Renin angiotensin aldosterone system altered in resistant hypertension in Sub-Saharan African diabetes patients without evidence of primary hyperaldosteronism

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Abstract

Background: The renin-angiotensin-aldosterone system may be altered in patients with resistant hypertension. This study aimed to evaluate the relation between renin-angiotensin-aldosterone system activity and resistant hypertension in Cameroonian diabetes patients with resistant hypertension.

Methods: We carried out a case-control study including 19 diabetes patients with resistant hypertension and 19 diabetes patients with controlled hypertension matched to cases according to age, sex and duration of hypertension since diagnosis. After collection of data, fasting blood was collected for measurement of sodium, potassium, chloride, active renin and plasma aldosterone of which the aldosterone-renin ratio was derived to assess the activity of renin-angiotensin-aldosterone system. Then, each participant received 2000 ml infusion of saline solution after which plasma aldosterone was re-assayed.

Results: Potassium levels were lower among cases compared to controls (mean: 4.10 ± 0.63 mmol/l vs. 4.47 ± 0.58 mmol/l), though nonsignificant (p = 0.065). Active renin, plasma aldosterone both before and after the dynamic test and aldosterone-renin ratio were comparable between cases and controls (all p values > 0.05). Plasma aldosterone significantly decreased after the dynamic test in both groups (p < 0.001), but no participant exhibited a post-test value > 280 pmol/l. We found a significant negative correlation between potassium ion and plasma aldosterone (r = -0.324; p = 0.047), the other correlations being weak and unsignificant.

Conclusion: Although this study failed to show an association between RH and primary hyperaldosteronism in our context, there was a hyperactivity of renin-angiotensin-aldosterone system. Moreover, this study confirms the importance of potassium dosage when screening the renin-angiotensin-aldosterone system.

Keywords
Resistant hypertension, diabetes, hyperaldosteronism, renin-angiotensin-aldosterone system, Cameroon

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Background

Although recognized as the most important modifiable risk factor for cardiovascular diseases (CVD), hypertension remains the leading cause of CVD and deaths worldwide.1 With a worldwide prevalence of almost 22%, hypertension is responsible for about 10.4 million deaths annually.1 In Sub-Saharan Africa, Ataklte et al.2 reported a prevalence of hypertension varying between 14.7% and 69.9%. This heavy burden may have negative consequences as a large proportion of hypertensive subjects may remain undiagnosed, untreated and/or poorly managed, being thus, at high risk of morbidity and mortality from potentially avoidable complications of hypertension such as stroke and heart disease.2,3

Hypertension and diabetes coexist in 40 to 60% of type 2 diabetes patients.4,5 Diabetes patients have been shown to have a 1.5–3 times increased prevalence of hypertension compared to people without diabetes,5 with half of the former diagnosed with hypertension at time of diagnosis. This co-existence accelerates the occurrence of related micro- and macro-vascular complications, and CVD as well.4 Evidence has shown that a proper control of diabetes patients’ blood pressure (BP) may prevent or delay the occurrence of these complications.6,7 However, achieving an adequate BP control in diabetes subjects remains challenging as less than half of them attain BP targets.5

Resistant hypertension (RH) has been increasingly identified as a reason for uncontrolled BP. It is defined as a failure to achieve the target BP values (<140/90 mmHg) despite a BP lowering regimen comprising at least three antihypertensive drugs at maximal doses, including a diuretic.8 Reports from several studies estimate the prevalence of RH at ~8%–16% across American and European countries.9,10 A recent report revealed a prevalence around 12% in Africa.11 RH is common in certain groups such as diabetes and chronic kidney disease patients,12 and has been associated with a high prevalence of vital organ damage and an increased incidence of cardiovascular events.3

A close relationship exists between RH and fluid retention.13 In this regard, international guidelines for RH management alongside recent studies have claimed, among effective strategies, to reduce salt intake and vulgarize the use of diuretics among BP lowering medications.14,15 Moreover, the renin-angiotensin-aldosterone system (RAAS), especially its renal and vasomotor components, plays a preponderant role in BP regulation.16 Activation of the RAAS leads notably to vasoconstriction and fluid and sodium retention.16 The pathophysiological analysis of various elements of the RAAS is fundamental when exploring patients with RH.16 Determining the concentration of angiotensinogen and renin, the angiotensin converting enzyme activity, and aldosterone concentration can be performed by various methods. However, only plasma renin and aldosterone assays are useful in current practice for the detection of hyperaldosteronism, with the aldosterone/plasma renin activity ratio (ARR) ’proposed as the most sensitive test to detect hyperaldosteronism.14,16

In Cameroon and to the best of our knowledge, no study has assessed the RAAS activity in resistant hypertensive subjects with diabetes. This study sought to evaluate the relationship between the RAAS activity and RH in Cameroonian diabetes patients.

