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Original Article

Evaluation of selected trace metals in some hypertensive subjects in a tertiary health institution in Southwest Nigeria.

Anaelechi J. Onuegbu[1], Olugbenga E. Ayodele[2] , Olubunmi G. Ayelagbe[3*], Madu J. Olisekodiaka[1], Rasheeda A. Abiola[4], Ubuo K. Amah[1], Isaac L. Ukeh[5]

[1]Department of Chemical Pathology, College of Medicine, Nnamdi Azikiwe University, Awka, Nigeria. [2]Department of Medicine, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. [3]Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. [4]Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. [5]International Institute of Tropical Agriculture (IITA), Idi-Ose, Ibadan, Nigeria

*Corresponding Author: Olubunmi G. Ayelagbe, Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. E-mail: bunmi.ayelagbe@gmail.com

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ABSTRACT: Published reports on the possible roles of trace metals in the aetiology of primary hypertension have not been consistent. This study investigated the possible aetiological role of zinc (Zn), manganese (Mn), copper (Cu) and selenium (Se) in primary hypertension. Atomic absorption spectrophotometry (AAS) was used to determine the serum levels of Zn, Cu, Mn and Se in 45 patients with primary hypertension (stage I and stage II) and 47 apparently healthy control subjects (normotensives and pre-hypertensives). Both patients and control subjects were classified based on the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7). The weight, height and blood pressure of all subjects were measured and their body mass indices (BMI) computed. The mean serum zinc concentration was significantly higher in the patients with hypertension than in the control subjects (135.78 ± 9.10 vs 130.80 ± 12.50 μg/ml, p = 0.032). However, serum levels of copper (68.16 ± 3.72 vs 68.53 ± 5.33 μg/dl, p = 0.697), manganese (63.11 ± 4.40 vs 62.87 ± 4.59 μg/dl, p = 0.800) and selenium (75.91 ± 5.66 vs 78.13 ± 5.92 μg/L, p = 0.070) were not statistically different between the patients and the control subjects. This study did not show any gender-, age- or obesity-related differences in serum level of zinc, copper, manganese and selenium. Elevated level of serum zinc may play an aetiological role in subjects with primary hypertension. However, further studies will be necessary to define the roles of trace elements in the aetiology of primary hypertension in these individuals.

KEYWORDS: hypertension; zinc; manganese; copper; selenium.

INTRODUCTION

Hypertension is one of the most prevalent diseases in developed and developing countries. The estimated total number of adults with hypertension in the year 2000 was 972 million: 333 million in economically developed countries, and 639 million in economically developing countries. This number is projected to increase by 60 % to a total of 1.56 billion in the year 2025. Most of the increase (i.e. a rise of 80 % from 639 million to 1.15 billion) will be in economically developing countries like Nigeria (Kearney *et al.*, 2005). Hypertension is an important modifiable risk factor for strokes, congestive heart failure, end-stage renal disease and vascular diseases (Flack *et al.*, 1995).

Hypertension, is defined in adults (≥18 years of age) as a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure of ≥ 90 mmHg (Chobanian *et al.*, 2003; Mancia *et al.*, 2007). Hypertension can be primary or secondary. Primary (essential) hypertension refers to elevated blood pressure with no clear identifiable cause. Secondary hypertension on the other hand, refers to elevated blood pressure resulting from a specific and potentially treatable cause such as renal disease, endocrine causes or drugs

(Camm & Bunce, 2005). Though primary hypertension has no identifiable cause, there are known predisposing factors. The predisposing factors to primary hypertension include obesity, sedentary lifestyle, high salt intake, low calcium intake, low potassium intake, increasing age, low birth weight, familial predisposition, autonomic imbalance, and likely trace metals (Whelton, 1994).

Trace metals make up less than 0.01 per cent of the body's dry weight (Crook, 2006). They may be essential or non–essential micronutrients. Sources of trace metals include both plant and animal foods. Plant foods tend to be rich sources of trace elements such as copper and manganese while animal products provide most of the zinc in diets (Anderson & Zlotkin, 2010). A total of 28 elements have been documented to play a role in blood pressure control. However, the role of these elements in the aetiology and control of blood pressure has not been fully elucidated (Loyke, 2002). The individual elements react directly and indirectly in a variety of metabolic and structural activities known to participate in blood pressure regulation[9]. Contradictory results have been published about the relation between copper, selenium, zinc, manganese and blood pressure (Taittonen *et al*., 1997). Hypertension is the most common manifestation of hypercupremia. The World Health Organization (1974) warned that high levels of copper in the tissues are positively correlated with hypertension.

