cases being normocytic. Moreover, the prevalence of anaemia increased with increasing severity of CKD, from 37.2% at stage 3 to 82.4% at stage 4-5.

Table 1: Clinical characteristics of the study population overall and by CKD (MDRD and CKD-EPI) status

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
Age (years)	50 (37-61)	49 (36-59)	68 (62-74)	<0.0001	50 (36-60)	69 (63-77)	<0.0001
Gender (n,% male)	389 (24.9)	372 (25.3)	17 (18.1)	0.215	373 (24.6)	16 (34.0)	0.093
Anthropometry							
Weight (kg)	72.0 (59.2-85.5)	71.9 (59.0-85.5)	74.0 (64.6-85.8)	0.2058	72.0 (59.2-85.5)	73.5 (64.1-85.7)	0.6903
WC (cm)	91.8 (78.5-103.5)	91.2 (77.8-103.0)	97.7 (89.0-105.8)	0.0001	91.5 (78.1-103.5)	96.0 (87.8-106.5)	0.0225
HC (cm)	102.8 (92.5-113.5)	102.5 (92.1-113.5)	104.3 (96.5-114.2)	0.1138	102.8 (92.5-113.8)	101.5 (95.8-111.5)	0.9439
BMI (kg/m^2)	28.4 (22.9-34.2)	28.3 (22.7-34.1)	30.3 (26.1-35.1)	0.0096	28.4 (22.9-34.2)	28.3 (24.7-34.4)	0.3836
Biochemical analysis							
Fasting blood glucose (mmol/l)	5.0 (4.6-5.7)	5.0 (4.6-5.6)	5.3 (5.0-6.9)	<0.0001	5.0 (4.6-5.6)	5.3 (5.0-7.7)	0.0014
2-hour glucose (mmol/l)	6.0 (4.9-7.6)	6.0 (4.8-7.5)	7.2 (5.8-9.2)	<0.0001	6.0 (4.8-7.5)	7.5 (5.7-9.2)	0.0034
HbA1c (%)	5.8 (5.4-6.3)	5.7 (5.4-6.2)	6.2 (5.9-7.1)	<0.0001	5.8 (5.4-6.2)	6.4 (5.9-7.3)	<0.0001
Fasting insulin (IU/l)	6.7 (4.3-11.1)	6.7 (4.2-10.9)	8.4 (5.3-12.4)	0.0089	6.7 (4.2-10.9)	9.0 (5.3-12.4)	0.0323
2-hour insulin (IU/l)	38 (20.6-71.8)	37.5 (19.8-69.8)	62.0 (30.3-105.6)	0.0002	37.8 (20.3-70.5)	63.5 (32.6-105.2)	0.0072
HOMA-IR (MU)	1.6 (0.9-2.9)	1.6 (0.9-2.8)	2.1 (1.2-3.9)	0.0004	1.6 (0.9-2.8)	2.4 (1.3-3.8)	0.0026
hsCRP (µg/ml)	4.0 (1.6-8.8)	4.0 (1.6-8.8)	4.7 (2.7-9.3)	0.0492	4.0 (1.6-8.8)	4.8 (2.4-7.5)	0.4268
TG (mmol/l)	1.2 (0.9-1.7)	1.2 (0.9-1.7)	1.6 (1.2-2.3)	<0.0001	1.2 (0.9-1.7)	1.8 (1.1-2.4)	0.0001
TC (mmol/l)	5.1 (4.4-5.9)	5.1 (4.3-5.9)	5.4 (4.8-6.4)	0.0024	5.1 (4.4-5.9)	5.3 (4.4-6.0)	0.2226
LDL-C (mmol/l)	3.1 (2.5-3.8)	3.1 (2.5-3.8)	3.2 (2.7-4.3)	0.0668	3.1 (2.5-3.8)	3.1 (2.5-3.9)	0.9444
HDL-C (mmol/l)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	0.7106	1.3 (1.1-1.5)	1.3 (1.1-1.4)	0.5132
Creatinine (µmol/l)	60 (52-70)	59 (51-68)	111.5 (89.0-140.5)	<0.0001	59 (51-69)	140 (124-209)	<0.0001
eGFR ($ml/min/1.73m^2$)	-	104.0 (88.0-121.0)	48.2 (33.7-55.4)	<0.0001	113.9 (101.4-126.5)	44.7 (26.4-49.6)	<0.0001

Blood pressure measures							
Mean SBP (mmHg)	125 (111-141)	125 (110-140)	142 (121-162)	<0.0001	125 (111-140)	150 (128-181)	<0.0001
Mean DBP (mmHg)	81 (72-90)	81 (72-90)	81 (74-95)	0.2114	81 (72-90)	85 (73-95)	0.2185
Pulse pressure (BPM)	70 (62-79)	70 (62-79)	70 (60-81)	0.9932	70 (62-79)	73 (62-82)	0.3861
Co-morbidities							
Overweight (BMI≥25kg/m^2; n.%)	361 (23.2)	335 (22.9)	26 (29.5)	0.139	348 (23.1)	13 (28.9)	0.348
Obese (BMI≥30kg/m^2; n,%)	662 (42.6)	617 (42.1)	45 (51.1)	0.085	642 (42.5)	20 (44.4)	0.771
Pre-diabetes (n, %)	238 (15.2)	226 (15.4)	12 (12.8)	0.671	233 (15.4)	5 (10.6)	0.436
T2D (n, %)	297 (19.0)	259 (17.6)	38 (40.4)	<0.0001	272 (17.9)	25 (53.2)	<0.0001
Hypertension (n, %)	567 (36.3)	517 (35.2)	50 (53.2)	<0.0001	537 (35.4)	30 (63.3)	<0.0001

Data is presented as median (25th-75th percentiles) and percentages. WC, waist circumference; HC, hip circumference; BMI, body mass index; HbA1c, Glycated haemoglobin; HOMA-IR, Homeostatic model assessment-insulin resistance; MU, mass units; hsCRP, high sensitivity C-reactive protein; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoproteins; HDL-C, high-density lipoproteins; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; IFG/IGT, impaired fasting glucose and impaired glucose tolerance; T2D, type 2 diabetes mellitus; MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKD, chronic kidney disease.

