*British Journal of Medicine & Medical Research*
*3(2): 428-441, 2013*

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# Serum and Urine Electrolyte Profiles during Amlodipine and Hydrochlorothiazide Combination Therapy in Nigerian Patients with Essential Hypertension

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors GBSI and EKIO designed the study, wrote the protocol and the first draft of the manuscript. Authors OOBI and SII referenced the paper, performed the statistical analysis and managed the analysis of the study. All authors read and approved the final manuscript.*

***Research Article***

***Received 2nd November 2012***
***Accepted 5th January 2013***
***Published 19th February 2013***

## ABSTRACT

**Aims:** To evaluate changes in electrolyte profiles during combination treatment with amlodipine (AML) and hydrochlorothiazide (HCZ) in hypertensive Nigerians.
**Study Design:** Randomized, open-label, prospective, two-centre, outpatient, 48-week study.
**Methodology:** We enrolled 90 male and female Nigerians aged 31-86 years with uncomplicated essential hypertension (blood pressure [BP] > 160/90 ≤ 180/120mmHg). Patients, who were 30 each (15males [M] and 15females [F]) in AML, HCZ and AML-HCZ groups, were treated, respectively, with 5mg AML for 6 weeks (wks) and the dose increased to 10mg till wk 12 (monotherapy) after which HCZ 25mg was added; HCZ 25mg till wk 6 (monotherapy) after which AML 5-10mg was added; and AML 5-10mg +

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HCZ 25mg. Body mass index (BMI), BP, 24h urine volume, serum and urine electrolytes ($Na^+$, $K^+$, $Cl^-$) were assessed at baseline and at the end of wks 1, 3, 6, 12, 24, 36 and 48 during treatment.

**Results:** The 3 regimens comparably significantly (*P*= .05) reduced BP. Diuresis was greatest and significant (*P*= .05) in HCZ group. A time dependent significant (*P*< .0001) hyponatraemic changes were observed in all subgroups except AML M subgroup such that the mean maximum M/F decrease in AML, HCZ and AML-HCZ groups, respectively, were *5.07/14.74, 17.40/16.40* and *10.93/16.86* mmol/L. A parallel significant (*P*< .01) increase in urine $Na^+$ was observed in all groups with maximum mean M/F increase in AML, HCZ and AML-HCZ groups being, respectively, *26.00/24.40*, *28.07/40.94* and *30.47/27.67* mmol/L. A baseline hypokalaemia was observed in all groups except in the AML M subgroup. Significant (*P*< .0001) M/F hypokalaemic changes were *0.23/0.35*, *0.76/0.53* and *0.18/0.19* mmol/L for AML, HCZ and AML-HCZ groups, respectively. Corresponding significant (*P*< .0001) M/F increase in urine $K^+$ were *4.60/5.71*, *10.67/18.60* and *8.2/9.3* mmol/L for AML, HCZ and AML-HCZ groups, respectively. Significant (*P*= .05) disproportionate chloraemia was observed at baseline in all groups. The observed significant (*P*< .0001) M/F hypochloraemic changes in AML, HCZ and AML-HCZ groups were, respectively, *10.60/11.46*, *25.60/26.94* and *22.93/17.67*. A significant (*P* < .0001) parallel hyperchloriuria was evident in all groups and M/F values in AML, HCZ and AML-HCZ groups were, respectively, *8.09/6.46*, *26.00/39.86* and *24.53/18.00* mmol/L.

**Conclusion:** Long-term AML and HCZ combination therapy, though effective, is associated with biochemical changes – $Na^+$, $K^+$ and $Cl^-$ depletion, thus making serum electrolytes monitoring and $K^+$ supplementation or concomitant use of a $K^+$-sparing diuretic clinically imperative.

*Keywords: Serum and urine electrolytes; amlodipine and hydrochlorothiazide; antihypertensive combination therapy; Nigerians; essential hypertension.*

## 1. INTRODUCTION

Worldwide, hypertension remains a common and treatable risk factor for cardiovascular (CV) morbidity and mortality affecting approximately one billion individuals [1-2]. Among blacks, hypertension particularly poses serious health risks because this population presents with earlier onset and more severe forms of the disease than non-blacks [3-4]. In Nigeria, hypertension is the commonest non-communicable disease representing the commonest CV cause of hospitalisation and mortality among the indigenous people [5-6].

Even though awareness and treatment of hypertension have increased over the years, substantial improvements in BP control rates are still lacking, with about two thirds of hypertensive adults aged 35-65 years failing to reach the BP target of < 140/90 mmHg [6-7]. Rather than a protracted vicious circle of ever increasing monotherapy dosages, with the potential for more adverse effects, the rapid achievement of BP goals through combination therapy of hypertension reduces patient frustration as well as increases trust in the therapeutic relationship and hence improves adherence [8].

