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ABO/Rh Blood Groups and Clinical Characteristics among Patients with Chronic Kidney Disease in Luanda, Angola

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Abstract

Introduction: CKD is an important public health concern in SSA, the risk is generally strongly associated with traditional risk factors such as age and chronic diseases, such as diabetes and hypertension. Objective: investigate the relationship between ABO/Rh blood groups and clinical characteristics among patients with CKD in Luanda, Angola.

Methodology: a cross-sectional study in patients diagnosed with CKD and undergoing hemodialysis at Josina Machel-Maria Hospital.

Results: 140 patients with CKD were enrolled in this study. About 55.7% (78/140) of patients were from the ORh+ blood group, followed by ARh^+ (20%wh), and BRh^+(17,8%), who lived in urban (52.2%) and rural (30.7%) areas, born in the north of Angola (67.1%), doing hemodialysis treatment between 6 years-10 years (50.7%), the underlying disease was hypertension (68.6%) and malaria(13,6%), the highest mean age was ABRh+ while the lowest age was in ORh^- patient. The statistical evaluation showed no relationship between the studied demographic and clinical characteristics, data, and blood groups ($p>0.05$).

Conclusion: CKD is predominant in young people, ORh^+ blood group, followed by ARh^+ and BRh^+, indiscriminately of the gender, especially individuals with low academic level, from urban and peri-urban regions, most patients have been on dialysis for less than 10 years, where the most common underlying diseases were hypertension and malaria.

Keywords: Chronic kidney disease • ABO/Rh blood groups • Clinical characteristics • Angola

Introduction

Chronic Kidney Disease (CKD) is considered a common condition characterized by the gradual loss of kidney function, with a high rate of morbidity and mortality, mainly in Low and Middle Income Countries ($LMIC_s$). Currently, CKD is ranked the 12th leading cause of death worldwide and is particularly common among people living in LMICs, such as in sub-Saharan Africa (SSA) regions [1]. SSA is a vast and heterogeneous region with a population of nearly one billion and markedly vast genetic diversity, where different social-economic factors contribute to a rapid transition in the burden of disease, including chronic diseases like CKD, and in Angola, a country located in SSA region, with fast population growth, around 32.5 Million on 2021, follow this pattern [1, 2].

There is evidence that African descendants are poorer and less educated when compared with the overall population. Studies of this population group in other countries like the United States suggest that CKD is more prevalent in African descent when compared with other ethnic groups [3-5]. CKD is an important public health concern in SSA, the risk is generally strongly associated with traditional risk factors such as age and chronic diseases, such as diabetes and hypertension [6, 7]. In a cross-sectional population study, which involved more than 8,000 participants, at six centers in four African countries, the overall prevalence of CKD was reported to be 10.7% [8].

It was observed in a meta-analysis with 98 432 subjects from 98 studies in Africa, that an overall prevalence of CKD in stages 1 to 5 and 3 to 5 of 15.8% and 4,6% for the general population, respectively [9]. This evidence highlights the urgent need for appropriate public health policy strategies and health systems interventions that support integrated screening and prevention strategies for CKD in infectious and noncommunicable disease management programs. It is also worth mentioning that LMIC regions present some risk factors including contaminated water supply, occupational exposures, use of over-the-counter pain relievers, and traditional medications [7-9]. Consistent epidemiological studies about CKD in Angola are limited. However, the Angolan Society of Nephrology has registered 1,809 patients undergoing hemodialysis in 2020.

ABO blood group antigens exhibit alternative phenotypes, genetically derived glycoconjugate structures located on the red cell surface that play a key role in physiological and pathological cell conditions [10, 11]. Recently our team showed interactions between ABO/Rh blood groups and several Chronic Diseases among Angolan Patients. We found that patients with the B blood group aged over 50 years, mainly females, are more susceptible to hypertension; male and female patients in blood groups O^+ and AB^+ are more susceptible to nephrotic syndrome and sickle cell anemia, especially males patients; it was also shown that patients in group O, followed by patients in blood groups A and B, mainly males, are more susceptible to CKD [12-14]. These data show that susceptibility and severity to certain diseases might be associated with ABO/Rh blood groups, so there is an urgent need to better understand and identify other risk factors associated with CKD in hemodialysis patients. In this study, we investigate the relationship between ABO/Rh blood groups with social and clinical conditions among Angolan patients diagnosed with CKD and undergoing hemodialysis in Luanda, the capital city of Angola.