Methods

Study design, setting and participants

This was a hospital-based case-control study conducted from August 2012 to January 2013 at the National Obesity Centre of the Yaoundé Central Hospital, Cameroon. This centre has a clinical research laboratory where the dynamic tests and blood samplings were conducted. The blood specimens were then transferred to the Biochemistry Laboratory of the Yaoundé University Teaching Hospital for biochemical assays.

Participants were type 2 diabetes patients seen during out-patient consultations at the study site, and enrolled after they voluntarily agreed to take part in the study. Cases were known hypertensive patients followed-up during at least six months, with a BP ≥140/90 mmHg, on at least three fully dosed antihypertensive drugs including a diuretic.8 To confirm RH, the patient measured his/her BP at home at least four times within 24 h for three consecutive days. At each of these occasions, BP was measured at rest three times at a 5-min interval. The patient was eligible if all these BP measurements were ≥140/90 mmHg.

Controls were known hypertensive patients followed-up during at least six months, with a BP <140/90 mmHg, on not more than three antihypertensive drugs.8 The participant’s BP lowering medication (either a case or a control) must have been unchanged during at least the last three months. Controls were matched to cases with regard to age, sex and duration of hypertension since diagnosis.

We excluded participants aged above 75 years, those with an estimated glomerular filtration rate (eGFR) ≤30 ml/min/1.73 m², patients with heart failure, pregnant women, those breastfeeding or currently taking oral contraceptive pills, patients presenting an acute complication of diabetes, and all those with a known history of Cushing syndrome, hyperthyroidism or pheochromocytoma. We also excluded all patients not rigorously taking their antihypertensive drugs as prescribed. The minimal sample size in each group was calculated assuming a 10% variation of the aldosterone/active renin ratio (ARR) and a precision of 5%,17 hence a minimum of 16 subjects per group.
Data collection

We used a structured and pretested questionnaire to record data on socio-demographic characteristics and medical history of the participant. A physical examination was subsequently undertaken during which BP, body weight (to the nearest kilogram), and height (to the nearest centimeter) were measured. Body mass index (BMI) was then derived as weight (kg) / height × height (m). Of note, BP was measured three times at a rest and a 5-min constant interval; the mean of the last two measurements was considered.

Blood collection and dynamic test

For the static exploration of RAAS, blood samples were collected in the morning (between 8 and 10 a.m.) following an overnight fasting, after 10 min of rest at a seated-position, without interruption of the current antihypertensive therapy. Blood was aseptically collected by venipuncture of the brachial vein in an EDTA tube for the measurement of plasma aldosterone (PA) and active renin mass concentration (ARC), and in a dry tube for the dosage of electrolytes (Na+, K+, Cl−), serum creatinine and urea. Additionally, a fasting capillary glycemia was measured using a One Touch Verio (Lifescan Inc., Milipitas, California, USA) glucose meter, and a urinary dipstick was performed to search for proteinuria.

Afterwards, the dynamic test was undertaken, aiming at inducing hypervolemia and inhibiting the production of renin. The participant received 2000 ml of 0.9% normal saline solution intravenous infusion within 4 h. Subsequently, another blood sample was performed for PA measurement.

Serum and plasma (from both samplings) were immediately separated by centrifugation at 3000 r/min within 5 min, aliquoted, put on ice and transported to the biochemistry laboratory where electrolytes, serum urea and creatinine were measured without delay, and plasma, kept at -20°C for further assessment of active renin and aldosterone before and after the dynamic test.

Biochemical assays

PA was measured by standard procedures using an ELISA kit (Alpoo immunoassays, Salem, NH), with reference values of 70–882 pmol/l. The concentration of plasma active renin was also measured by standard related procedures using an ELISA kit (reference EIA – 5125, DRG Instruments GmbH, Germany) with reference values of 0.81–128 ng/l. The ARR was subsequently derived. Sodium, potassium and chloride ions were assayed using an iometer (iLyte NA+/K+/CL− system Ion Selective electrode analyzer, Instrumentation Laboratory, USA). The reference values were respectively 137–151 mmol/l, 3.5–5.8 mmol/l, and 98–109 mmol/l. Serum urea and creatinine were measured using colorimetric methods with reagents from Hospitex Diagnostics (Osmannoro, Sesto Fiorentino (FI), Italy) and Labcare Diagnostics (Mumbai, India), respectively; the eGFR was then calculated using the Cockroft and Gault formula.18