Thus, treatment of essential hypertension has largely evolved from single drug therapy to combination of drugs exhibiting different mechanisms of action [9-12]. Particularly for those patients with stage 2 hypertension (or BP > 20/10 mmHg above goal), it is recommended that treatment begins with a combination of two drugs from different classes [2,13-14]. In

fact, data from different countries show that up to 85% of hypertensive patients may need multiple medications to help control their BP [9-12,15]. In Nigeria, calcium channel blockers (CCBs) such as AML and thiazides such as hydrochlorothiazide (HCZ) are popular antihypertensive agents for combination therapy. This is because apart from their effectiveness in specifically addressing the low renin, salt-sensitive and volume dependent hypertension [3-4, 16] that is prevalent among the people, these drugs are comparatively cheap, available and tolerable [17-20]. However, many authors [17,21-23] have expressed safety concerns over increasing evidence of adverse effects including electrolyte derangement, such as hyponatraemia, disproportionate hypochloraemia, alkalosis and hypokalaemia produced during diuretic therapy. Apart from attendant metabolic problems, according to McKibbin et al. [24] and Siscovick et al. [25], this condition predisposes to increase CV risk and lack of protection against coronary mortality.

Although CCBs are generally reported to be metabolically neutral [26], their intrinsic natriuretic and diuretic properties [27] may aggravate the electrolyte disturbances caused by thiazides [23]. Till date, information regarding the effects of AML and HCZ combination treatment on electrolyte profiles is scarce. For the aforementioned reasons and based on our earlier observations [17,28-29], we studied the long-term effects of AML and HCZ combination therapy on serum and urine electrolytes in hypertensive Nigerians.

## 2. MATERIALS AND METHODS

### 2.1 Study Population

We enrolled into the study 90 male and female Nigerians with newly diagnosed essential hypertension (stages 1 and 2) aged 31-86 years and were attending Central Hospital and Osigbemhe Hospital both in Auchi in Edo State of Nigeria between March 2008 and March 2009. The sample size was estimated based on the number of Nigerians that are believed to be hypertensive [3]; and to detect a difference of 2 units in mean change in the measured variables, between both treatment arms with a power equal to 90% using a one sample t-test at a one-sided significance level of .05, requires 30 patients per group. Eligible participants had qualifying hypertension of BP > 160/90 and ≤ 180/120 mmHg measured on at least 2 occasions in lying/supine, sitting and standing positions using standardized methods [30]. Excluded were patients with identifiable cause of the hypertension, clinical evidence of cerebrovascular, cardiac, renal, hepatic, gastrointestinal or endocrinologic disease, hypersensitivity to AML and HCZ or related drugs, history of smoking, alcohol intake, substance abuse or mental illness. Also excluded were patients needing any concomitant medication eg digitalis, non-steroidal anti-inflammatory drugs, psychotropic drugs, monoamine oxidase inhibitors or oral contraceptives, that may interact with the trial drugs and pregnant or lactating females.

The research protocol was reviewed and approved by the Ethics Committees of Irrua Specialist Teaching Hospital Irrua, Nigeria (Ambrose Alli University College of Medicine Teaching Hospital) and Central Hospital Auchi, Nigeria. After suitable explanation of the study protocol in lay language, all literate patients gave informed written consent and the illiterates thumb-printed the consent form before the beginning of the study.

## 2.2 Study Design

Subjects were examined by a standardized pre-tested questionnaire seeking information on demographic data, the history of hypertension, current drugs if any, educational and social status, dietary habits, smoking and alcohol intake, etc. The 90 patients were randomized to three groups (AML, HCZ and AML-HCZ groups) each comprising 30 patients divided into subgroups of 15 M + 15 F using computer program-generated random numbers. AML group was treated initially with AML 5 mg and the dose was doubled after 6 weeks if BP was not controlled. Then after 12 wks (end of monotherapy), HCZ 25 mg was added if goal BP was not still achieved. HCZ group was treated initially with HCZ 25 mg for 6 weeks (end of monotherapy), after which AML 5 mg was added if BP was uncontrolled. The dose of AML was doubled after 12 wks if BP was still not normalized. AML-HCZ group was initially treated with AML 5 mg + HCZ 25 mg. If BP was not controlled after 6 weeks, the dose of AML was doubled.

The outpatient treatment lasted 48 weeks. The patients were monitored closely and outcome measures evaluated at baseline before treatment as well as at the end of wks 1, 3, 6, 12, 24, 36 and 48. Unequivocal patient identification was possible via a patient identification list consisting of the patient number, first name and surname.

## 2.3 Measurements of Heights (m), Weights (wt) (kg) and BP (mmHg)

A stadiometer scale (Seca model, UK) was used for measuring height, with no shoes on; and a beam balance (Hackman, UK) was used to measure wt while on light clothing. BMI was computed as wt divided by height squared. SBP and DBP were measured with a standard mercury sphygmomanometer (Riester Diplomat Presameter, Germany) using standardized methods [30] at the sitting, standing and supine positions; always between 8am and 10am. All constricting clothing on the upper arm was removed before any measurement and subjects were discouraged from talking or moving during measurements. The first phase of the Korotkov sound was regarded as the SBP while the fifth phase was regarded as the DBP. During measurement, readings were taken two consecutive times with an interval of at least one minute and the average recorded. During the study, subjects were not told the results of BP measurement.