Since agricultural activities in the dry land semi-arid areas in general and study area, Fedis, Deder and Shinile woredas in particular, are influenced and controlled by seasonal rain, the study of meteorological drought analysis is carried out season-wise using climate based drought index (SPI).These index as proxies of drought, and crop and/or vegetation health condition are vital in drought assessment and hence, they are applied in this study. Problem Statement is Ethiopia's climate is influenced by general atmospheric and oceanic factors that affect the weather system and climatic factors such as drought has significant impact on agricultural output [11]. This makes Ethiopia vulnerable to climate variability. Climate variability over the last three decades resulted in drought and famines in Ethiopia and several other countries in Africa [12]. Due to greater reliance on climate sensitive sectors such as agriculture, Ethiopia is vulnerable to the risk of climate variability and change such as MD. MD is a period of abnormally dry weather sufficiently for the lack of precipitation to cause a serious hydrological imbalance and carries connotations of a moisture deficiency [13]. In Eastern Ethiopia, the major impact of MD is manifested through rainfall variability and recurrent drought due to climate change.

Methodology

Study design and setting

This was a cross-sectional study carried out with adult patients diagnosed with CKD and undergoing hemodialysis at Josina Machel Maria Pia Hospital, a tertiary level health unit, located in Luanda Angola, in 1st semester of 2020. The sample was collected by simple random, among a population of 350 patients who are treated at this hospital. After explaining the purpose of this research, 140 eligible patients were asked to sign a study-specific consent form and have been enrolled in this study. The study was reviewed and approved by the Human Research Ethics Committee of the Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto (nr. 187/GD/ISCISA/UAN/2020) and authorized by the Pedagogical and Scientific Department of Josina Machel hospital (nr. 210/DPC/HJM/2020). All patients gave their informed consent before their inclusion in the study. All patients gave their informed consent before their inclusion in the study.

Patient's enrolment criteria and sample collection

Only patients with confirmed CKD and who are followed up in hemodialysis treatment were included in the study. Sociodemographic and clinical data such as age, gender, ABO/Rh blood group, social history, clinical history, and family history were obtained from all patients. An estimated 2 mL blood sample was collected for each patient using the venipuncture technique and the samples were placed in test tubes containing Ethylene-Di-Amine-Tetra-Acetic Acid (EDTA). The samples were placed in 3 wells and the posterior was associated with anti-A, Anti-B, and Anti-D reagents (Immucor, Portugal). The determination of the blood group was performed using the microplate technique according to the manufacturer's instructions, which is an agglutination test between the patient's serum and the anti-A, Anti-B, and Anti-D reagents of each well for phenotypic identification of blood groups (ABO and Rh).

Statistical analysis

Descriptive statistics were calculated using the statistical program SPSS v20.0 (IBM SPSS Statistics, USA), and the results presented in graphs were developed using the Sigmaplot 12.0 (Systat Software, Inc.) The Chi-square (X^2) test was used to assess the relationship between categorical variables. All reported p-values are two-tailed and deemed significant when $p<0.05$.

Results

Demographic characteristics and ABO/Rh blood group distribution

The demographic characteristics and ABO/Rh blood group distribution among the studied population are shown in Table 1. A total of 140 patients with CKD were enrolled in this study. About 55.7% (78/140) of patients were from the ORh^+ blood group, followed by ARh^+ (20%), and BRh^+ (17.8%). In general, the Rh^- blood group was the less prevalent, being that only two of the patients were Rh^- (from blood groups A and O). The majority of the population with CKD was younger than 51 years, concentrating especially on the age group between 41 years-50 years old (36.4%), and it was found that individuals over 21 had an average time of dialysis of more than 6 years, which may demonstrate that the diagnosis of the disease in young people seems to be common. The male gender presented a slightly higher number of individuals (51.5%) living with CKD, although there seemed to be no difference between the mean age as well as the time of dialysis among men and women. Most individuals with CKD (90%) had primary education (47.9%) and secondary school (41.2%). It was evident that the majority of individuals with CKD lived in urban (52.2%) and rural (30.7%) areas, however, individuals from the urban region had the highest mean age and duration of haemodialysis treatment to about residents of peri urban and rural areas. Most of the individuals included in the study reported being born in the north of Angola (67.1%) and the central region of Angola (30.7%), on the other hand, an interesting fact was that individuals from other countries, although a minority (2.1%), had a higher mean age (59 years ± 12.7 years old) and shorter dialysis time (6 years ± 3.7 years) compared to those from Angola (below 47 years of age and over 6.4 years of dialysis). Regarding blood groups in the demographic assessment, except for individuals from peri urban areas and of foreign origin where the ARh^+ group equaled the ORh^+ group, in all other conditions the ORh^+ group maintained the predominance over the other blood groups. The statistical evaluation showed no relationship between the demographic data studied and the blood groups ($p>0.05$).