Table 2: Haematological profile of study population overall and by CKD (MDRD and CKD-EPI) status

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
RBC ($x10^6$/µl)	4.7 (4.3-5.0)	4.7 (4.4-5.0)	4.3 (3.9-4.7)	<0.0001	4.7 (4.4-5.0)	4.2 (3.8-4.7)	<0.0001
WBC ($x10^6$/µl)	7.5 (6.2-9.1)	7.4 (6.2-9.1)	7.7 (6.5-9.2)	0.5704	7.4 (6.2-9.1)	7.9 (6.3-9.3)	0.5458
N/L (ratio)	2.0 (1.5-2.6)	1.9 (1.5-2.5)	2.5 (1.7-3.5)	<0.0001	1.9 (1.5-2.5)	2.7 (2.0-3.7)	<0.0001
Lymphocyte count ($x10^9$/l)	2.2 (1.8-2.80)	2.2 (1.8-2.8)	1.9 (1.4-2.5)	0.0001	2.2 (1.8-2.8)	1.8 (1.4-2.4)	<0.0001
Monocyte count ($x10^9$/l)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.1389	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.9446
Neutrophil count ($x10^9$/l)	4.5 (3.4-5.7)	4.5 (3.3-5.6)	5.0 (3.7-5.9)	0.0255	4.5 (3.4-5.6)	5.1 (4.3-6.1)	0.0297
Basophil count ($x10^9$/l)	0.1 (0.1-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.283	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.1366
Eosinophil count ($x10^9$/l)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1579	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1223
Platelet count ($x10^9$/l)	271 (227-322)	271 (228-322)	277 (214-324)	0.9417	271 (228-322)	266 (197-313)	0.2211
Haematocrit (volume %)	41 (39-44)	41 (39-44)	38 (35-41)	<0.0001	41 (39-44)	37 (34-41)	<0.0001
MCV (fl/cell)	89 (85-93)	89 (85-93)	89 (86-92)	0.8150	89 (85-93)	89 (86-91)	0.4748
MCH (pg/cell)	29 (28-31)	29 (28-31)	29 (28-30)	0.1399	29 (28-31)	29 (28-30)	0.057
MCHC (g/dl)	33 (32-33)	33 (32-33)	33 (32-33)	0.1471	33 (32-33)	32 (32-33)	0.1156
RDW (%)	14.2 (13.5-15.0)	14.1 (13.4-15.0)	14.5 (13.7-15.6)	0.0601	14.1 (13.4-15.0)	14.3 (13.8-15.5)	0.0673
Hb (g/dl)	13.5 (12.6-14.4)	13.5 (12.7-14.5)	12.2 (11.2-13.3)	<0.0001	13.5 (12.6-14.4)	11.9 (11.1-13.2)	<0.0001
Anaemia (n, %)	289 (18.48)	249 (16.9)	40 (45.5)	<0.0001	263 (17.3)	26 (57.8)	<0.0001
Microcytic	83 (28.7)	83 (33.3)	0 (0.0)	-	83 (31.6)	0 (0.0)	-
Normocytic	180 (62.3)	141 (56.6)	39 (97.5)	-	155 (58.9)	25 (96.2)	-
Macrocytic	26 (9.0)	25 (10.0)	1 (2.5)	-	25 (9.5)	1 (3.8)	-

Data are presented as median (25th-75th percentiles) and percentages. RBC, red blood cells; WBC, white blood cells; N/L ratio, lymphocyte to neutrophil ratio; MCV, mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular

haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Association between the different haematological indices and eGFR

The age and gender-adjusted associations between the different haematological indices and eGFR, estimated by means of the MDRD and CKD-EPI equations, are presented in Table 3. Based on the CKD-EPI, however not the MDRD equation, eGFR was positively associated with all the RBC indices, including total RBC count, haemoglobin and haematocrit levels. eGFR was not associated with total WBC count, however a lower lymphocyte count was associated with a lower eGFR and N/L ratio was inversely associated with eGFR. Furthermore, male gender was significantly associated with all haematological measures, except basophil count and eosinophil count, and age was inversely associated with all RBC indices, lymphocytes, neutrophils, platelet count, MCHC and positively associated with RDW.

Table 3: Linear regression coefficients, adjusted for age, gender (Model 1) and eGFR (MDRD and CKD-EPI-derived) (Models 2) for the prediction of haematological-derived measures

Haematological-derived measures	MODEL 1							MODEL 2.1				MODEL 2.2			
	Age			Gender			R^2	eGFR (MDRD)			R^2	eGFR (CKD-EPI)			R^2
	β	95% CI	p	β	95% CI	p		β	95% CI	p		β	95% CI	p	
RBC (x10^3/µl)	-2.8	-4.5 to -1.2	0.001	327.4	269.6 to 385.3	<0.0001	0.08	0.3	-0.7 to 1.3	0.541	0.08	3.2	1.5 to 5.0	<0.0001	0.09
Haematocrit (%)	-0.2	-0.3 to -0.0	0.018	40.2	35.3 to 45.1	<0.0001	0.15	0.0	-0.1 to 0.1	0.709	0.15	0.3	0.1 to 0.4	<0.0001	0.16
Hb (g/l)	-0.1	-0.1 to -0.0	0.002	14.2	12.5 to 15.9	<0.0001	0.16	0.0	-0.0 to 0.0	0.907	0.16	0.1	0.0 to 0.1	<0.0001	0.16
WBC (x10^3/µl)	-15.1	-22.3 to -7.8	<0.0001	-431.9	-690.8 to -173.0	0.001	0.01	-0.5	-4.8 to 3.9	0.834	0.01	-1.7	-9.7 to 6.3	0.678	0.01
N/L (%)	-0.1	-3.8 to 3.5	0.941	136.2	5.6 to 266.7	0.041	0.00	-0.1	-0.4 to 0.1	0.214	0.00	-6.3	-10.3 to -2.3	0.002	0.01
Lymphocyte count (x10^6/l)	-2.9	-5.2 to -0.5	0.017	-257.10	-341.0 to -173.2	<0.0001	0.02	0.7	-0.8 to 2.1	0.364	0.02	3.0	0.4 to 5.6	0.022	0.03
Monocyte count (x10^6/l)	-0.8	-1.4 to -0.2	0.005	91.6	71.2 to 112.0	<0.0001	0.05	0.3	-0.1 to 0.6	0.114	0.05	0.5	-0.1 to 1.1	0.122	0.05
Neutrophil count (x10^6/l)	-10.9	-16.8 to -5.1	<0.0001	-291.8	-500 to -82.8	0.006	0.01	-1.1	-4.6 to 2.4	0.542	0.01	-4.7	-11.1 to 1.7	0.150	0.01
Basophil count (x10^6/l)	1.6	-8.4 to 11.5	0.759	-187.9	-541.9 to 166.1	0.298	0.00	0.7	-5.3 to 6.6	0.822	0.00	-8.3	-19.2 to 2.6	0.136	0.00
Eosinophil count (x10^6/l)	-0.5	-1.1 to 0.0	0.067	15.9	-4.9 to 36.7	0.135	0.00	-0.4	-0.7 to 0.0	0.071	0.00	-0.6	-1.2 to 0.1	0.074	0.00
Platelet count (x10^9/l)	-0.4	-0.6 to -0.1	0.003	-33.0	-42.0 to -24.0	<0.0001	0.03	0.1	-0.0 to 0.3	0.088	0.04	0.1	-0.0 to 0.3	0.088	0.04
MCV (fL/100cell)	1.4	-1.0 to 3.7	0.255	232.2	148.1 to 316.2	<0.0001	0.02	-0.2	-1.6 to 1.2	0.761	0.02	0.1	-2.5 to 2.7	0.946	0.02
MCH (pg/100cell)	-0.2	-1.1 to 0.7	0.698	95.3	63.3 to 127.4	<0.0001	0.02	-0.1	-0.7 to 0.4	0.646	0.02	0.1	-0.9 to 1.1	0.881	0.02
MCHC (g/l)	-0.1	-0.01 to -0.0	<0.0001	2.3	0.9 to 3.8	0.002	0.02	-0.0	-0.0 to 0.0	0.227	0.02	-0.0	-0.1 to 0.0	0.664	0.01
RDW (%)	0.1	0.0 to 0.1	0.004	-1.9	-3.7 to -0.0	0.05	0.01	0.1	0.0 to 0.1	<0.0001	0.02	0.1	0.0 to 0.1	0.025	0.01