## 2.4 Pharmacotherapy Intervention

AML group was treated initially with AML 5 mg and the dose was doubled after 6 weeks if BP was not controlled. Then after 12 wks (end of monotherapy), HCZ 25 mg was added if goal BP was not still achieved. HCZ group was treated initially with HCZ 25 mg for 6 weeks (end of monotherapy), after which AML 5 mg was added if BP was uncontrolled. The dose of AML was doubled after 12 wks if BP was still not normalized. AML-HCZ group was initially treated with AML 5 mg + HCZ 25 mg. If BP was not controlled after 6 weeks, the dose of AML was doubled. The outpatient treatment lasted 48 weeks. The patients were monitored closely and outcome measures evaluated at baseline before treatment as well as at the end of wks 1, 3, 6, 12, 24, 36 and 48 during treatment. Unequivocal patient identification was possible via a patient identification list consisting of the patient number, first name and surname.

The study medications AML and HCZ are licensed for long-term treatment of hypertension so that dangerous side effects due to the medicaments were not to be expected. AML 5mg

and 10mg tablets (Amlovar$^{R}$), were donated by Neimeth International Pharmaceuticals Ikeja, Nigeria: NAFDAC Reg No A4-0333; Manufacturing Date 07-2007 and Expiry Date 07-2010. HCZ 25 mg tablets (Esidrex$^{R}$) were donated by Novartis Pharma SAS Nigerian Representative, NAFDAC Reg No OL-3705, Manufacturing Date 08-2007 and Expiry Date 08-2010.

## 2.5 Course of Study and Methods for Recording Efficacy and Safety

All patients were advised to maintain their usual diet (weight-maintaining no-salt-added diet) and regular physical activity but to avoid undue stress throughout the duration of the study. They were instructed to take their drugs every morning. Each patient was observed for about 2 hours after taking medication drug for the first time. Adherence in respect of intake of medication was encouraged by interviewing patients through phone calls, sporadic visits and pill counts outside the view of patients. To preclude white-coat effect, observer bias and to accurately assess the efficacy of the drugs, patients were followed up repeatedly at weeks 1, 3,6,12, 24, 36, and 48. At each visit, volunteered or spontaneous report of adverse events were assessed for severity and association with treatment; and the attending physicians/investigators also recorded any adverse events they observed themselves or elicited from the patient through careful interrogation like “How do you feel?” No patient withdrew from the study because of adverse events.

Response to therapy as regards the BP measurement was defined as a decrease in the mean trough sitting SBP and DBP of 10 mmHg or a drop to < 90 mmHg with reduction of > 5 mmHg. BP was regarded as controlled if the DBP was < 90 mmHg and SBP < 140 mmHg. The effects of treatment on the various variables (except height) were assessed by comparing the values at each visit with the pretreatment baseline values.

## 2.6 Collection of Samples and Analysis

### 2.6.1 Urine

Each subject collected a 24h urine sample into a plastic container from Sunday 7am to Monday 7am at baseline (week 0) before treatment and on the evaluation days. The need to carefully collect all urine passed was well emphasized. The volume of 24h urine was measured with a measuring cylinder and recorded on each evaluation day as well as urine $Na^{+}$, $K^{+}$ and $Cl^{-}$ which were measured with an ion-selective electrolyte analyser branded Biolyte 2000 (BioCare Corporation, Hsinchu 300, Taiwan).

### 2.6.2 Blood

At baseline and at the end of weeks 1, 3, 6, 12, 24, 36, and 48, 10ml of venous blood was obtained from every patient by peripheral venepuncture into a plain sterile bottle. From the prepared serum sample of each subject serum $Na^{+}$, $K^{+}$ and $Cl^{-}$ were assayed using ion-selective electrolyte analyser.

## 2.7 Statistical Analysis

All data are presented as mean ± SEM or mean ± SD (for age, height and weight) using the general linear model procedure (PROC GLM) of the statistical analysis system (SAS) (2004). Where significant differences were noticed, mean separation was carried out using Duncan

Multiple Range Test. Correlation between two sets of variables was determined using Spearman's rank correlation. *P* = .05 was regarded as significant in all cases.

## 3. RESULTS AND DISCUSSION

Table 1 shows that at baseline (week 0), there was no statistically significant difference observed in the M vs F patients in the AML, HCZ and AML-HCZ groups, respectively, with regard to the means of ages, BMIs as well as the SBP and DBP. Most of the patients had significant (stage 2) hypertension. The analysis excluded the data for a M patient in the AML group who travelled and so could not report for evaluation at week 48; a F from AML group who became pregnant and so was withdrawn from the study between weeks 36 and 48 as well as a M patient whose week 36 serum specimen was lost in the AML-HCZ group.

