

C-reactive protein, trace element and lipid profile in cardiovascular disease.

ABSTRACT

Background: The increase in consumption of industrial processed food, economic instability consequent to global economic recession; job losses; increase incidence of diabetes and chronic kidney disease; increase steroids use; dyslipidemia; and obesity has been implicated in the global increase in hypertension and corresponding heart diseases. This study evaluates the levels of C-reactive protein, trace element and lipid profile in cardiovascular disease between January 2016 and August 2017.

Method: A total of one hundred participants consisting of 50 patients and 50 control subjects (mean age 56.12 ± 11.4 and 59.8 ± 10.8 years respectively) attending medical outpatient clinic of the Ogun state general hospital Abeokuta on account of hypertensive heart diseases and other cardiovascular related illness were recruited for the study. Relations that accompanied the patients to the clinic and some workers of the hospital were recruited as controls. A structured questionnaire was administered to obtain some demographic data from all the participants. Blood pressure was measured using mercurial sphygmomanometer, venous blood was collected from all the participants, allowed to clot and centrifuged to obtain serum for the estimation of C-reactive protein, total cholesterol, triglycerides, HDL-C, Zinc, Magnesium and Selenium.

Result: There was no significant changes observed in age, and height ($p = 0.371$ and 0.258 respectively); a statistically significant increase in weight, BMI, SBP, DBP, serum total

23 cholesterol, LDLC, VLDLC, triglyceride, AI, CRI and CRP ($p = 0.000$); and a statistically
24 significant decrease in serum HDLC, trace element (Zn, Mg, and Se) ($p = 0.000$) among the
25 hypertensive patients when compared with the control subjects.

26 Conclusion: Base on the result from this study, CVD is associated with abnormalities in
27 inflammatory markers, lipid profile, and antioxidant micronutrients.

28 **Key words:** Cardiovascular disease, hypertension, lipid profile, micronutrient, antioxidants

29 1.0 INTRODUCTION

30 Hypertension is defined as systolic blood pressure higher than 130mmHg and diastolic blood
31 pressure higher than 90mmHg [1]. It is a measure of pressure differences between the effect
32 of blood flow on vascular walls and resistance from the vascular walls. Hypertensive heart
33 disease refers to heart conditions caused by high blood pressure. The heart working under
34 increased pressure causes different heart disorders such as heart failure, thickening of the
35 heart muscle, coronary artery disease, and other conditions. The high blood pressure makes it
36 difficult for the heart to pump blood thus necessitating regular hard work against resistance
37 and causes compensatory but detrimental hypertrophic changes in the left atrium, left
38 ventricle and coronary arteries. There is resultant cardiac muscle dysfunction with the
39 involvement of proinflammatory cytokines [2].

40 The global increase in hypertension and corresponding heart diseases may be connected with
41 the increase in consumption of industrial processed food which has impacted the amount of
42 salt in diets worldwide; economic instability consequent to global economic recession; job
43 losses; increase incidence of diabetes and chronic kidney disease; increase steroids use;
44 dyslipidemia; and overweight or obesity as a result of physical inactivity.

45 There is convincing evidence that oxidative stress play important roles in the development of
46 cardiovascular disease (CVD) [3]. Oxidative stress increases low density lipoprotein
47 cholesterol (LDLC) peroxidation, thereby increasing its uptake by macrophages with

48 increased foam cell formation and atherosclerosis. The connection between the various heart
 49 disease risk factors and the clinical features of CVD such as congestive cardiomyopathy,
 50 myocardial ischemic injury and hypertensive heart disease is oxidative stress [4. 5].
 51 Consequently, there is inflammatory response which is an important factor in the initiation
 52 and progression of atherosclerosis and atherothrombosis.
 53 Several trace elements have been implicated in the pathogenesis of CVD [6]. Low serum Zn
 54 levels have been reported to be associated with increased cardiovascular mortality [6].
 55 To evaluate the disease and status of emerging risks in patients, various physical factors and
 56 other biomarkers alone or in combination are used as risk predictors. Traditional risk factors
 57 and biophysical factors commonly used to estimate cardiovascular risk include hypertension,
 58 diabetes, cigarette smoking, heavy alcohol ingestion, elevated total cholesterol, elevated
 59 LDLC, reduced high-density lipoproteins cholesterol (HDL), elevated triglycerides and
 60 family history, each can predict premature coronary heart diseases [7]. Pro-inflammatory risk
 61 factors include oxidized LDLC, cytokines (interleukin-1, tumour necrosis factor- α),
 62 intercellular adhesion molecule-1, selectins, interleukin-6 and C-reactive protein (CRP). The
 63 contributory role of CRP, trace elements and lipid either solely or in various combinations in
 64 CVD therefore necessitated this study.