Data presented as β-coefficient, 95% confidence interval and adjusted-R^2. Analysis are adjusted for age and gender. RBC, red blood cells; WBC, white blood cells; MCV, L/N ratio, lymphocyte to neutrophil ratio; mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease. Model 1 = age + gender; Model 2.1 = age + gender + eGFR (MDRD); Model 2.2 = age + gender + eGFR (CKD-EPI)

DISCUSSION

In this community-based sample of mixed-ancestry South Africans, we have shown that the haematological profile of individuals with reduced eGFR (<60ml/min/1.73m^2) are substantially impaired compared to those with normal kidney function, giving rise to the high prevalence of anaemia in this screen-detected CKD population. Furthermore, despite eGFR being positively associated with RBC indices, indicative of the severity of kidney function impairment, the disease state had no effect on the morphology of the RBC. Lastly, we confirmed that a chronic pro-inflammatory state exists in participants with CKD.

This study, which is in accordance with other studies in Africa and other developing countries [36-42], has shown that CKD is associated with significant impairment in RBC indices. Indeed, we have shown that total RBC count, haemoglobin concentration and percentage haematocrit were substantially reduced in participants with eGFR below 60ml/min/1.73m^2, compared to those with normal kidney function, independent of age and gender. Since erythropoietin is produced mainly by the proximal tubule of the nephron, kidney function decline will result in a decline in erythropoietin production and as a consequence result in decreased haemoglobin synthesis, leading to a fall in total RBC count [17]. This significant reduction in RBC, inevitably gives rise to anaemia [14]. Indeed, our study and numerous other studies have shown that the severity of anaemia increases with disease progression; with most of these studies showing anaemia at least twice as prevalent in participants with CKD, compared to the general adult population [37]. Furthermore, we found that 17% of the sample population with normal kidney function had haemoglobin levels <13.5g/dL and <12g/dL for men and women, respectively. However, this is not uncommon in Africa as previous studies have found that Africa has a high prevalence of anaemia caused by iron deficiency. In South Africa in particular, the South African National Health and Nutrition Examination Survey (SANHANES-1) [43] showed that 22% and 12.2% of adult females and males have anaemia.

The activation of the immune system, caused by inflammation, increases white blood cell counts [23]; emphasising the potential of WBC indices as a surrogate marker of inflammation in CKD [20]. Our study showed that despite no correlation between total WBC and reduced kidney function, CKD was associated with higher neutrophil and lower lymphocyte counts; both of which are

independently associated with the promotion of atherosclerosis [44 45] and poor cardiovascular outcomes [46]. N/L ratio, which combines the predictive power of both increased neutrophil count and decreased lymphocyte count [47], was associated with reduced eGFR in our study, as also found in other studies [23 48 49]. Indeed previous studies, which included CKD patients on haemodialysis [23 48] and pre-dialysis [49], showed that an increased N/L ratio was associated with known inflammatory markers such as tumor necrosis factor (TNF)-α [23], interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) levels [49]. These studies demonstrated that these well-established markers of inflammation were independent factors for predicting N/L ratio, thus presenting N/L ratio as an inflammatory biomarker for CKD patients. Since full blood count analysis are done routinely and a relatively affordable and easy measure to acquire, these findings are especially valuable taking into account the severely resource limited setting found in Africa and other low and middle-income countries.

Our study has a few limitations. This study was conducted in only one geographical area, which may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa. Furthermore, this was a community-based sample with high female to male participation, however the latter being a common trend in South African population studies. Our study also used a single serum creatinine measure to determine the grade of kidney function and did not include estimates of albuminuria. Albuminuria, in particular, is required for clinical and aetiological diagnosis of CKD, as this information is important particularly in the interpretation of eGFR greater that 60ml/min/1.73m^2 where inaccuracies of the eGFR equations are greatest. It is however a common practice in community-based studies to diagnose CKD using a single measurement of serum creatinine. Furthermore, we did not investigate other haematinic deficiencies, such as vitamin B12 and iron deficiencies, which if present however, are less likely to affect haematological profile in a differential way in people with and without CKD. However, despite these limitations, we are not aware of other studies that have assessed the haematological profile of individuals with reduced kidney function in a population-based setting in Africa, even more specific, individuals of mixed-ancestry. Furthermore, we studied a community with a high burden of obesity, hypertension and diabetes, reflective of the current burden in Africa. This study provides much needed evidence for the association between the haematological profile and

CKD as population-based data on the haematological profile of people with CKD in Africa, are very limited.

In conclusion, the findings from our study are valuable as full blood count analyses are done routinely and are relatively affordable, taking into account the severely resource limited setting found in Africa and other low and middle-income countries. Furthermore, though it still remains unclear whether the advocated kidney function estimators provide accurate estimates of CKD burden in African populations [50], the correlation of these estimates, with deteriorating profile of blood cell counts, suggests that these advocated GFR estimates, particularly the CKD-EPI equation, to some extent, measure kidney function in African populations.

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Research Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003, respectively). The study was conducted in accordance with the Declaration of Helsinki. All participants voluntary signed written informed consent after all the procedures were fully explained in the language of their choice. Permission to conduct the study was also obtained from relevant authorities including the city and community authorities.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests

Funding

The South African Medical Research Council (SAMRC) funded this research project with funds from National Treasury under its Economic Competitiveness and Support Package (MRC-RFA-UFSP-01-2013/VMH Study).

Authors' contribution

Study conception and funding acquisition (TEM, APK, RTE), operationalization and supervision of the data collection (TEM), data analysis and interpretation (CG, APK), drafting the manuscript (CG, APK), critical revision of the manuscript and approval of the final version (all co-authors).

Acknowledgements

We are grateful to the Cape Town VMH study investigation team and population of Bellville-South for their participation.

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BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025694.R1
Article Type:	Research
Date Submitted by the Author:	10-Sep-2018
Complete List of Authors:	George, Cindy; South African Medical Research Council, Non-Communicable Diseases Research Unit Matsha, Tandi; Cape Peninsula University of Technology, Department of Biomedical Sciences Erasmus, Rajiv; University of Stellenbosch, Chemical Pathology Kengne , AP; South African Medical Research Council, Non-Communicable Diseases Research Unit; University of Cape Town
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Renal medicine
Keywords:	Africa, HAEMATOLOGY, Chronic Kidney Disease