**Table 1. Demographic characteristics and baseline blood pressures of subjects**

| | | Male | | Female | |
|---|---|---|---|---|---|
| **Group** | **Characteristics** | **Range** | **Mean±SD/SEM*** | **Range** | **Mean±SD/SEM** |
| **AML** | Age (yrs) | 31-80 | 60.80±14.03 | 37-80 | 63.60±8.02 |
| | Height (m) | 1.59-1.74 | 1.66±0.04 | 1.56-1.76 | 1.66±0.07 |
| | Weight (kg) | 62-88 | 76.77±8.94 | 62-90.4 | 79.80±8.85 |
| | BMI (kg/m$^2$) | 24.84-29.75 | 26.50±0.80 | 25.48-30.13 | 27.50±0.31 |
| | SBP (mmHg) | 150-175 | 162.50±2.82 | 150-180 | 165.67±3.00* |
| | DBP (mmHg) | 100-115 | 103.00±1.75 | 90-115 | 105.33±2.04* |
| **HCZ** | Age (yrs) | 45-86 | 63.80±12.02 | 48-80 | 65.47±10.15 |
| | Height (m) | 1.61-1.80 | 1.69±0.05 | 1.12-1.76 | 1.67±0.04 |
| | Weight (kg) | 68-89 | 75.37±6.27 | 60-80 | 73.44±6.30 |
| | BMI (kg/m$^2$) | 26.25-27.47 | 26.50±0.32 | 23.17-26.67 | 24.50±0.42 |
| | SBP (mmHg) | 160-180 | 170.33±2.09* | 160-180 | 169.00±1.90* |
| | DBP (mmHg) | 95-115 | 104.67±1.50* | 90-115 | 104.67±1.98* |
| **AML – HCZ** | Age (yrs) | 42-78 | 62.13±11.89 | 48-87 | 69.93±11.60 |
| | Height (m) | 1.58-1.72 | 1.64±0.04 | 1.59-1.82 | 1.67±0.06 |
| | Weight (kg) | 65-87.5 | 74.33±7.07 | 65-90.2 | 77.21±8.67 |
| | BMI (kg/m$^2$) | 26.10-29.66 | 27.50±0.38 | 25.79-27.33 | 26.00±0.56 |
| | SBP(mmHg) | 150-180 | 165.00±4.57* | 155-180 | 167.50±4.44* |
| | DBP(mmHg) | 100-115 | 106.00±1.48* | 90-120 | 107.33±2.01* |

*Characteristics and BPs in the groups are not significantly different; AML, Amlodipine; HCZ, Hydrochlorothiazide, AML-HCZ, Amlodipine-hydrochlorothiazide combination; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; *, Standard error of mean; (N = 30 [15M + 15F] per group)*

Duration and treatment effect on the SBP and DBP were significant (*P* = .05) and gender effect was also significant (*P* < .001), for the differences in M values were higher than in F. The 3 treatment regimens comparably significantly (*P* = .05) reduced BP. At week 48 M vs F reductions in SBP/DBP in AML, HCZ and AML-HCZ groups were, respectively, 37.88/30.86 vs 37.75/31.76, 37.66/28.67 vs 34.43/27.34 and 33.00/30.33 vs 31.67/31.33 mmHg. Diuresis

was greatest and significant (*P*= .05) in HCZ group at week 1 with M vs F AML, HCZ and AML-HCZ levels being, respectively, 1523.33+/-57.34 vs 1486.00+/-56.09, 1621.33+/-36.49 vs 1586.67+/-37.01 and 1506.00+/-42.26 vs 1514.67+/-45.30ml.

The effects of treatment drugs on serum $Na^+$ ($SNa^+$) are presented in Table 2. AML M subgroup had a significant (*P* = .05), lower pretreatment values than their counterparts. However, this difference disappeared during treatment. A time-dependent significant (*P* < .0001), hyponatraemic changes were observed in all groups except AML M subgroup such that the mean maximum M/F decrease (mmol/L) in AML, HCZ and AML-HCZ groups, respectively, were 5.07/14.74 at wk 12, 17.40/16.40 at wk 12 and 10.93/16.86 at wk 24. However after this period, $SNa^+$ stabilized, decreasing towards baseline values. Treatment and gender effects were significant (P < .0001 and P < .02, respectively) in all groups. $SNa^+$ was positively correlated with urine $Na^+$ ($UNa^+$) (r= .2091, *P* = .0001). A parallel significant (*P* < .01) increase in $UNa^+$ was observed in all groups such that maximum mean M/F increase (mmol/L) in AML, HCZ and AML-HCZ groups were, respectively, 26.00/24.40 at wk 6, 28.07/40.94 at wk 12 and 30.47/27.67 at wk 24 (Table 3). The hypernatriuria decreased soon after with the urine loss decreasing towards baseline. Treatment effect was significant (P < .03) in week 6 (M subgroups) while gender effect was not.

The effects of treatment drugs on serum $K^+$ ($SK^+$) are shown in Table 4. A baseline relative hypokalaemia was observed in all groups except in the AML M subgroup. Significant (*P* < .0001) M/F hypokalaemic changes (mmol/L) were 0.23/0.35, 0.76/0.53 and 0.18/0.19 for AML, HCZ and AML-HCZ groups, respectively. The drug effect was significant (*P* < .0001) in all groups, while the gender effect was not. As displayed in Table 5, corresponding parallel time dependent significant (*P* < .0001) M/F increase (mmol/L) in urine $K^+$ ($UK^+$) excretion were 4.60/5.71 at wk 12 (M) and at wk 48 (F), 10.67/18.60 at wk 12 and 8.2/9.3 at wk 12 (M) and 48 (F) for AML, HCZ and AML-HCZ groups, respectively. Thus, treatment effect was significant (P< 0.0001) in all groups, while the gender effect was not.