Melanin, the natural pigment found in the skin seems to be a factor in the aetiology of some forms of hypertension, most especially in the darker skinned population. The physical property of melanin causes it to bind to heavy metals (Larsson & Tjalve, 1978). A strong positive correlation between high serum copper and hypertension have been reported in the dark skinned population with the assumption that copper excess might be a strong factor in the aetiology of hypertension (Pfeiffer & Mailloux, 1987). An earlier study by Creason *et al.* (1976) found that blood copper level is significantly higher in hypertensive black military recruits as compared to white military recruits. Thus copper, through its ability to be sequestered by melanin and its strong correlation with hypertension, may well lie at the heart of the high prevalence of hypertension in black population. Copper depletion experiments with men and women have revealed increased blood pressure (Klevay, 2000).

Zinc deficiency may cause arteries to become hard, brittle and often inflamed instead of soft and flexible and this loss of flexibility is expected to raise the blood pressure, in particular the systolic pressure. Zinc has been reported to lower blood pressure in some hypertensive patients (Pfeiffer & Lamola, 1983). The study also observed that oral doses of manganese causes a rise in blood pressure in patients over 40 years of age. Some other reports have shown that deficiency of Cu, Se, Zn and Mn might be associated with an increased risk of hypertension (Russo *et al*., 1998; Hajjar & Kotchen, 2003). On the other hand, Taneja and Mandal (2007) demonstrated high level of zinc in some hypertensives. However, another study (Ekmekci *et al*., 2003) did not observe any significant difference between serum zinc level of subjects with essential hypertension and normal controls.

Our literature search shows that there is a dearth of published reports on the relationship between trace elements and blood pressure in Nigeria and Africa in general. In this study, we report our study of the level of trace elements in normal subjects without hypertension and patients with newly diagnosed hypertension, and assessed the possible causal role of trace metals in the aetiology of hypertension in Nigerian Africans. The objectives of this study were to (1) determine the level of copper, selenium, zinc and manganese in the serum of patients with primary hypertension and subjects without hypertension; and (2) to determine if there is a relationship between the concentration of these trace metals and the level of blood pressure in patients with hypertension.

MATERIALS AND METHODS

Study Location

This study was carried out in Osogbo, a city with a population of approximately 450,000 people and located in the heart of South Western Nigeria.

Subjects

A total of ninety–two (92) subjects participated in the study. The subjects were grouped into two categories comprising of forty five (45) patients with primary hypertension attending the medical clinic of the Ladoke Akintola University Teaching Hospital, Osogbo and forty–seven (47) control subjects. The age ranged between 30 to 74 years. Informed consent was obtained from each of the subjects after the study was explained to them. All the control subjects had normal blood pressure according to the JNC 7 classification of hypertension. Excluded from the study were subjects that have glycosuria, are less than 30 years and those that had abnormal urinalysis or urinary sediments that may suggest the presence of kidney disease. Test subjects with newly diagnosed hypertension, not known to be on any anti-hypertensive drugs and who were not on any special diets were selected for the study.

Data were collected on each patient using a structured questionnaire. Body mass index (BMI) was calculated using the formula: weight/height^2 (kg/m^2). All patients were screened for glycosuria, and those with a history of other illnesses other than hypertension were excluded from the study.