Table 1. Demographic characteristics and ABO/Rh blood group distribution among patients with CKD in Luanda, Angola.

Demographic	Blood Group [n(%)]						Age (Dialysis)		P-value
	ARh+	ARh-	BRh+	ABRh+	ORh+	ORh-	Sub-Total	Years (Dialysis Years)	(X^2)
	28(20,0)	1(0,7)	25(17,8)	7(5,1)	78(55,7)	1(0,7)	140(100)	Mean ± SD	
Age									
≤ 20	0(0,0)	0(0,0)	3(37,5)	0(0,0)	5(62,5)	0(0,0)	8(5,7)	14,3 ± 3,8 (3,0 ± 1,7)	0,158
21-30	3(20,0)	0(0,0)	1(6,7)	2(13,3)	9(60,0)	0(0,0)	15(10,7)	26,9 ± 2,9 (6,7±2,7)	
31-40	9(32,1)	0(0,0)	5(17,9)	0(0,0)	13(46,4)	1(3,6)	28(20,0)	35,8 ± 3,3 (6,1 ± 3,3)	
41-50	11(21,6)	1(2,0)	11(21,6)	0(0,0)	28(54,9)	0(0,0)	51(36,4)	45,1 ± 2,9 (6,7 ± 3,1)	
≥ 51	5(13,2)	0(0,0)	5(13,2)	5(13,2)	23(60,5)	0(0,0)	38(27,2)	58,7 ± 6,1 (7,1 ± 4,4)	
Gender									
Male	15(20,8)	1(1,4)	13(18,1)	4(5,6)	38(52,8)	1(1,4)	72(51,5)	43,9 ± 13,5(6,6 ± 4,0)	0,811
Female	13(19,1)	0(0,0)	12(17,6)	3(4,4)	40(58,8)	0(0,0)	68(48,5)	42,4 ± 12,7(6,4 ± 3,1)	
Education									
Basic	11(16,4)	0(0,0)	13(19,4)	6(9,0)	36(53,7)	1(1,5)	67(47,9)	45,1 ± 14,5(6,1 ± 3,4)	0,303
Secondary	12(20,3)	1(1,7)	12(20,3)	1(1,7)	33(55,9)	0(0,0)	59(42,1)	40,0 ± 10,5(7,1 ± 2,9)	
University	5(35,7)	0(0,0)	0(0,0)	0(0,0)	9(64,3)	0(0,0)	14(10,0)	47,4 ± 15,3(6,1 ± 6,1)	
Residence									
Urban	14(19,2)	1(1,4)	12(16,4)	3(4,1)	43(58,9)	0(0,0)	73(52,2)	44,9 ± 12,7(7,2 ± 3,6)	0,349
Peri-urban	9(37,5)	0(0,0)	4(16,7)	2(8,3)	9(37,5)	0(0,0)	24(17,1)	40,9 ± 13,4(6,3 ± 3,5)	

Rural	5(11,6)	0(0,0)	9(20,9)	2(4,7)	26(60,5)	1(2,3)	43(30,7)	41,5 ± 13,3(5,3 ± 3,3)	
Birth Region									
North	18(19,1)	0(0,0)	15(16,0)	6(6,4)	55(58,5)	0(0,0)	94(67,1)	41,1 ± 13,8(6,4 ± 3,7)	0,693
Center	9(20,9)	1(2,3)	9(20,9)	1(2,3)	22(51,2)	1(2,3)	43(30,7)	46,6 ± 9,8 (6,8 ± 3,5)	
Foreigners	1(33,3)	0(0,0)	1(33,3)	0(0,0)	1(33,3)	0(0,0)	3(2,1)	59,0 ± 12,7(6,0 ± 1,0)	