Significant (*P* = .05) disproportionate chloraemia was observed at baseline in the groups (Table 6). The observed significant (*P* < .0001) maximum M/F hypochloraemic changes (mmol/L) in AML, HCZ and AML-HCZ groups were, respectively, 10.60/11.46 at wk 36 (M) and at wk 48 (F), 25.60/26.94 at wk 12 and 22.93/17.67 at wk 24. Treatment effect among groups was significant (*P* < .0001) while the gender effect was not. A significant (*P* < .0001) parallel hyperchloriuria was evident in all groups and maximum M/F increase (mmol/L) in AML, HCZ and AML-HCZ groups were, respectively, 8.09/6.46, 26.00/39.86 and 24.53/18.00 (Table 7). Treatment effect was significant (*P* < .0001) but the gender effect was not.

**Table 2. Effects of AML and HCZ combination therapy on serum Na+ (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | 137.27±2.25$^{b}$ | 147.60±1.88$^{a}$ | 144.00±1.78$^{a}$ | 146.87±2.60 | 146.20±2.32 | 149.53±2.71 | 0.025$^{*}$ |
| 1 | 135.67±2.57 | 140.67±2.23$_{A}$ | 141.40±1.52 | 145.00±2.89 | 139.73±1.81$_{A}$ | 145.13±2.25 | |
| 3 | 133.73±2.52 | 132.40±1.99$_{B}$ | 138.47±1.46$_{A}$ | 141.73±2.41$^{a}{}_{A}$ | 131.40±1.74$^{b}{}_{B}$ | 139.07±2.31$^{a}{}_{A}$ | |
| 6 | 132.20±2.42 | 132.80±1.72$_{B}$ | 135.67±1.42$_{B}$ | 140.53±2.21$^{a}{}_{A}$ | 130.47±1.54$^{b}{}_{B}$ | 135.00±2.25$_{B}$ | |
| 12$^{\$}$ | 132.40±2.33 | 130.20±1.66$_{B}$ | 135.27±1.61$_{B}$ | 132.13±7.18$_{B}$ | 128.60±1.87$_{B}$ | 133.80±1.89$_{B}$ | |
| 24$^{\$}$ | 134.33±1.82 | 131.40±1.52$_{B}$ | 133.07±1.31$_{B}$ | 137.80±1.61$^{a}{}_{B}$ | 130.47±1.35$^{b}{}_{B}$ | 132.67±1.64$^{b}{}_{B}$ | |
| 36 | 134.33±1.49 | 132.87±1.34$_{B}$ | 134.27±1.22$_{B}$ | 135.86±1.55$^{a}{}_{B}$ | 130.20±1.20$^{b}{}_{B}$ | 134.67±1.31$^{a}{}_{B}$ | |
| 48 | 135.29±1.48 | 132.67±1.23$_{B}$ | 133.73±0.78$_{B}$ | 135.43±1.37$^{a}{}_{B}$ | 130.73±1.12$^{b}{}_{B}$ | 134.00±0.98$_{B}$ | |

*Significant differences within columns are indicated by AB and within rows by ab (P< 0.05): Significant treatment effect occurred in females more than males and except in AML male subgroup, treatment regimens induced significant time-dependent hyponatraemia; *, P = 0.05; other abbreviations are as used in Table 2; (N = 15 per subgroup)*

**Table 3. Effects of AML and HCZ combination therapy on urine Na+ (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | 256.00±6.56 | 265.20±6.98 | 256.00±6.20 | 253.20±11.18 | 247.33±9.32 | 261.60±5.67 | 0.030$^{*}$ |
| 1 | 266.73±6.42 | 284.20±6.35$_{A}$ | 265.13±5.89 | 258.40±11.62 | 280.07±8.20$_{A}$ | 271.33±5.86 | |
| 3 | 275.33±5.89 | 289.20±5.25$_{A}$ | 270.80±6.22 | 269.33±11.76$_{A}$ | 282.73±8.38$_{A}$ | 277.73±5.87 | |
| 6 | 282.00±9.65$_{A}$ | 287.07±4.49$_{A}$ | 276.53±10.26 | 274.60±11.67$_{A}$ | 285.47±8.11$_{A}$ | 283.40±5.93$_{A}$ | |
| 12$^{\$}$ | 276.87±6.91$^{a}$ | 293.27±4.20$^{b}{}_{A}$ | 279.00±5.84$_{A}$ | 277.00±11.58$_{A}$ | 288.27±7.92$_{A}$ | 286.80±7.05$_{A}$ | |
| 24$^{\$}$ | 274.47±6.91 | 288.00±4.13$_{A}$ | 286.47±5.20$_{A}$ | 268.27±12.18 | 269.20±7.98 | 289.27±5.29$_{A}$ | |
| 36 | 267.47±5.71 | 286.13±3.70$_{A}$ | 274.00±5.14 | 263.07±12.62 | 264.27±7.65 | 276.73±5.12 | |
| 48 | 270.07±4.62 | 278.00±3.63 | 268.80±4.45 | 262.14±11.04 | 269.80±7.67 | 275.80±4.50 | |

*Significant differences within columns are indicated by AA and within rows by ab (P = .05): Treatment effect was significant in males (week 12), and significant hypernatriuria occurred in all subgroups; *, P< 0.05; other abbreviations are as used in Table 2; (N = 15 per group)*