Statistical methods

Data were coded, entered and analyzed using SPSS version 20.0 (IBM SPSS Inc., Chicago, Illinois, USA). Results are presented as count (proportion), and mean ± standard deviation (SD) or median [interquartile range (IQR)] where appropriate. Variable comparisons used the Chi-square test for categorical variables, and the Student t test or nonparametric equivalents (Mann–Whitney test or the Signed Rank Wilcoxon test) for continuous variables. The Spearman correlation coefficient (r) served to investigate any association between quantitative variables. A p value < 0.05 was used to characterize statistically significant results.

Results

We enrolled a total of 38 participants: 19 cases and 19 matched-controls. The sex distribution was equal in the two groups with a male/female sex ratio of 1.4 (Table 1). Ages ranged from 42 to 75 years.

Table 1. Summary of categorical variables.

| Variable | Cases (%) | Controls (%) | Total (%) |
|----------|-----------|--------------|-----------|
| Sex | | | |
| Male | 11 (57.9) | 11 (57.9) | 22 (57.9) |
| Female | 8 (42.1) | 8 (42.1) | 16 (42.1) |
| Number of antihypertensive drugs | | | |
| 1 | 0 (0.0) | 6 (31.6) | 6 (15.8) |
| 2 | 0 (0.0) | 10 (52.6) | 10 (26.3) |
| 3 | 11 (57.9) | 3 (15.8) | 14 (36.8) |
| 4 | 8 (42.1) | 0 (0.0) | 8 (21.1) |
| Antihypertensive drugs currently taken | | | |
| Calcium channel blockers | 18 (94.7) | 10 (52.6) | 28 (73.7) |
| ACE inhibitors | 18 (94.7) | 13 (68.4) | 31 (81.6) |
| Beta-blockers | 13 (68.4) | 2 (10.5) | 15 (39.5) |
| Angiotensin II receptor blockers | 2 (10.5) | 0 (0.0) | 2 (5.3) |
| Diuretics | 14 (73.7) | 10 (52.6) | 24 (63.2) |
| Proteinuria | | | |
| Yes | 11 (57.9) | 12 (63.2) | 23 (60.5) |
| No | 8 (42.1) | 7 (36.8) | 15 (39.5) |

ACE: angiotensin converting enzyme.
Hypertension is a complex pathology which can be essential or secondary. Specifically, RH may be the

The mean values of serum urea, serum creatinine, eGFR, sodium, potassium, chloride, plasma active renin, ARR, and PA before and after the dynamic test are depicted by Table 2. We found no difference in the distribution of these parameters between cases and controls (all p values > 0.05; Table 2). The ionic profile of our participants ranged within the reference values. Fifty percent of patients had their plasma active renin level <2 ng/l. After the dynamic test, PA was measured in 16 cases and their matched controls. Although we found no difference between cases and controls (p = 0.445), we observed a significant decrease in PA before and after the test in both cases and controls (all p values < 0.001; Table 2). But, no patient had a post-test PA >280 pmol/l.

There was no association between RH and primary hyperaldosteronism. Indeed, apart from the correlation between potassium and PA which was negative and significant (p = −0.324; p = 0.047), all other correlations were very weak and insignificant (Table 3).

**Discussion**

Hypertension is a complex pathology which can be essential or secondary. Specifically, RH may be the