Clinical characteristics and ABO/Rh blood group distribution

The clinical characteristics and ABO/Rh blood group distribution among the studied population are described in Table 2, where it can be seen that the majority of the population with CKD was already undergoing hemodialysis treatment between 6 years to 10 years (50.7%) and the mean age increased as the mean dialyze time increased. All the patients were undergoing treatment, however, a small number of individuals (12.9%) came to give up hemodialysis treatment, however, they returned to treatment when they realized that their health situation worsened. The average age among these individuals who came to give up treatment was greater than those who did not give up, however, the average time in treatment, seems to show that individuals with less time of treatment were more likely to give up on hemodialysis treatment. An extremely interesting data was that after hypertension, which was the most common underlying disease among patients with CKD (68.6%), malaria was the second underlying disease among patients included in the study (13.6%), and it was this group that had the lowest mean age (30.7 ± 16.8), even surpassing the data of diabetes (5.7%) which has been identified as one of the main causes of CKD in the world. Only a small number of individuals with CKD (36.4%) had a family history of the underlying disease (Hypertension and Diabetes). Patients without a family history of the disease (63.6%), had a lower mean age than individuals with a family history of chronic non-communicable disease. Only a very small number (2.9%) of CKD patients had a family history of CKD. Regarding the blood groups in the clinical evaluation, in all other conditions, the ORh$^+$ group maintained the predominance over the other blood groups. The statistical evaluation showed no relationship between the clinical characteristics and ABO/Rh blood groups among the studied population ($p>0.05$).

Table 2. Clinical characteristics and ABO/Rh blood group distribution among patients with CKD in Luanda, Angola.

Clinical characteristics	Blood Group [n(%)]						Years(Dialyse)		P-value
	ARh$^+$	ARh$^-$	BRh$^+$	ABRh$^+$	ORh$^+$	ORh$^-$	Sub-Total	Years(Month)	(X^2)
	28(20,0)	**1(0,7)**	**25(17,8)**	**7(5,1)**	**78(55,7)**	**1(0,7)**	**140 (100)**	**Mean ± SD**	
Dialysis time (years)									
≤5	12(22,2)	0(0,0)	8(14,8)	3(5,6)	30(55,6)	1(1,9)	54(38,6)	40,4 ±15,2(2,9 ± 1,5)	0,898
06-Oct	13(18,3)	1(1,4)	15(21,1)	4(5,6)	38(53,5)	0(0,0)	71(50,7)	44,3 ± 11,3(7,9 ± 1,4)	
≥11	3(20,0)	0(0,0)	2(13,3)	0(0,0)	10(66,7)	0(0,0)	15(10,7)	47,1 ± 11,7(12,6 ± 3,3)	
Withdrawal of treatment									
Yes	1(5,6)	0(0,0)	4(22,2)	1(5,6)	11(61,1)	1(5,6)	18(12,9)	45,8 ± 14,4(4,8 ± 3,3)	0,093
No	27(22,1)	1(0,8)	21(17,2)	6(4,9)	67(54,9)	0(0,0)	122 (87,1)	42,7 ± 12,9(6,7 ± 3,6)	
Baseline diseases									
Malaria	4(21,1)	0(0,0)	2(10,5)	0(0,0)	13(68,4)	0(0,0)	19(13,6)	30,7 ± 16,8(6,2 ± 5,3)	0,844
Hypertension	21(21,9)	1(1,0)	20(20,8)	4(4,2)	49(51,0)	1(1,0)	96(68,6)	45,2 ± 10,5(6,9 ± 3,2)	
Diabetes	2(25,0)	0(0,0)	1(12,5)	1(12,5)	4(50,0)	0(0,0)	8(5,7)	56,0 ± 11,1(3,2 ± 2,1)	
Others	1(5,9)	0(0,0)	2(11,8)	2(11,8)	12(70,6)	0(0,0)	17(12,1)	39,4 ± 12,7(5,8 ± 3,4)	
Family history of baseline diseases									
Yes	10(19,6)	1(2,0)	10(19,6)	2(3,9)	28(54,9)	0(0,0)	51(36,4)	45,8 ± 10,6(6,7 ± 2,9)	0,753
No	18(20,2)	0(0,0)	15(16,9)	5(5,6)	50(56,2)	1(1,1)	89(63,6)	41,6 ± 14,1(6,3 ± 3,9)	
Family history of chronic kidney disease									
Yes	1(25,0)	0(0,0)	0(0,0)	0(0,0)	3(75,0)	0(0,0)	4(2,9)	44,7 ± 4,9(8,5 ± 2,4)	0,932
No	27(19,9)	1(0,7)	25(18,4)	7(5,1)	75(55,1)	1(0,7)	136 (97,1)	43,1 ± 13,3(6,4 ± 3,6)	