**Table 4. Effects of AML and HCZ combination therapy on serum K+ (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | 3.54±0.11$^{b}$ | 4.23±0.10$^{a}$ | 3.68±0.10$^{b}$ | 3.65±0.09$^{b}$ | 3.91±0.06$^{a}$ | 3.61±0.11$^{b}$ | 0.061$^{NS}$ |
| 1 | 3.54±0.10$^{b}$ | 3.97±0.09$^{a}{}_{A}$ | 3.66±0.10$^{b}$ | 3.55±0.09$^{b}$ | 3.82±0.06$^{a}$ | 3.61±0.11$^{ab}$ | |
| 3 | 3.35±0.00$^{b}$ | 3.84±0.09$^{a}{}_{A}$ | 3.61±0.10$^{a}$ | 3.49±0.09$_{A}$ | 3.69±0.06$_{A}$ | 3.52±0.10 | |
| 6 | 3.28±0.07$^{b}$ | 3.67±0.09$^{a}{}_{A}$ | 3.56±0.01$^{a}$ | 3.44±0.08$_{A}$ | 3.54±0.07$_{A}$ | 3.51±0.11 | |
| 12$^{\$}$ | 3.26±0.07$^{b}$ | 3.59±0.08$^{a}{}_{B}$ | 3.54±0.10$^{a}$ | 3.39±0.07$_{A}$ | 3.47±0.06$_{A}$ | 3.47±0.11 | |
| 24$^{\$}$ | 3.34±0.08 | 3.51±0.07$_{B}$ | 3.43±0.11 | 3.36±0.07$_{A}$ | 3.43±0.05$_{A}$ | 3.43±0.11 | |
| 36 | 3.32±0.31 | 3.47±0.07$_{B}$ | 3.55±0.09 | 3.34±0.06$_{A}$ | 3.41±0.06$_{A}$ | 3.42±0.10 | |
| 48 | 3.31±0.09 | 3.45±0.07$_{B}$ | 3.50±0.09 | 3.30±0.06$_{B}$ | 3.39±0.05$_{B}$ | 3.42±0.10 | |

*Significant differences within columns are indicated by AB and within rows by ab (P = .05): Baseline relative hypokalaemia, significant treatment effects (more marked in males) and time-dependent hypokalaemic changes are demonstrated; other abbreviations are as used in Table 2; (N = 15 per subgroup)*

**Table 5. Effects of AML and HCZ combination therapy on urine K+ (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | 84.33±3.53$^{a}$ | 78.93±1.79 | 75.60±2.83$^{b}$ | 82.93±3.00 | 78.00±1.92 | 75.47±3.20 | 0.419$^{NS}$ |
| 1 | 85.93±3.52 | 87.53±1.89$_{A}$ | 79.73±2.79 | 84.40±2.81 | 86.20±1.83 | 79.60±3.24 | |
| 3 | 87.27±3.10 | 88.87±1.98$_{A}$ | 82.00±2.82 | 86.07±2.76$^{b}$ | 90.27±1.58$^{a}{}_{A}$ | 81.47±3.21$^{b}$ | |
| 6 | 88.53±3.10 | 87.20±1.53$_{A}$ | 83.47±2.59 | 85.67±2.81 | 94.73±1.44$_{A}$ | 83.80±2.73 | |
| 12$^{\$}$ | 88.93±3.18 | 89.60±1.82$_{A}$ | 83.80±2.54 | 86.20±3.00 | 96.60±1.47$_{A}$ | 84.60±2.73 | |
| 24$^{\$}$ | 86.10±2.99 | 86.13±1.92$_{A}$ | 82.47±2.41 | 85.60±2.65 | 84.87±1.48 | 84.40±2.71 | |
| 36 | 85.20±2.95 | 86.27±1.45$_{A}$ | 81.86±2.31 | 86.64±2.52 | 83.27±1.33$^{a}$ | 81.53±2.51$^{b}$ | |
| 48 | 85.79±1.58 | 85.67±1.31$_{A}$ | 80.67±2.13 | 88.64±2.11$^{a}$ | 84.63±1.41$^{a}$ | 78.87±2.74$^{b}$ | |

*Significant differences within columns are indicated by AA and within rows by ab (P = .05): Significant treatment effect and time-dependent increase in urine K+ excretion maximal at week 12 and more marked in HCZ and AML-HCZ subgroups are indicated; other abbreviations are as used in Table 2; (N = 15 per subgroup)*