HAEMATOLOGICAL PROFILE OF CHRONIC KIDNEY DISEASE IN A MIXED-ANCESTRY SOUTH AFRICAN POPULATION: A CROSS-SECTIONAL STUDY

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Word count: 3085

Abstract word count: 294

References: 50

Tables: 3

ABSTRACT

Objectives: The objectives were to characterise the haematological profile of screen-detected chronic kidney disease (CKD) participants and to correlate the complete blood count measures with the commonly advocated kidney function estimators. **Methods:** The current cross-sectional study utilized data, collected between February 2015 and November 2016, of 1564 adults of mixed-ancestry, who participated in the Cape Town Vascular and Metabolic Health study. Kidney function was estimated using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. CKD was defined as eGFR <60ml/min/1.73m^2, and anaemia as haemoglobin level <13.5g/dL (men) and <12g/dL (women). **Results:** Based on the MDRD and CKD-EPI equations, the crude prevalence of CKD was 6% and 3%. Irrespective of the equation used, median red blood cell (RBC) indices were consistently lower in those with CKD compared to those without CKD (all p<0.0001). Despite not showing any significant difference in total white blood cell (WBC) count between the two groups, the number of lymphocytes were lower (p=0.0001 and p<0.0001 for MDRD and CKD-EPI, respectively) and neutrophil count (both p≤0.0297) and the ratio of lymphocytes to neutrophil (both p<0.0001) higher in the CKD group compared to those without CKD; with the remaining WBC indices similar in the two groups. The platelet count was similar in both groups. Of the screen-detected CKD participants, 45.5% (MDRD) and 57.8% (CKD-EPI) were anaemic, with the prevalence increasing with increasing severity of CKD, from 37.2% (stage 3) to 82.4% (stages 4-5). Furthermore, CKD-EPI-estimated kidney function, but not MDRD, was positively associated with RBC indices. **Conclusion:** Though it remains unclear whether common kidney function estimators provide accurate estimates of CKD in Africans, the correlation of their estimates with deteriorating RBC profile, suggests that advocated estimators, to some extent approximate kidney function in African populations.

Key words: chronic kidney disease; haematology; Africa

Strengths and limitations of the study

- The first study to characterize the haematological profile of individuals with reduced kidney function in a population-based setting in Africa, even more specific, individuals of mixed-ancestry
- We studied a community with a high burden of obesity, hypertension and diabetes, reflective of the current burden in Africa.
- This study was conducted in only one geographical area, which may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa.
- Our study was based on a single serum creatinine measure to determine CKD and did not include estimates of albuminuria. Albuminuria, which are required for clinical and aetiological diagnosis of CKD, as this information is important particularly in the interpretation of eGFR greater that 60ml/min/1.73m^2 where inaccuracies of the eGFR equations are greatest

BACKGROUND

Chronic kidney disease (CKD) is a major global public health problem [1], estimated to affect more than 10% of the general adult population and up to 50% of some high-risk subpopulations, such as the elderly [2], those with non-communicable diseases (NCD), including type 2 diabetes mellitus (T2D) and hypertension, and communicable diseases (CD), including human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) [3 4]. Africa is currently experiencing the double burden of NCDs and CDs, which are all driving the increasing burden of CKD on the continent [5]. However, the exact burden of CKD in Africa has yet to be fully elucidated [6-9], in part due to the absence of appropriate estimates for predicting reduced kidney function in individuals from African ancestry [9 10].

CKD encompasses a wide range of physiological processes altered by the progressive decline in glomerular filtration rate (GFR) [11 12]. Haematological parameters, particularly red blood cell (RBC) indices, are most commonly affected [13], giving rise to anaemia. Anaemia is the most common, consistent and severe of the various haematological abnormalities, and has been shown to be a very common condition in black Africans [14]. Although anaemia may be found at any stage of CKD, the severity of anaemia increases with CKD progression [15], resultantly affecting nearly all patients with end-stage renal disease (CKD stage 5) [13]. The predominant cause of anaemia in CKD is failure of the kidneys to produce enough endogenous erythropoietin, which accompanies the fall in GFR [16 17]. Untreated, prolonged anaemia is strongly predictive of all-cause and cardiovascular mortality, as well as reduced quality of life and increased morbidity in patients with CKD [13 18]. Untreated anaemia can also accelerate the decline in renal function by causing renal haemodynamic alterations and tissue hypoxia [15]. Other potentially affected haematological parameters in CKD, of which the association with CKD is not yet fully characterized, include total and differential white blood cell (WBC) counts. Persistent, low-grade inflammation is an essential part of the aetiology of CKD and has been recognized as such since the late 1990s, when it was linked to cardiovascular disease (CVD) and mortality [19]. Recently, the ratio of neutrophil-to-lymphocyte count (N/L) has been proposed as a novel measure of inflammation in distinct populations and has been shown to have prognostic value [20]; particularly for mortality risk in patients with myocardial infarction and heart failure [21 22]. However, studies on the relationship of N/L ratio with reduced eGFR are limited [23]. Thus, despite recent advances in the aetiology

governing the development and progression of CKD, population-based data on the haematological profile of people with CKD in Africa, are scanty.

We therefore aimed to characterise the haematological profile of screen-detected CKD participants in a community-based sample, and to correlate the complete blood count measures with two commonly advocated kidney function estimators of CKD in urban South Africans of mixed-ancestry.

METHODS

Study setting and population

The current study utilized data from the ongoing Cape Town Vascular and Metabolic Health (VMH) study, an extension of the Cape Town Bellville-South study, which has been described in detail previously [24]. Bellville-South, with a population of approximately 29,301, is a township formed in the late 1950s, located in the metropolitan city of Cape Town, South Africa. The population consists predominantly of individuals of mixed-ancestry (coloured) (76%), followed by black Africans (18.5%), with only 1.5% of the population being of Caucasian and Asians ancestry. The data collection for the current analysis took place between February 2015 and November 2016 during a community-based survey involving only mixed-ancestry South Africans. The study was approved by the Research Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003, respectively). The study was conducted in accordance with the Declaration of Helsinki. All participants voluntary signed written informed consent after all the procedures were fully explained in the language of their choice.

Participant involvement

The participants were not involved in the design or recruitment process of this study. However, permission to conduct the study was obtained from relevant authorities including the city and community authorities.

Questionnaires and physical examination

All interviews and physical examinations took place at a research clinic on the campus of Cape Peninsula University of Technology, located within the study suburb. All consenting participants received a standardized interview, explained in great detail elsewhere [25]. Physical examination involved blood pressure (BP) determination, measured according to the World Health Organisation (WHO) guidelines [26], using a semi-automatic digital blood pressure monitor (Omron M6 comfort-preformed cuff BP Monitor), placed on the right arm in sitting position and at rest for at least 10 min. Three measures were taken of which the average of the lowest two was used in all analyses. Body weight (to the nearest 0.1 kg) was measured with the participant in light clothing and without shoes, using an Omron body fat meter HBF-511 digital bathroom scale, which was calibrated and standardized using a weight of known mass. Height (to the nearest centimetre) was measured with a stadiometer, with subjects standing on a flat surface. Body mass index (BMI) was calculated as weight per square meter (kg/m^2). Waist circumference (WC) was measured with a non-elastic tape measure at the level of the narrowest part of the torso, as seen from the anterior view. Anthropometric measurements were performed three times and the average used for analysis.