ABO/Rh blood group distribution by mean age and time of dialysis

The ABO/Rh blood group distribution by mean age and time of dialysis are shown in Figure 1. The highest mean age was identified in the ABRh$^+$ while the lowest age was observed in the ORh$^-$ patient (31 years). On the other hand, the highest dialysis time was of the single ARh$^-$ patient (more than 8 years of hemodialysis treatment), and the shortest dialysis time was observed in the single ORh$^+$ individual (less than 1 year). Among the Rh+ individuals, the longest treatment time was verified in the individuals of group B (over 7 years) and the short treatment time observed in patients of group AB (less than 6 years). Regarding gender, there was no difference in the number of male and female patients concerning the blood groups studied and an interesting fact is that the two Rh- patients found in the study, were male.

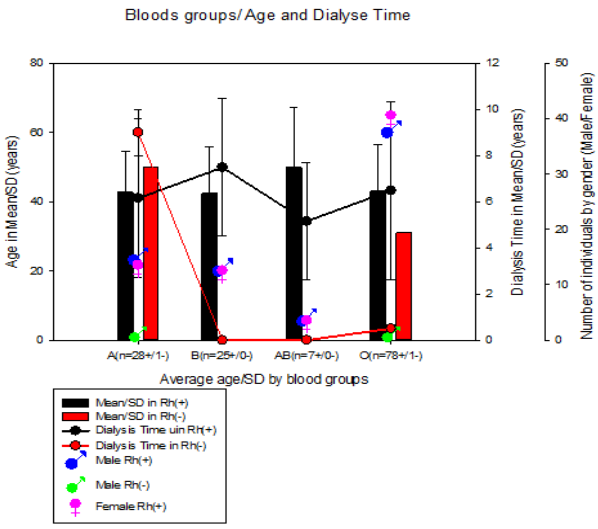

Figure 1. ABO blood groups distribution by mean age and time of dialysis among patients with CKD in Luanda, Angola.

Discussion

Interestingly, the data on blood groups and sociodemographic characteristics found in patients with CKD seem to differ a little from other analytical studies carried out by our team to assess the frequency of ABO/Rh blood groups and the social status of hypertensive patients in Luanda, where most patients were blood group B (36.4%) and blood group O (33.3%), Rh+ (84.8%), and the majority were female (72.7%), most hypertensive patients lived in rural (43.4%) or peri-urban areas (35.4%), were self-employed (73.87%) and the mean age was 50.6 years ± 10.4 years [12] (Table 1). In a previous study carried out by our team with patients treated at another hemodialysis unit to assess the sociodemographic conditions of Angolan patients with new pathologies

after hemodialysis treatment, we found that among the patients studied (n=100), the illiterate represented 17%, with education basic 27%, medium 26%, and university students 5%, where single individuals represent 60% most of them 47% were self-employed and 55% of them acquired new pathologies after starting hemodialysis treatment [15]. The data from the present study are similar to the data reported in 2017, to identify whether ABO blood groups are associated with renal outcomes in patients with IgA nephropathy, it was found that there were 27.4% of patients in group A, 29.7% in group B, 10.1% in group AB, and 32.8% in group O [16]. In a study developed in Nigeria, in patients with acute kidney disease, 70% were male and the mean age was 36.32 years ± 13.3 years old, where the ABO blood group in patients with acute kidney disease indicated high significance (p=0.001) rejecting the null hypothesis that the degree of acute kidney disease is independent of ABO blood group and accepting that the degree of acute kidney disease depends on the ABO blood group [17]. The data of the present study regarding educational levels differ from the findings of a study carried out in Iran where the authors conclude that higher educational levels were associated with the occurrence of CKD over a decade of follow-up, and this finding can be attributed to unhealthy lifestyle behaviors in this population group. Data by place of residence and region of birth appear to agree with a study that showed that in high-income countries, lower socioeconomic status is associated with a higher risk of kidney disease due to behavioral and metabolic risk factors and reduced access to care, while in LMICs, the burden imposed by this poverty-related kidney disease is even greater, due to associated infections, hazardous work, poor education, and poor maternal health [18].