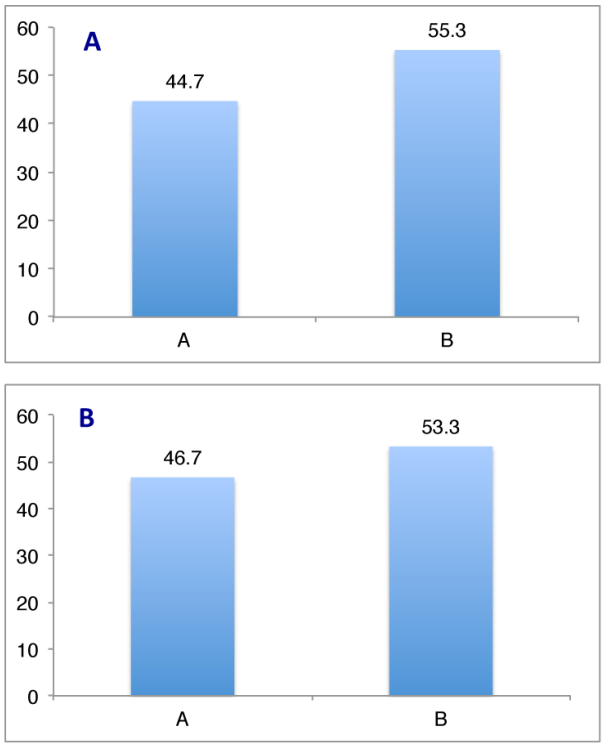

Figure 1: (A) Control subjects classified according to the JNC 7 report. A = < 120 / 80 mm Hg; B = ≥ 120 / 80 mm Hg but < 140 / 90 mm Hg (B) Patients with primary hypertension classified according to the JNC 7 report A = ≥ 140 / 90 mm Hg but < 160 / 100 mm Hg; B = ≥ 160 / 100 mm Hg

Table 1: Statistical comparison of baseline demographic and clinical characteristics between patients and control subjects.

CHARACTERISTICS	PATIENTS N = 45 (Mean ± SD)	CONTROLS N = 47 (Mean ± SD)	p – value
Age (years)	50.98 ± 10.37	42.28 ± 8.12	0.001*
Weight (kg)	73.47 ± 15.55	73.28 ± 15.19	0.953**
Height (m)	1.66 ± 0.11	1.76 ± 0.09	0.043*
BMI (kg/m^2)	26.75 ± 5.48	25.38 ± 5.51	0.238**
SBP (mmHg)	151.99 ± 18.72	116.60 ± 9.79	0.001*
DBP (mmHg)	97.62 ± 10.62	77.01 ± 7.52	0.001*

BMI = Body Mass Index; SBP = systolic blood pressure;
DBP = diastolic blood pressure; ** = not significant; * = significant

Blood pressure was taken using a validated, digital automatic blood pressure monitor A&D UA–767 (Rogoza *et al.*, 2000) after the patient had rested for five minutes and after ensuring that he or she was not on any anti–hypertensive drug or alcohol or coffee within 30 minutes of measuring the blood pressure. Three blood pressure readings were taken at intervals of two minutes for each clinic visit, and the average of the last two readings was calculated. The diagnosis of hypertension was made when the average diastolic blood pressure ≥90 mmHg or when the average systolic blood pressure is ≥140 mmHg on at least two occasions (JNC VI). 5ml of venous blood was drawn from each subject and control into plain serum bottles. The blood sample was centrifuged at 4000rpm for 15 minutes at room temperature to separate the serum. The serum was dispensed into another plain bottle and stored at -20^{0}C until analysis. The serum concentration of copper, manganese, zinc and selenium were determined with flame atomic absorption spectrophotometer using a direct method as described by Kaneko (1999). The measurement was performed on a Beck (200) atomic absorption spectrophotometer.

Statistical Analysis

The SPSS software package was used for statistical analysis and graphical representation by Microsoft excel software. Continuous variables were summarized and displayed as means ± standard deviation (SD). Differences between continuous variables were assessed using ANOVA (analysis of variance). Comparison of means was done using the student's t–test. Pearson correlation coefficient was used to measure the level of association between variables. A p value of < 0.05 was considered to be statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristics

The comparison of the baseline demographic and clinical characteristics in subjects indicates that the mean age, systolic and diastolic blood pressures were significantly higher in patients than in controls. The BMI was not significantly altered (Table 1)

Table 2: Statistical comparison of biochemical parameters between the patients and control subjects.

BIOCHEMICAL PARAMETERS	PATIENTS N = 45 (Mean ± SD)	CONTROLS N = 47 (Mean ± SD)	P – value
Zn (μg/ml)	135.78 ± 9.10	130.80 ± 12.50	0.032*
Cu (μg/dl)	68.16 ± 3.72	68.53 ± 5.33	0.697**
Mn (μg/dl)	63.11 ± 4.40	62.87 ± 4.59	0.800**
Se (μg/L)	75.91 ± 5.66	78.13 ± 5.92	0.070**

* = significant; ** = not significant

Table 3: Comparison between blood pressure and biochemical parameters of the study population with normal blood pressure as compared with those with pre-hypertension, stage i and stage ii hypertension