Biochemical analysis and calculations

All biochemical analyses took place at an ISO 15189 accredited Pathology practice (Path-Care, Reference Laboratory, Cape Town, South Africa). Blood samples were collected from all participants after an overnight fast, and two hours after a 75g oral glucose tolerance test (OGTT) following the WHO recommendations [27]. Plasma glucose levels and haemoglobin A1c (HbA1c) were measured by enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa) and high performance liquid chromatography (Biorad Variant Turbo, BioRad, South Africa), respectively. Insulin was determined by a paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). Triglycerides (TG), total cholesterol (TC), and high-density lipoproteins (HDL-C) were analysed using the Roche Modular auto analyser and enzymatic colorimetric assays, and low-density lipoproteins (LDL-C) were calculated using the Friedewald formula [28]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: HOMA-IR = [fasting insulin concentration (mIU/l) × fasting plasma glucose (mmol/l)/22.5. Serum concentration of high sensitivity C-reactive protein (hsCRP) (Immun Diagnostik AG, Bensheim, Germany) was analysed using commercially available ELISA

kits according to the manufacturer's protocols. Serum creatinine was measured by the modified Jaffe-Kinetic method (Beckman AU, Beckman Coulter, South Africa). Creatinine assays at our Partner pathology service are standardized to the internationally accepted reference method (isotope dilution mass spectrophotometry [IDMS]) since 2009 and eGFR estimators applicable to standardised creatinine values were used. Kidney function was assessed using serum creatinine-based estimators of glomerular filtration rate (eGFR), namely, the 4-variable Modification of Diet in Renal Disease (MDRD) equation [29] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [30]. The African-American ethnicity correction factor was omitted from the eGFR calculation, as the South African Renal Society CKD guidelines promotes the exclusion of the correction factor, except in the case of black Africans. Full blood counts, including total RBC, total WBC, lymphocytes count and percentage, monocyte count and percentage, neutrophil count and percentage, basophil count and percentage, eosinophil count and percentage, haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width, and platelets, were measured on a Coulter LH 750 hematology analyzer (Beckman Coulter, South Africa).

Classification of renal function and co-morbidities

Staging of kidney function was based on the National Kidney Foundation Disease Outcomes Quality Initiative (NKF-KDOQI) classification [31]. An eGFR<60 ml/min/1.73 m^2 was used to define CKD (or CKD stage 3–5). Anaemia was defined using the National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines (haemoglobin level <13.5g/dL for men and <12g/dL for women) [32] and further classified into micro-, normo- and macrocytic based on the MCV. Microcytic anaemia was defined as an MCV of <80fL, normocytic as 100-80 fL, and macrocytic as >100fL [33]. Hypertension was based on either a history of diagnosed hypertension (receiving medications for hypertension) or screen-detected hypertension. The latter being classified if they had a SBP≥140mmHg and/or DBP≥90 mmHg [34]. Diabetes status was based on a history of diagnosed diabetes or screen-detected diabetes. OGTT glucose values were used to classify the glucose tolerance status of participants as recommended by WHO [35] as: (1) normal glucose tolerance (fasting plasma glucose (FPG) <6.1 mmol/l and 2-h glucose <7.8 mmol/l); (2) pre-diabetes including impaired fasting glycaemia (IGT, 6.1≤FPG< 7.0 mmol/l), impaired glucose

tolerance (IGT, 7.8<2-h glucose< 11.1 mmol/l) and the combination of both; and (3) diabetes (FPG≥7.0 mmol/l and/or 2-h glucose≥11.1 mmol/l). A BMI≥25kg/m^2 and BMI≥30kg/m^2 was classified as overweight obese, respectively.

Statistical analysis

All statistical analyses were performed using STATA version 13 (Statcorp, College Station, TX) and statistical significance was based on a p-value <0.05. General characteristics of the participants are summarized as count and percentage for qualitative variables and median and 25th-75th percentiles for quantitative variables. Group comparisons used chi-squared test for qualitative variables, and Wilcoxon rank-sum test for quantitative variables, respectively. Multiple linear regression models were used to assess the independent association between eGFR and haematological indices, while adjusting for age and gender.

RESULTS

Participant characteristics

The initial study sample comprised 1,647 participants. Of those, 83 were excluded due to missing data on serum creatinine or any of the variables required to estimate kidney function, including age and gender. The general characteristics and the haematological profile of the study population are summarised in Tables 1 and 2, respectively. The final sample included 1,564 participants, of which 24.9% were male, with a group median age of 50 years. The crude prevalence of CKD was 6% and 3%, based on the MDRD and CKD-EPI equations respectively. Of those participants with MDRD-diagnosed CKD, 80.7%, 14.8% and 4.5% where in stages 3, 4 and 5, respectively. Similarly, of those diagnosed by means of the CKD-EPI equation, 68.9%, 24.4% and 6.7% where in stages 3, 4 and 5, respectively. MDRD-diagnosed CKD participants had higher creatinine levels (111.5 vs. 59 µmol/l; p<0.0001) and lower eGFR (48.2 vs. 104 ml/min/1.73m^2; p<0.0001), were on average older (68 vs. 49 years; p<0.0001), with a higher WC (97.7 vs. 91.2 cm; p=0.0001), BMI (30.3 vs. 28.3 kg/m^2; p=0.0096), and SBP (142 vs. 125 mmHg; p<0.0001), compared to participants with normal kidney function. Furthermore, MDRD-diagnosed CKD participants had higher fasting and 2-hour blood glucose (5.3 vs. 5.0 mmol/l; p<0.0001 and 7.2 vs. 6.0 mmol/l; p<0.0001, respectively), HbA1c levels (6.2 vs. 5.7%; p<0.0001), fasting and 2-hour insulin levels (8.4 vs. 6.7 IU/l; p=0.0089 and 62.0 vs. 37.5 IU/l; p=0.0002, respectively), higher HOMA-IR index

(2.1 vs. 1.6; p=0.0004), hsCRP (4.7 vs. 4.0 µg/ml; p=0.0492), TG (1.6 vs. 1.2 mmol/l; p<0.0001) and TC (5.4 vs. 5.1 mmol/l; p=0.024); with similar LDL-C (3.2 vs. 3.1 mmol/l; p=0.0668) and HDL-C levels (1.3 vs. 1.3 mmol/l; p=0.7106) compared to those without CKD. When sub-dividing the groups based on CKD diagnosed by the CKD-EPI equation, similar differences were observed, with the exception of BMI, hsCRP and TC, which showed no difference between the groups (28.3 vs. 28.4 kg/m^2; p=0.384, 4.8 vs. 4.0 µg/ml; p=0.4268, 5.3 vs. 5.1 mmol/l; p=0.2226, respectively). Participants with reduced kidney function, both MDRD and CKD-EPI-diagnosed, had a similar prevalence of overweight and obesity, however had a higher prevalence of hypertension and T2D, despite similar prevalence of pre-diabetes (IFG and IGT) between the two groups.

The red blood cell indices, including RBC count, haematocrit and haemoglobin levels were consistently lower in CKD participants compared to the group with normal kidney function (all p<0.0001), irrespective of the eGFR equation used. Conversely, the morphology of the RBC's were not different, as similar values for MCV, MCH, MCHC and RDW were observed between CKD participants and the participants with normal kidney function. Despite not showing any significant difference in total WBC count between the two groups, the number of lymphocytes were lower and neutrophil count and the ratio of lymphocytes to neutrophil higher in the CKD group compared to those individuals with normal kidney function; with the remaining WBC indices similar in the two groups. The platelet count was similar in both groups. Furthermore, based on the K/DOQI guidelines, 45.5% (MDRD) and 57.8% (CKD-EPI) of the CKD participants had anaemia, with the majority of cases being normocytic. Moreover, the prevalence of anaemia increased with increasing severity of CKD, from 37.2% at stage 3 to 82.4% at stage 4-5.