**Table 6. Effects of AML and HCZ combination therapy on serum Cl- (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | $91.20\pm4.51^{b}$ | $120.80\pm3.29^{a}$ | $111.33\pm4.53^{a}$ | $89.60\pm4.71^{c}$ | $123.07\pm2.08^{a}$ | $106.07\pm6.42^{b}$ | $0.183^{NS}$ |
| 1 | $87.13\pm5.37^{b}$ | $112.67\pm4.07^{a}$ | $105.13\pm4.57^{a}$ | $87.67\pm4.68^{b}$ | $116.13\pm2.42^{a}{}_{A}$ | $99.40\pm5.78^{b}$ | |
| 3 | $88.47\pm3.99^{b}$ | $107.07\pm3.40^{a}{}_{A}$ | $98.33\pm4.48_{A}$ | $86.87\pm5.00^{b}$ | $105.00\pm2.67^{a}{}_{B}$ | $95.00\pm5.38$ | |
| 6 | $86.47\pm3.95^{b}$ | $98.33\pm2.91^{a}{}_{A}$ | $93.67\pm4.09_{A}$ | $84.53\pm5.04^{b}$ | $96.60\pm2.05^{a}{}_{C}$ | $91.47\pm4.70$ | |
| $12^{\$}$ | $85.53\pm3.98^{b}$ | $95.20\pm2.81^{a}{}_{A}$ | $95.67\pm4.05_{A}$ | $85.00\pm3.66$ | $96.13\pm2.25_{C}$ | $88.93\pm4.93_{A}$ | |
| $24^{\$}$ | $83.00\pm3.27^{b}$ | $96.67\pm2.97^{a}{}_{A}$ | $88.40\pm4.36^{a}{}_{B}$ | $83.87\pm3.53^{b}$ | $98.80\pm1.99^{a}{}_{C}$ | $88.40\pm4.36^{b}{}_{A}$ | |
| 36 | $80.60\pm3.04^{b}$ | $95.53\pm3.35^{a}{}_{A}$ | $94.00\pm3.18^{a}{}_{A}$ | $80.64\pm3.61^{b}$ | $100.80\pm2.29^{a}{}_{C}$ | $89.27\pm4.13^{b}{}_{A}$ | |
| 48 | $83.10\pm2.73^{b}$ | $97.20\pm3.23^{a}{}_{A}$ | $94.67\pm3.12^{a}{}_{A}$ | $78.14\pm2.34^{c}$ | $100.73\pm1.87^{a}{}_{C}$ | $89.10\pm4.09^{b}{}_{A}$ | |

*Significant differences within columns are indicated by ABC and within rows by abc (P = .05): A baseline disproportionate chloraemia, significant treatment effects and hypochloraemic changes are shown; other abbreviations are as used in Table 2; (N = 15 per subgroup)*

**Table 7. Effects of AML and HCZ combination therapy on urine Cl- (mmol/L) in hypertensive subjects for 48 weeks**

| | Treatment subgroups (Male) | | | Treatment subgroups (Female) | | | |
|---|---|---|---|---|---|---|---|
| **Week** | **AML** | **HCZ** | **AML-HCZ** | **AML** | **HCZ** | **AML-HCZ** | **Gender effect** |
| 0 | $160.20\pm14.45^{b}$ | $217.93\pm9.36^{a}$ | $207.40\pm2.98^{a}$ | $152.47\pm10.64^{b}$ | $200.67\pm8.57^{a}$ | $208.13\pm13.60^{a}$ | $0.969^{NS}$ |
| 1 | $172.67\pm14.34^{b}{}_{B}$ | $241.27\pm8.14^{a}{}_{C}$ | $213.60\pm2.00^{a}{}_{C}$ | $155.60\pm10.90^{b}$ | $242.00\pm7.88^{a}{}_{A}$ | $228.40\pm12.80^{a}{}_{C}$ | |
| 3 | $177.27\pm14.79^{b}{}_{B}$ | $267.47\pm7.38^{a}{}_{B}$ | $238.00\pm3.48^{a}{}_{B}$ | $163.60\pm10.78^{b}{}_{A}$ | $260.13\pm7.48^{a}{}_{A}$ | $241.93\pm12.40^{a}{}_{B}$ | |
| 6 | $189.93\pm14.88^{b}{}_{A}$ | $265.13\pm9.36^{a}{}_{B}$ | $241.93\pm12.67^{a}{}_{A}$ | $165.27\pm10.93^{b}{}_{A}$ | $264.00\pm7.73^{a}{}_{A}$ | $251.60\pm12.43^{a}{}_{A}$ | |
| $12^{\$}$ | $174.27\pm14.81^{b}{}_{B}$ | $277.07\pm8.71^{a}{}_{A}$ | $241.13\pm12.08^{a}{}_{A}$ | $173.07\pm12.45^{b}{}_{A}$ | $269.00\pm7.96^{a}{}_{A}$ | $258.07\pm11.75^{a}{}_{A}$ | |
| $24^{\$}$ | $170.10\pm13.07^{b}{}_{B}$ | $249.67\pm9.57^{a}{}_{C}$ | $246.20\pm11.72^{a}{}_{A}$ | $156.93\pm11.65^{b}$ | $238.60\pm8.25^{a}{}_{B}$ | $266.00\pm12.05^{a}{}_{A}$ | |
| 36 | $162.00\pm12.01^{b}{}_{B}$ | $247.07\pm8.77^{a}{}_{C}$ | $232.92\pm12.21^{a}{}_{B}$ | $158.86\pm9.99^{b}$ | $244.67\pm8.62^{a}{}_{B}$ | $248.13\pm12.29^{a}{}_{B}$ | |
| 48 | $168.29\pm10.01^{b}{}_{B}$ | $243.93\pm7.41^{a}{}_{C}$ | $231.93\pm11.50^{a}{}_{B}$ | $158.93\pm8.00^{b}$ | $240.53\pm8.72^{a}{}_{B}$ | $226.13\pm12.61^{a}{}_{C}$ | |