BIOCHEMICAL PARAMETERS	NORMOTENSIVE N = 21 MEAN ± SD	PRE-HYPERTENSION N = 26 MEAN ± SD	p^a	STAGE I N = 21 MEAN ± SD	p^b	STAGE II N = 24 MEAN ± SD	p^c	p^d	p^e	p^f
Zn (µg/ml)	129.81 ± 13.17	133.27 ± 9.43	0.249**	133.29 ± 8.05	0.289**	141.33 ± 11.84	0.012*	0.994**	0.025*	0.027*
Cu (µg/dl)	69.07 ± 5.53	67.93 ± 4.62	0.395**	67.29 ± 3.68	0.205**	69.50 ± 3.23	0.803**	0.596**	0.291**	0.093**
Mn (µg/dl)	62.14 ± 4.85	64.20 ± 3.85	0.075**	63.86 ± 4.41	0.205**	60.50 ± 4.15	0.312**	0.769**	0.009*	0.040*
Se (µg/L)	78.24 ± 6.08	76.90 ± 5.57	0.380**	76.62 ± 5.92	0.351**	75.25 ± 6.14	0.161**	0.864**	0.405**	0.533**

p^a = p-value for comparison between study participants with normal blood pressure and those with pre-hypertension, p^b = p-value for comparison between study participants with normal blood pressure and those with stage I hypertension, p^c = p-value for comparison between study participants with normal blood pressure and those with stage II hypertension, p^d = p-value for comparison between study participants with pre-hypertension and those with stage I hypertension, p^e = p-value for comparism between study participants with pre-hypertension and those with stage II, p^f = p-value for comparison between study participants with stage I hypertension and those with stage II hypertension, * = significant, ** = not significant.

Among the controls, 21 participants (44.7%) had blood pressure of less than 120/80 mmHg while 26 participants (55.3%) had blood pressure of between ≥120/80 mmHg and <140/90 mmHg (Fig 1A). 21 (46.7%) of patients reported a blood pressure of between ≥140/90 mmHg to <160/100 mmHg while 24 patients (53.3%) had a BP of ≥160/100 mmHg (Figure 1B)

Biochemical Parameters

Patients with hypertension had significantly higher serum zinc than control subjects ($p<0.05$). Mean serum copper, magnesium and selenium did not differ significantly between patients and controls (Table 2). It was also observed that serum zinc increased progressively from those with normal BP to stage II hypertensives. Significant differences were particularly seen when those with normal BP were compared with stage II hypertensives ($p<0.05$) and between stage I and stage II hypertensives ($p<0.05$) (Table 3). There were also significant differences between the mean serum manganese of subjects with pre-hypertension and those with stage II hypertension ($p < 0.01$), and serum manganese of subjects with stage I hypertension and those with stage II hypertension ($p < 0.05$). Comparison of biochemical parameters in both the control and patient groups with different classes of BMI (Tables 4 & Table 5) did not reveal any significant relationship between the level of trace metals across the categories of BMI in individual groups.

DISCUSSION

This study showed that the mean serum levels of zinc in subjects with hypertension was significantly higher than that in the control subjects. This seemed to increase progressively from prehypertensives to those with stage II hypertension. The result is consistent with that obtained by Henrotte *et al.* (1990) and Davydenko *et al.* (1995) who found a significant association between high level of zinc in serum and primary hypertension. Excess serum level of zinc is thought to cause an increase in intracellular zinc within the cell which in turn causes a rise of free calcium ion level in the smooth muscular layer of blood vessels, with consequent

vasoconstriction, increased peripheral resistance and elevated blood pressure. Tubek (2005) and Tomat *et al.* (2005) on the other hand found decreased level of serum zinc in hypertension when compared to controls. These authors showed that deficiency in serum zinc cause diminished nitric oxide activity with consequent elevation of blood pressure. The findings of Tubek (2005) and Tomat *et al.* (2005) also suggest however that zinc deficiency may be involved in the aetiology of primary hypertension which is contradictory to the finding of the present study. It appears that the severity of hypertension affects zinc levels or vice versa and this might in part explain this contradiction. In addition, Tubek (2005) used recently weaned animal models but Tomat *et al.* (2005) also demonstrated that zinc efflux

Table 4: Comparison of biochemical parameters between classes of BMI of control subjects