### Table 2. Clinical and biochemical parameters.

| Variable                     | Cases                     | Controls                  |
|------------------------------|---------------------------|---------------------------|
| **Mean ± SD**                | **Median [IQR]**          | **Median [IQR]**          | **Mean ± SD**                |
| Age (years)                  | 63.6 ± 7.5                | 64 [60–69]                | 0.725                      | 64 [60–68]                |
| BMI (kg/m²)                  | 29.3 ± 5.5                | 28.9 [26.0–30.9]          | 0.650                      | 28.3 [24.7–31.1]          |
| Duration of hypertension (years) | 13.7 ± 9.1               | 12.5 [3.8–22]             | 0.255                      | 8 [5–14]                |
| Duration of diabetes (years) | 10.7 ± 8.0                | 7 [4.5–20]                | 0.438                      | 10 [8–17]                |
| Number of visits per year    | 6.2 ± 3.2                 | 5 [4–6]                   | 0.004                      | 4 [2–4]                |
| SBP (mmHg)                   | 159.2 ± 16.5              | 157 [146–164]             | <0.001                     | 131 [125–136]           |
| DBP (mmHg)                   | 86.3 ± 13.5               | 83 [79–93]                | 0.004                      | 78 [74–80]              |
| Fasting capillary glucose (g/l) | 1.87 ± 0.74            | 1.73 [1.34–2.40]          | 0.028                      | 1.10 [0.90–1.88]         |
| Serum urea (g/l)             | 0.45 ± 0.28               | 0.39 [0.26–0.57]          | 0.361                      | 0.35 [0.30–0.45]         |
| Serum creatinine (mg/l)      | 12.94 ± 3.99              | 12.0 [9.9–15.4]           | 0.821                      | 12.8 [11.1–15.0]         |
| eGFR (ml/min)                | 70.34 ± 28.82             | 53.5 [49.5–92.7]          | 0.528                      | 67.73 [50.87–80.36]      |
| Sodium (mmol/l)              | 139.56 ± 1.95             | 139.8 [138.0–141.3]       | 0.450                      | 139.6 [139.1–141.3]      |
| Potassium (mmol/l)           | 4.10 ± 0.63               | 4.01 [3.57–4.51]          | 0.065                      | 4.43 [4.07–4.89]         |
| Chloride (mmol/l)            | 106.92 ± 3.52             | 108.1 [104.8–109.3]       | 0.184                      | 108.5 [106.7–110.2]      |
| PA (pmol/l)                  | 591.52 ± 364.2            | 454.44 [343.84–914.76]    | 0.298                      | 587.44 [273.56–1429.68]  |
| Plasma active renin (ng/l)   | 15.08 ± 24.4              | 1.43 [0.81–19.46]         | 0.884                      | 2.15 [0.81–21.16]        |
| ARR (pmol/ng)                | 319.63 ± 325.1            | 255.80 [209.5–561.04]     | 0.531                      | 250.27 [54.15–800.93]    |
| PA after the dynamic test (pmol/l) | 15.61 ± 27.6         | 6.72 [5.75–10.89]         | 0.445                      | 7.94 [4.93–18.41]        |

**SD:** standard deviation; **IQR:** interquartile range; **BMI:** body mass index; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure; **eGFR:** estimated glomerular filtration rate; **ARR:** aldosterone/active renin ratio; **PA:** plasma aldosterone
The mean aldosterone in this study (591.52 ± 364.2 pmol/l for cases and 820.44 ± 837.36 pmol/l for controls) was lower than what has been previously reported in a Cameroonian hypertensive population by Youmbissi et al.24 (1105.23 ± 718.7 pmol/l). Likewise, our results are lower than those from some western studies conducted among Black and White Americans with primary hyperaldosteronism: 532.61 ± 296.8 and 432 ± 221.9 pmol/l, respectively.22 Our results could be explained by the fact that due to ethical issues alongside their status of high risk patients, our participants were asked not to stop their antihypertensive drugs. Indeed, Youmbissi et al.24 recruited in their study only patients whose interruption of their antihypertensive medication would be without any danger. Besides, we observed elevated concentrations of PA in controls, remembering that they were placed on no more than three antihypertensive drugs (15.9% on three medications) compared to cases who were taking at least three antihypertensive drugs (57.9% on three drugs and 42.1% on four drugs). Nonetheless, the difference in PA between cases and controls was not statistically significant (p = 0.298), even after the dynamic test (p = 0.455).

The mean concentrations of plasma active renin were: 15.08 ± 24.4 ng/l and 16.56 ± 35.12 ng/l, respectively for cases and controls. Mirroring these findings, Corbin et al.25 found a mean of 19.7 ± 2.9 ng/l in their group of hypertensive patients.25 Moreover, 50% of our study population had their level of plasma active renin < 2 ng/l, which corroborates what has been observed by Corbin et al.25 in their group of patients with confirmed primary hyperaldosteronism.

Besides, the mean levels of ARR were 319.63 ± 325.1 pmol/ng in the RH group, and 392.54 ± 365.54 pmol/ng in controls, with no difference between the two groups (p = 0.531). Our results are higher than those from Corbin et al.25 in the group of hypertensive subjects (37, 8 ± 2.9 pmol/ng), but on the contrary, are lower when compared to what was obtained in the group of patients with primary hyperaldosteronism in the same study (519 ± 99 pmol/ng).25 In Corbin et al.’s study, all hypertensive subjects with an associated co-morbidity were automatically excluded; hence, these authors probably underestimated the ARR as opposed to our study which included subjects with diabetes and RH.