Table 1: Clinical characteristics of the study population overall and by CKD (MDRD and CKD-EPI) status

Variables		MDRD			CKD-EPI		
	Total (n=1564)	Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
Age (years)	50 (37-61)	49 (36-59)	68 (62-74)	<0.0001	50 (36-60)	69 (63-77)	<0.0001
Gender (n,% male)	389 (24.9)	372 (25.3)	17 (18.1)	0.215	373 (24.6)	16 (34.0)	0.093
Anthropometry							
Weight (kg)	72.0 (59.2-85.5)	71.9 (59.0-85.5)	74.0 (64.6-85.8)	0.2058	72.0 (59.2-85.5)	73.5 (64.1-85.7)	0.6903
WC (cm)	91.8 (78.5-103.5)	91.2 (77.8-103.0)	97.7 (89.0-105.8)	0.0001	91.5 (78.1-103.5)	96.0 (87.8-106.5)	0.0225
HC (cm)	102.8 (92.5-113.5)	102.5 (92.1-113.5)	104.3 (96.5-114.2)	0.1138	102.8 (92.5-113.8)	101.5 (95.8-111.5)	0.9439
BMI (kg/m^2)	28.4 (22.9-34.2)	28.3 (22.7-34.1)	30.3 (26.1-35.1)	0.0096	28.4 (22.9-34.2)	28.3 (24.7-34.4)	0.3836
Biochemical analysis							
Fasting blood glucose (mmol/l)	5.0 (4.6-5.7)	5.0 (4.6-5.6)	5.3 (5.0-6.9)	<0.0001	5.0 (4.6-5.6)	5.3 (5.0-7.7)	0.0014
2-hour glucose (mmol/l)	6.0 (4.9-7.6)	6.0 (4.8-7.5)	7.2 (5.8-9.2)	<0.0001	6.0 (4.8-7.5)	7.5 (5.7-9.2)	0.0034
HbA1c (%)	5.8 (5.4-6.3)	5.7 (5.4-6.2)	6.2 (5.9-7.1)	<0.0001	5.8 (5.4-6.2)	6.4 (5.9-7.3)	<0.0001
Fasting insulin (IU/l)	6.7 (4.3-11.1)	6.7 (4.2-10.9)	8.4 (5.3-12.4)	0.0089	6.7 (4.2-10.9)	9.0 (5.3-12.4)	0.0323
2-hour insulin (IU/l)	38 (20.6-71.8)	37.5 (19.8-69.8)	62.0 (30.3-105.6)	0.0002	37.8 (20.3-70.5)	63.5 (32.6-105.2)	0.0072
HOMA-IR (MU)	1.6 (0.9-2.9)	1.6 (0.9-2.8)	2.1 (1.2-3.9)	0.0004	1.6 (0.9-2.8)	2.4 (1.3-3.8)	0.0026
hsCRP (µg/ml)	4.0 (1.6-8.8)	4.0 (1.6-8.8)	4.7 (2.7-9.3)	0.0492	4.0 (1.6-8.8)	4.8 (2.4-7.5)	0.4268
TG (mmol/l)	1.2 (0.9-1.7)	1.2 (0.9-1.7)	1.6 (1.2-2.3)	<0.0001	1.2 (0.9-1.7)	1.8 (1.1-2.4)	0.0001
TC (mmol/l)	5.1 (4.4-5.9)	5.1 (4.3-5.9)	5.4 (4.8-6.4)	0.0024	5.1 (4.4-5.9)	5.3 (4.4-6.0)	0.2226
LDL-C (mmol/l)	3.1 (2.5-3.8)	3.1 (2.5-3.8)	3.2 (2.7-4.3)	0.0668	3.1 (2.5-3.8)	3.1 (2.5-3.9)	0.9444
HDL-C (mmol/l)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	1.3 (1.1-1.5)	0.7106	1.3 (1.1-1.5)	1.3 (1.1-1.4)	0.5132
Creatinine (µmol/l)	60 (52-70)	59 (51-68)	111.5 (89.0-140.5)	<0.0001	59 (51-69)	140 (124-209)	<0.0001
eGFR ($ml/min/1.73m^2$)	-	104.0 (88.0-121.0)	48.2 (33.7-55.4)	<0.0001	113.9 (101.4-126.5)	44.7 (26.4-49.6)	<0.0001

Blood pressure measures							
Mean SBP (mmHg)	125 (111-141)	125 (110-140)	142 (121-162)	<0.0001	125 (111-140)	150 (128-181)	<0.0001
Mean DBP (mmHg)	81 (72-90)	81 (72-90)	81 (74-95)	0.2114	81 (72-90)	85 (73-95)	0.2185
Pulse pressure (BPM)	70 (62-79)	70 (62-79)	70 (60-81)	0.9932	70 (62-79)	73 (62-82)	0.3861
Co-morbidities							
Overweight (BMI≥25kg/m^2; n.%)	361 (23.2)	335 (22.9)	26 (29.5)	0.139	348 (23.1)	13 (28.9)	0.348
Obese (BMI≥30kg/m^2; n,%)	662 (42.6)	617 (42.1)	45 (51.1)	0.085	642 (42.5)	20 (44.4)	0.771
Pre-diabetes (n, %)	238 (15.2)	226 (15.4)	12 (12.8)	0.671	233 (15.4)	5 (10.6)	0.436
T2D (n, %)	297 (19.0)	259 (17.6)	38 (40.4)	<0.0001	272 (17.9)	25 (53.2)	<0.0001
Hypertension (n, %)	567 (36.3)	517 (35.2)	50 (53.2)	<0.0001	537 (35.4)	30 (63.3)	<0.0001

Data is presented as median (25th-75th percentiles) and percentages. WC, waist circumference; HC, hip circumference; BMI, body mass index; HbA1c, Glycated haemoglobin; HOMA-IR, Homeostatic model assessment-insulin resistance; MU, mass units; hsCRP, high sensitivity C-reactive protein; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoproteins; HDL-C, high-density lipoproteins; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; IFG/IGT, impaired fasting glucose and impaired glucose tolerance; T2D, type 2 diabetes mellitus; MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKD, chronic kidney disease.