BIOCHEMICAL PARAMETERS	BMI 1 = 28 2 = 10 3 = 9	CONTROLS N = 47 Mean ± SD	p^a	p^b	p^c
	1	130.34 ± 12.49			
Zn (μg/ml)	2	133.90 ± 15.91	0.727**	0.476**	0.399**
	3	128.99 ± 8.34			
	1	63.46 ± 4.63			
Mn (μg/dl)	2	60.80 ± 4.57	0.941**	0.126**	0.231**
	3	63.33 ± 4.30			
	1	77.21 ± 5.20			
Se (μg/L)	2	79.60 ± 7.88	0.306**	0.286**	0.934**
	3	79.33 ± 5.72			
	1	69.57 ± 4.85			
Cu (μg/dl)	2	67.30 ± 6.24	0.140**	0.246**	0.819**
	3	66.67 ± 5.55			

BMI = body mass index, ** = not significant, **1** = (15 - 24) kg/m^2, **2** = (25 - 29) kg/m^2, **3** = (30 - 50) kg/m^2, **Zn** = zinc, **Mn** = manganese, **Cu** = copper, **Se** = selenium, p^a = p-value for comparison between study participants with normal BMI and those that are obese, p^b = p-value for comparison between study participants with normal BMI and those that are over-weight, p^c = p-value for comparison between study participants that are over-weight and those with obesity, **N** = total number of patients/subjects.

Table 5: Comparison of biochemical parameters between classes of BMI of patients

BIOCHEMICAL PARAMETERS	BMI 1 = 28 2 = 15 3 = 13	PATIENTS N = 45 Mean ± SD	p^a	p^b	p^c
	1	136.71 ± 9.76			
Zn (μg/ml)	2	133.67 ± 5.27	0.327**	0.291**	0.941**
	3	137.00 ± 11.64			
	1	63.71 ± 5.10			
Mn (μg/dl)	2	62.33 ± 3.85	0.561**	0.402**	0.787**
	3	63.23 ± 4.21			
	1	75.12 ± 4.95			
Se (μg/L)	2	77.67 ± 7.16	0.324**	0.246**	0.912**
	3	74.92 ± 4.41			
	1	67.76 ± 3.98			
Cu (μg/dl)	2	67.73 ± 4.28	0.308**	0.983**	0.285**
	3	69.15 ± 4.28			

BMI = body mass index, ** = not significant, **1** = (15 - 24) kg/m^2, **2** = (25 - 29) kg/m^2, **3** = (30 – 50) kg/m^2, **Zn** = zinc, **Mn** = manganese, **Cu** = copper, **Se** = selenium, p^c = p-value for comparison between study participants with normal BMI and those that are obese, p^b = p-value for comparison between study participants with normal BMI and those that are over-weight, p^a = p-value for comparison between study participants that are over-weight and those with obesity, **N** =total number of patients/subjects.

rate from lymphocytes decreased with severe hypertension but increased with mild hypertension. From the foregoing, the role of zinc in the aetiology of primary hypertension appears yet to be fully established. However, This study did not find any significant statistical difference in serum zinc, selenium, manganese and copper in relation to obesity (BMI). Although Ghayour-Mobarhan *et al.* (2005) did not find any significant difference between obese and non-obese subjects with regard to selenium which is similar to the findings of this study, Tungtrongchitr *et al.* (2003) however found lower serum zinc levels and higher serum copper in obese compared with non-obese subjects while Taneja *et al.* (1996) found significantly higher serum zinc levels in obese subjects compared to control group. Manganese intake has been associated with an increased risk of hypertension in a representative sample of the adult Korean population (Lee & Kim, 2011). This study revealed that the serum levels of manganese in the patients with hypertension was higher than that of the control subjects but the difference was not statistically significant. However, a relationship between those with stage 1 and stage 2 hypertension, with regards to manganese was observed. Significant lower manganese level was seen in stage 2 hypertensives compared to stage 1 patients. Overall, there was no consistency in manganese level across the various stages of hypertension in this study. Even though it has been reported that copper deficiency is associated with impaired endothelium-dependent arterial relaxation that may result in hypertension causing extensive damage to arteries (Fell *et al.*, 1980), there was no statistically significant change in the plasma copper level of hypertensive subjects in the present study.

In conclusion, this study showed that serum levels of zinc are significantly higher in patients with primary hypertension. There were insignificant changes in the concentrations of maganese, copper and selenium in patients with hypertension compared to subjects without hypertension. In addition, there were no obesity-related differences in serum zinc, copper, manganese and selenium levels. These findings show that it is important to continue with further research to elucidate the possible roles of these trace elements in the aetiology of primary hypertension.

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