After infusion of the saline solution, we observed a significant reduction in PA both in cases and controls (p < 0.001), but no patient presented a titer > 280 pmol/l which has been set as the threshold for a biological confirmation of primary hyperaldosteronism following infusion of a saline solution.26,27 Our study indicates perhaps that this threshold needs to be reconsidered in our settings. More studies in this regard are therefore warranted.

### Table 3. Correlations.

| Variables                  | Coefficient of correlation (p) |
|---------------------------|--------------------------------|
|                           | Cases | Controls | Cases + Controls |
| Potassium – Aldosterone   | −0.24 (0.321) | −0.37 (0.119) | −0.324 (0.047) |
| Potassium – Active renin   | −0.036 (0.883) | −0.106 (0.665) | −0.14 (0.401) |
| Potassium – ARR            | −0.064 (0.794) | −0.178 (0.466) | −0.011 (0.946) |
| Aldosterone – Active renin | −0.019 (0.937) | 0.178 (0.466)  | 0.11 (0.51)    |

ARR: aldosterone/active renin ratio.
Moreover, there have been some claims that the black subject presents a particular RAAS at the level of the kidneys, and this could contribute to explain our findings. Indeed, the widely known RAAS, and that we have explored in the present study is the circulating RAAS, which is responsible for the regulation of the vascular tonus and intravascular volume. Apart from this classical one, 16 other localizations of histological forms of RAAS have been identified among which the kidney.28 Like all other tissue RAAS, the renal RAAS is autonomous, and the concentrations of its components may be thereby very different from plasmatic ones. In this regard, Price and Fisher have clearly demonstrated that in black subjects, there is a low plasmatic renin activity, contrasting with an increased activity of the renal RAAS which could perhaps explain why more frequent and more severe cases of hypertension are recorded among blacks.29,30 Although this study failed to show any association between RH and primary hyperaldosteronism, based on the just-exposed facts, we cannot conclude with conviction that this association does not exist in our setting. Further studies are therefore needed, focused at exploring all the components of the RAAS in our patients, especially the renal one. Unfortunately, this study presents some flaws. Although we had a group of diabetes patients with RH and a group of matched diabetes controls with controlled hypertension, a third group of diabetes patients without hypertension could have possibly enabled us to better investigate the relation between RH and hyperaldosteronism in our context. Additionally, our small sample size could perhaps explain why we obtained very few statistically significant results. Furthermore, the measurement of aldosterone and renin in our study was performed at rest in a seated position during about 10 min (instead of at least 1 hour of rest in the supine position); this may have rendered our measurements somewhat inaccurate. Another limitation of this study lies in the fact that we did not stop the BP lowering medications of our patients, especially those which can affect the plasma renin activity or the PA concentration. We are not certain to what extent this might have affected our results and their current interpretation. Nonetheless, the dynamic test was undertaken in both cases and controls to overcome this difficulty, in conformity with Umpierrez et al.22

Conclusion
This study showed relative lower levels of kaliemia in diabetes patients with RH in comparison with their well-controlled counterparts, though the difference was not significant. Besides, we observed a negative relation between serum potassium and PA. However, this study failed to show any association between RH and primary hyperaldosteronism. Despite the stigma of primary hyperaldosteronism in our study population, there was a hyperactivity of RAAS in hypertensive patients, and even more in patients with RH. This hyperactivity of the RAAS had an electrolytic impact. Furthermore, our study confirmed the importance of potassium dosage when screening the RAAS. Further studies are needed in our milieu, which will explore all the components of RAAS and perhaps lead to the identification of a local threshold for biological confirmation of primary hyperaldosteronism.

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Ethical approval and consent to participate
Before starting the study, an authorization was obtained from authorities of the Yaoundé Central Hospital and the Yaoundé University Teaching Hospital, and an ethical clearance was delivered by the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, Cameroon. All the procedures used in the present study were in conformity with the current revision of the Helsinki Declaration. All participants were informed of the various aspects of the study, and they were enrolled only after providing a signed consent form. No patient had to stop his/her treatment during the study.

Guarantor
The guarantor is Prof Eugene Sobngwi.

Contributorship
Study conception: BEEM, VJAM, ES. Data collection: BEEM. Laboratory analysis: BEEM, VJAM, ES. Data analysis and Manuscript drafting: BEEM, VJAM, JRNN, RDN, MKM, JCK, JJNN, ES. Critical revision of manuscript: JRNN, JJNN, JCK, ES. All the authors approved the final version of the manuscript and consented for its publication.

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