Table 2: Haematological profile of study population overall and by CKD (MDRD and CKD-EPI) status

Variables	Total (n=1564)	MDRD			CKD-EPI		
		Without CKD (n=1470)	CKD (n=94)	p-value	Without CKD (n=1517)	CKD (n=47)	p-value
RBC (x10^6/µl)	4.7 (4.3-5.0)	4.7 (4.4-5.0)	4.3 (3.9-4.7)	<0.0001	4.7 (4.4-5.0)	4.2 (3.8-4.7)	<0.0001
WBC (x10^6/µl)	7.5 (6.2-9.1)	7.4 (6.2-9.1)	7.7 (6.5-9.2)	0.5704	7.4 (6.2-9.1)	7.9 (6.3-9.3)	0.5458
N/L (ratio)	2.0 (1.5-2.6)	1.9 (1.5-2.5)	2.5 (1.7-3.5)	<0.0001	1.9 (1.5-2.5)	2.7 (2.0-3.7)	<0.0001
Lymphocyte count (x10^9/l)	2.2 (1.8-2.80)	2.2 (1.8-2.8)	1.9 (1.4-2.5)	0.0001	2.2 (1.8-2.8)	1.8 (1.4-2.4)	<0.0001
Monocyte count (x10^9/l)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.1389	0.5 (0.4-0.6)	0.4 (0.4-0.6)	0.9446
Neutrophil count (x10^9/l)	4.5 (3.4-5.7)	4.5 (3.3-5.6)	5.0 (3.7-5.9)	0.0255	4.5 (3.4-5.6)	5.1 (4.3-6.1)	0.0297
Basophil count (x10^9/l)	0.1 (0.1-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.283	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.1366
Eosinophil count (x10^9/l)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1579	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.1223
Platelet count (x10^9/l)	271 (227-322)	271 (228-322)	277 (214-324)	0.9417	271 (228-322)	266 (197-313)	0.2211
Haematocrit (volume %)	41 (39-44)	41 (39-44)	38 (35-41)	<0.0001	41 (39-44)	37 (34-41)	<0.0001
MCV (fl/cell)	89 (85-93)	89 (85-93)	89 (86-92)	0.8150	89 (85-93)	89 (86-91)	0.4748
MCH (pg/cell)	29 (28-31)	29 (28-31)	29 (28-30)	0.1399	29 (28-31)	29 (28-30)	0.057
MCHC (g/dl)	33 (32-33)	33 (32-33)	33 (32-33)	0.1471	33 (32-33)	32 (32-33)	0.1156
RDW (%)	14.2 (13.5-15.0)	14.1 (13.4-15.0)	14.5 (13.7-15.6)	0.0601	14.1 (13.4-15.0)	14.3 (13.8-15.5)	0.0673
Hb (g/dl)	13.5 (12.6-14.4)	13.5 (12.7-14.5)	12.2 (11.2-13.3)	<0.0001	13.5 (12.6-14.4)	11.9 (11.1-13.2)	<0.0001
Anaemia (n, %)	289 (18.48)	249 (16.9)	40 (45.5)	<0.0001	263 (17.3)	26 (57.8)	<0.0001
Microcytic	83 (28.7)	83 (33.3)	0 (0.0)	-	83 (31.6)	0 (0.0)	-
Normocytic	180 (62.3)	141 (56.6)	39 (97.5)	-	155 (58.9)	25 (96.2)	-
Macrocytic	26 (9.0)	25 (10.0)	1 (2.5)	-	25 (9.5)	1 (3.8)	-

Data are presented as median (25th-75th percentiles) and percentages. RBC, red blood cells; WBC, white blood cells; N/L ratio, lymphocyte to neutrophil ratio; MCV, mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular

haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Association between the different haematological indices and eGFR

The age and gender-adjusted associations between the different haematological indices and eGFR, estimated by means of the MDRD and CKD-EPI equations, are presented in Table 3. Based on the CKD-EPI, however not the MDRD equation, eGFR was positively associated with all the RBC indices, including total RBC count, haemoglobin and haematocrit levels. eGFR was not associated with total WBC count, however a lower lymphocyte count was associated with a lower eGFR and N/L ratio was inversely associated with eGFR. Furthermore, male gender was significantly associated with all haematological measures, except basophil count and eosinophil count, and age was inversely associated with all RBC indices, lymphocytes, neutrophils, platelet count, MCHC and positively associated with RDW.

Table 3: Linear regression coefficients, adjusted for age, gender (Model 1) and eGFR (MDRD and CKD-EPI-derived) (Models 2) for the prediction of haematological-derived measures

Haematological-derived measures	MODEL 1							MODEL 2.1				MODEL 2.2			
	Age			Gender			R^2	eGFR (MDRD)			R^2	eGFR (CKD-EPI)			R^2
	β	95% CI	p	β	95% CI	p		β	95% CI	p		β	95% CI	p	
RBC (x10^3/µl)	-2.8	-4.5 to -1.2	0.001	327.4	269.6 to 385.3	<0.0001	0.08	0.3	-0.7 to 1.3	0.541	0.08	3.2	1.5 to 5.0	<0.0001	0.09
Haematocrit (%)	-0.2	-0.3 to -0.0	0.018	40.2	35.3 to 45.1	<0.0001	0.15	0.0	-0.1 to 0.1	0.709	0.15	0.3	0.1 to 0.4	<0.0001	0.16
Hb (g/l)	-0.1	-0.1 to -0.0	0.002	14.2	12.5 to 15.9	<0.0001	0.16	0.0	-0.0 to 0.0	0.907	0.16	0.1	0.0 to 0.1	<0.0001	0.16
WBC (x10^3/µl)	-15.1	-22.3 to -7.8	<0.0001	-431.9	-690.8 to -173.0	0.001	0.01	-0.5	-4.8 to 3.9	0.834	0.01	-1.7	-9.7 to 6.3	0.678	0.01
N/L (%)	-0.1	-3.8 to 3.5	0.941	136.2	5.6 to 266.7	0.041	0.00	-0.1	-0.4 to 0.1	0.214	0.00	-6.3	-10.3 to -2.3	0.002	0.01
Lymphocyte count (x10^6/l)	-2.9	-5.2 to -0.5	0.017	-257.10	-341.0 to -173.2	<0.0001	0.02	0.7	-0.8 to 2.1	0.364	0.02	3.0	0.4 to 5.6	0.022	0.03
Monocyte count (x10^6/l)	-0.8	-1.4 to -0.2	0.005	91.6	71.2 to 112.0	<0.0001	0.05	0.3	-0.1 to 0.6	0.114	0.05	0.5	-0.1 to 1.1	0.122	0.05
Neutrophil count (x10^6/l)	-10.9	-16.8 to -5.1	<0.0001	-291.8	-500 to -82.8	0.006	0.01	-1.1	-4.6 to 2.4	0.542	0.01	-4.7	-11.1 to 1.7	0.150	0.01
Basophil count (x10^6/l)	1.6	-8.4 to 11.5	0.759	-187.9	-541.9 to 166.1	0.298	0.00	0.7	-5.3 to 6.6	0.822	0.00	-8.3	-19.2 to 2.6	0.136	0.00
Eosinophil count (x10^6/l)	-0.5	-1.1 to 0.0	0.067	15.9	-4.9 to 36.7	0.135	0.00	-0.4	-0.7 to 0.0	0.071	0.00	-0.6	-1.2 to 0.1	0.074	0.00
Platelet count (x10^9/l)	-0.4	-0.6 to -0.1	0.003	-33.0	-42.0 to -24.0	<0.0001	0.03	0.1	-0.0 to 0.3	0.088	0.04	0.1	-0.0 to 0.3	0.088	0.04
MCV (fL/100cell)	1.4	-1.0 to 3.7	0.255	232.2	148.1 to 316.2	<0.0001	0.02	-0.2	-1.6 to 1.2	0.761	0.02	0.1	-2.5 to 2.7	0.946	0.02
MCH (pg/100cell)	-0.2	-1.1 to 0.7	0.698	95.3	63.3 to 127.4	<0.0001	0.02	-0.1	-0.7 to 0.4	0.646	0.02	0.1	-0.9 to 1.1	0.881	0.02
MCHC (g/l)	-0.1	-0.01 to -0.0	<0.0001	2.3	0.9 to 3.8	0.002	0.02	-0.0	-0.0 to 0.0	0.227	0.02	-0.0	-0.1 to 0.0	0.664	0.01
RDW (%)	0.1	0.0 to 0.1	0.004	-1.9	-3.7 to -0.0	0.05	0.01	0.1	0.0 to 0.1	<0.0001	0.02	0.1	0.0 to 0.1	0.025	0.01