In the previous study that our team developed, 81% of the patients had been undergoing hemodialysis for less than 6 years and it was found that, unlike in the present study, hypertension and diabetes were the underlying diseases that most contributed to chronic renal failure in patients [15]. A study published in 2017 looking at whether ABO blood was associated with renal outcomes in patients with IgA nephropathy found that patients with IgAN blood group O or A have an independent increased risk of deterioration in renal function, which may be explained by an increased level of an inflammatory state. Although in the present study, the majority who gave up on treatment and returned, claimed that they gave up because they were tired of doing the treatment, even though they knew that there would be no cure, a study carried out in 2018, in the United States, concluded that the dropout rate of hemodialysis was twice as high as expected and that situations such as acute medical complications and frailty seemed to be determining factors for dropout, however, palliative care services were used in only a minority of patients [19]. In the present study, malaria was the second underlying disease that most contributed to CKD, this data has been increasingly observed, since data from two studies carried out by our research team in patients with malaria have shown that kidney damage in this group of patients ranges from 36% to 41%, especially in young individuals [20]. In a study carried out in India, in 4056 patients with CKD in 2020, where the mean age was 50.3 years ± 11.8 years old and 67.2% of the patients were male, the most common underlying diseases found were hypertensive (87%), diabetes (37%), CVD (22%), history of acute kidney injury (6.7%), and previous use of alternative drugs (23%) [21]. In the present study, only 2.9% of the individuals had a family history of CKD, data that differ from a study carried out in the Netherlands, where the prevalence of CKD was 1.19% (1,862 cases per 155,911) [22].

This study did not find B(B/AB) patients in Rh- the blood group, and the disease times per group . Figure 1 lead us to agree with a study carried out in 2017, where patients in the non-B antigen (O/A type) group were found to have lower baseline e-GFR, Systolic Blood Pressure (SBP), acid uric acid, lactate dehydrogenase, high-sensitivity C reactive protein, and tumor necrosis factor α compared with patients in group B antigen group (type B/AB), and also after a mean follow-up of 58 months, 14% of patients progressed to end-stage renal disease, including 18% in the non-B antigen group and 7.1% in the B antigen group [16]. In addition, these data allow us to agree with the authors who claim that the risks of kidney disease are also influenced by ethnicity, gender, location, lifestyle, increasing economic and health disparities, migration, demographic transition, unsafe working conditions, threats to environmental conditions, natural disasters, pollution and other factors that can thwart attempts to reduce morbidity and mortality from kidney disease [23]. The present study seems to differ from the study carried out in China, where they found that the frequency of blood group O was significantly lower in patients with end-stage of renal disease on dialysis [24]. Some limitations should be considered when interpreting our observations. The fact that our study was descriptive did not allow us to present aspects of causality. On the other hand, clinical outcome data, as well as the description of the degree of kidney injury, were not considered, which would be essential to have greater clarity if the ABO/Rh blood group together with the sociodemographic or clinical characteristics could predict the degree of kidney injury or the clinical outcome of patients with CKD. Despite these limitations, our findings show in detail for the first time the possible relationship between ABO/Rh blood groups, demographic, and/or clinical characteristics in patients with a certain degree of CDK. Therefore, future studies should explore the possible relationship between blood groups, demographic, clinical, and/or behavioral characteristics, with clinical outcomes among CDK patients. In addition, the description of the degree of kidney damage should also be considered to explore whether progression to different degrees of kidney damage is affected by the ABO/Rh blood groups.

Conclusion

It can be concluded from the study that Chronic Kidney Disease in Angola is predominant in the young people, with ORh^+ blood group, followed by ARh^+ and BRh^+ the disease, indiscriminately of the gender, especially individuals with low academic level, from urban and peri-urban regions, most patients have been on dialysis for less than 10 years, where the most common underlying diseases were hypertension and malaria in a group of individuals who mostly do not have a family history of the underlying disease or a familial disease. However, future studies should be carried out to delve deeper into the impact of blood group on predisposition to kidney disease, as well as on clinical outcomes among Angolan patients.

Declaration

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are within the paper..

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

- Conceptualization: OFSH, ENMS. Data curation: ENMS, EKC, SRS, MC, CAPS, CSS, EEV.
- Formal analysis: ENMS, EKC, SRS, MC, CAPS, CSS, EEV. Investigation: ENMS, OFSH.
- Project administration: ENMS, EKC, SRS, MC, CAPS, CSS, EEV. Supervision: ENMS.
- Validation: ENMS, EKC, SRS, MC, CAPS, CSS, EEV, OFSH. Writing – original draft: ENMS, EKC, CSS.
- Writing – review & editing: ENMS, EKC, SRS, MC, CAPS, CSS, EEV, OFSH.

All authors have seen and approved the submitted version of this manuscript.

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