*Significant differences within columns are indicated by ABC and within rows by ab (P = .05): Differences in baseline urine Cl- excretion, treatment effect and time-dependent increase in urine Cl- excretion are significant, other abbreviations are as used in Table 2; (N = 15 per subgroup)*

Hyponatraemic changes were comparable in all groups. The decrease in $SNa^+$ concentration and increase in $UNa^+$ concentration in all groups suggests that the patients were salt ($Na^+$) sensitive. The fact that the levels stabilized even though $UNa^+$ excretion continued implies a physiological adaptation and the possible influence of increased dietary salt intake since the diet was not controlled. Salt sensitivity, defined as a change in BP in response to changes in salt and water homeostasis is the hallmark of hypertension in majority of blacks, the obese and the elderly [31-32]. Large meta-analyses examining the results of studies on the impact of dietary salt on BP showed that there was a benefit of salt reduction in this category of hypertensives a substantial number of whom had to discontinue antihypertensive therapy or had their medications drastically reduced [33-34]. Since life-style is an important determinant of our physical health and its modification is an effective public health tool for successful treatment and control of hypertension [2,17,35]. The World Health Organisation/International Society of Hypertension Joint Committee has recommended that all individuals, particularly hypertensives and those at risk should adopt the low $Na^+$ DASH-diet [36].

A baseline relative hypokalaemia, which could compromise vascular function, was observed even before treatment. We reported in our previous communication [17] that, this could well be a vital factor in the pathogenesis of hypertension in this rural/sub-urban population. It is known that in various human populations, hypertension is closely correlated with low $K^+$ intake. Majority of subjects participating in this study were the elderly who perhaps because of poverty eat less $K^+$ and become susceptible to hypokalaemia and the few rich who eat more $Na^+$ and waste more $K^+$ [35]. Treatment type and duration significantly affected $SK^+$ such that M vs F $K^+$ loss by week 48 was 0.23 vs 0.35 (AML), 0.76 vs 0.52 (HCZ) and 0.18 vs 0.19 mmol/L (AML-HCZ). Thus HCZ caused a more significant $K^+$ depletion while AML-HCZ caused the least, demonstrating the advantage of combination therapy [10].

In a review of published series, the fall in $SK^+$ with diuretic therapy of hypertension was found to average 0.67 mmol/L [37]; and the percentage of diuretic-treated patients who develop hypokalaemia varies from 0 to 40%, the differences reflecting variable dietary $Na^+$ and $K^+$ intake, the degree of secondary aldosteronism invoked by diuretics and the concomitant use of other interacting drugs [17,36]. In this study, however, 83% of subjects, developed hypokalaemia and the average $SK^+$ loss approximated to 0.38 mmol/L. This observation underscores the importance of monitoring $SK^+$ before and during diuretic therapy and justifies the use of HCZ with a $K^+$- sparing diuretic [36]. The adverse consequences of hypokalaemia, $SK^+$ concentration < 3.5mmol/L, include cardiac arrhythmias, muscle weakness and other problems. It has been suggested that the adverse effects of thiazides on lipid levels and on glucose tolerance are, in part, a consequence of $K^+$ depletion. The ATP-sensitive $K^+$ channels are an essential component of glucose-dependent insulin secretion in pancreatic islet ß-cells. Because the initial response of increased insulin release by pancreatic ß -cells to glucose depends on normal $SK^+$ levels, diuretic-induced hypokalaemia may cause postprandial hyperglycaemia [38].

## 4. CONCLUSION

Long-term AML and HCZ combination therapy, though effective, is associated with adverse biochemical changes – $Na^+$, $K^+$ and $Cl^-$ depletion. The associated robust natriuresis and diuresis suggest that the patients were salt-sensitive and so may benefit from salt restriction as a lifestyle intervention to control hypertension. The observed significant positive correlations between SBP and $SNa^+$ as well as $UNa^+$ and between DBP and $SNa^+$, support the evidence that $Na^+$ is positively related to hypertension. The observed baseline hypokalaemia and inverse relationship between $SK^+$ and BP may be important in the

aetiopathogenesis of essential hypertension in the population. Thus, monitoring of serum electrolytes is clinically imperative in this population before and during diuretic therapy. Data from this study also imply that $K^+$ supplementation or the addition of a $K^+$-sparing diuretic may be indicated during treatment. To our knowledge, this is the first report of the effects of combination treatment with AML and HCZ on electrolyte profiles in hypertensive Nigerians. However, because of the relatively small sample size, further research with larger sample sizes is necessary to validate our results.

## ACKNOWLEDGEMENTS

The authors are indebted to the Managements of the hospitals and patients used for this study as well as Neimeth International Pharmaceuticals, Nigeria for donation of amlodipine (Amlovar$^R$) tablets and the Nigerian Representative of Novartis Pharma SAS France for donation of hydrochlorothiazide (Esidrex$^R$) tablets for the study.

## CONSENT

All authors declare that written informed consent was obtained from the patients before the commencement of this study.

## ETHICAL APPROVAL

All authors hereby declare that this research protocol has been reviewed and approved by the Ethics Committees of Irrua Specialist Teaching Hospital, Irrua, Nigeria as well as that of Central Hospital Auchi, Nigeria and the research has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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*The peer review history for this paper can be accessed here:*
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