Data presented as β-coefficient, 95% confidence interval and adjusted-R^2. Analysis are adjusted for age and gender. RBC, red blood cells; WBC, white blood cells; MCV, L/N ratio, lymphocyte to neutrophil ratio; mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; Hb, haemoglobin. MDRD, Modification of Diet in Renal Disease. Model 1 = age + gender; Model 2.1 = age + gender + eGFR (MDRD); Model 2.2 = age + gender + eGFR (CKD-EPI)

DISCUSSION

In this community-based sample of mixed-ancestry South Africans, we have shown that the haematological profile of individuals with reduced eGFR (<60ml/min/1.73m^2) are substantially impaired compared to those with normal kidney function, giving rise to the high prevalence of anaemia in this screen-detected CKD population. Furthermore, despite eGFR being positively associated with RBC indices, indicative of the severity of kidney function impairment, the disease state had no effect on the morphology of the RBC. Lastly, we confirmed that a chronic pro-inflammatory state exists in participants with CKD.

This study, which is in accordance with other studies in Africa and other developing countries [36-42], has shown that CKD is associated with significant impairment in RBC indices. Indeed, we have shown that total RBC count, haemoglobin concentration and percentage haematocrit were substantially reduced in participants with eGFR below 60ml/min/1.73m^2, compared to those with normal kidney function, independent of age and gender. Since erythropoietin is produced mainly by the proximal tubule of the nephron, kidney function decline will result in a decline in erythropoietin production and as a consequence result in decreased haemoglobin synthesis, leading to a fall in total RBC count [17]. This significant reduction in RBC, inevitably gives rise to anaemia [14]. Indeed, our study and numerous other studies have shown that the severity of anaemia increases with disease progression; with most of these studies showing anaemia at least twice as prevalent in participants with CKD, compared to the general adult population [37]. Furthermore, we found that 17% of the sample population with normal kidney function had haemoglobin levels <13.5g/dL and <12g/dL for men and women, respectively. However, this is not uncommon in Africa as previous studies have found that Africa has a high prevalence of anaemia caused by iron deficiency. In South Africa in particular, the South African National Health and Nutrition Examination Survey (SANHANES-1) [43] showed that 22% and 12.2% of adult females and males have anaemia.

The activation of the immune system, caused by inflammation, increases white blood cell counts [23]; emphasising the potential of WBC indices as a surrogate marker of inflammation in CKD [20]. Our study showed that despite no correlation between total WBC and reduced kidney function, CKD was associated with higher neutrophil and lower lymphocyte counts; both of which are independently associated with the promotion of atherosclerosis [44 45] and poor cardiovascular

outcomes [46]. N/L ratio, which combines the predictive power of both increased neutrophil count and decreased lymphocyte count [47], was associated with reduced eGFR in our study, as also found in other studies [23 48 49]. Indeed previous studies, which included CKD patients on haemodialysis [23 48] and pre-dialysis [49], showed that an increased N/L ratio was associated with known inflammatory markers such as tumor necrosis factor (TNF)-α [23], interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) levels [49]. These studies demonstrated that these well-established markers of inflammation were independent factors for predicting N/L ratio, thus presenting N/L ratio as an inflammatory biomarker for CKD patients. Since full blood count analysis are done routinely and a relatively affordable and easy measure to acquire, these findings are especially valuable taking into account the severely resource limited setting found in Africa and other low and middle-income countries.

Our study has a few limitations. This study was conducted in only one geographical area, which may not adequately reflect all the mixed ancestry population groups in Sub-Saharan Africa. Furthermore, this was a community-based sample with high female to male participation, however the latter being a common trend in South African population studies. Our study also used a single serum creatinine measure to determine the grade of kidney function and did not include estimates of albuminuria. Albuminuria, in particular, is required for clinical and aetiological diagnosis of CKD, as this information is important particularly in the interpretation of eGFR greater that 60ml/min/1.73m^2 where inaccuracies of the eGFR equations are greatest. It is however a common practice in community-based studies to diagnose CKD using a single measurement of serum creatinine. Furthermore, we did not investigate other haematinic deficiencies, such as vitamin B12 and iron deficiencies, which if present however, are less likely to affect haematological profile in a differential way in people with and without CKD. However, despite these limitations, we are not aware of other studies that have assessed the haematological profile of individuals with reduced kidney function in a population-based setting in Africa, even more specific, individuals of mixed-ancestry. Furthermore, we studied a community with a high burden of obesity, hypertension and diabetes, reflective of the current burden in Africa. This study provides much needed evidence for the association between the haematological profile and CKD as population-based data on the haematological profile of people with CKD in Africa, are very limited.

In conclusion, the findings from our study are valuable as full blood count analyses are done routinely and are relatively affordable, taking into account the severely resource limited setting found in Africa and other low and middle-income countries. Furthermore, though it still remains unclear whether the advocated kidney function estimators provide accurate estimates of CKD burden in African populations [50], the correlation of these estimates, with deteriorating profile of blood cell counts, suggests that these advocated GFR estimates, particularly the CKD-EPI equation, to some extent, measure kidney function in African populations.

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Research Ethics Committees of the Cape Peninsula University of Technology and Stellenbosch University (NHREC: REC—230 408–014 and N14/01/003, respectively). The study was conducted in accordance with the Declaration of Helsinki. All participants voluntary signed written informed consent after all the procedures were fully explained in the language of their choice. Permission to conduct the study was also obtained from relevant authorities including the city and community authorities.

Consent for publication

Not applicable

Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests

Funding

The South African Medical Research Council (SAMRC) funded this research project with funds from National Treasury under its Economic Competitiveness and Support Package (MRC-RFA-UFSP-01-2013/VMH Study).

Authors' contribution

Study conception and funding acquisition (TEM, APK, RTE), operationalization and supervision of the data collection (TEM), data analysis and interpretation (CG, APK), drafting the manuscript (CG, APK), critical revision of the manuscript and approval of the final version (all co-authors).

Acknowledgements

Poster presented at the 28th European Meeting on Hypertension and Cardiovascular Protection, held in Barcelona, June 8-11, 2018. As such, results have been published as an abstract. We are also grateful to the Cape Town VMH study investigation team and population of Bellville-South for their participation.

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