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66 **2.0 MATERIAL AND METHODS**

67 **2.1 LOCATION**

68 This study was carried out at the Ogun State General Hospital, Sokenu, Ijaye, Abeokuta,
 69 Ogun State, Nigeria. The patients were drawn from those attending medical outpatient clinics
 70 (MOP) of the hospital.

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73 **2.2 INFORMED CONSENT AND ETHICAL CONSIDERATION**

74 After explaining details of the study to both the patients and control subjects, informed
75 consent was obtained from each of them before the commencement of the research. Ethical
76 approval was also obtained from the Health Research Ethics Committee of the hospital.

77 **2.3 SUBJECT AND CONTROL SELECTION**

78 A total of one hundred participants (consisting of 50 patients and 50 control subjects; both
79 male and female inclusive) attending medical outpatient clinic (MOP) of the hospital on
80 account of hypertensive heart diseases and other cardiovascular related illness were recruited
81 for the study. The controls were non hypertensive, non-active cigarette smokers, and non-
82 chronic alcohol consumers selected from relations that accompanied the patients to the clinic
83 and among workers of the hospital. Patients and control subjects were matched for age and
84 sex.

85 A structured questionnaire was administered on both the patients and the control subjects to
86 obtain some demographic data such as age, sex, and social habits (cigarette smoking and
87 alcohol intake). Anthropometric measurements (height and weight) were carried out, and the
88 body mass index (BMI) was computed using the formula: $BMI = \text{Weight}/\text{Height}^2$ (kg/m²).
89 Blood pressure was measured in both the patients and control subjects using mercurial
90 sphygmomanometer while participants were sitting with their arm rested. The systolic and
91 diastolic blood pressure was determined using the first and fifth phases of korotkof sound
92 respectively. Hypertension was described as systolic and diastolic blood pressure greater than
93 140mmHg and 90mmHg respectively [1, 8].

94 **2.4 COLLECTION AND STORAGE OF SAMPLES**

95 Ten milliliter (10ml) of fasting venous blood (8-10hr overnight fast) was aseptically collected
96 from antecubital fossa vein of both the patients and controls. The blood sample was
97 dispensed into sterile plain bottles and allowed to clot. The samples were then centrifuged at

3000 rpm for 5 minutes and serum separated into sterile plain bottles. Two mililiter (2ml) of serum sample was used to analyse for C-reactive protein immediately after collection while the remaining serum sample was stored at -20°C until analysed (for total cholesterol, triglycerides, HDL-C, Zinc, Magnesium and Selenium).

Fasting plasma lipid profiles (~~triglyceride~~, total cholesterol, HDL-C) were assayed enzymatically with commercial test kits obtained from Randox Laboratories, Crumlin, England using standard methods [9, 10, 11]. LDLC was computed using the formula $LDL-C = Total\ Cholesterol - 0.2(Triglyceride) - HDLC$ [9] while the atherogenic index (LDLC/HDL-C ratio) and coronary risk index (total cholesterol/HDL-C ratio) were calculated for all samples [12, 13]. Serum C-reactive protein was also determined [14]. Trace element (Zinc, Magnesium, and Selenium) were measured by colorimetric and atomic absorption spectrophotometric techniques.

2.5 STATISTICAL ANALYSIS

The obtained data was analysed using SPSS version 21. Continuous variables were expressed as Mean $\pm$ Standard Error of Mean (Mean $\pm$ SEM). The differences between mean values were compared using Independent student t-test.

3.0 RESULTS

Result on biophysical parameters shows that the mean age of the hypertensive patients and the control subjects were 56.12 ± 11.4 and 59.8 ± 10.8 years respectively. There was no statistically significant difference in the mean age and height. A statistically significant increase in weight, BMI, SBP and DBP was observed among the hypertensive patients when compared with the control subjects (Table I).

Results of biochemical parameters showed a statistically significant increase in serum total cholesterol, LDLC, VLDLC, triglyceride, AI, CRI and CRP among the hypertensive patients

when compared with the control subjects. Also, a statistically significant decrease in serum HDLC was observed among the hypertensive patients when compared with the control subjects.

Result of trace element showed that serum trace element (Zn, Mg, and Se) of the hypertensive patients is significantly reduced as compared to that of control subjects (Table 3).

Table 1: Biophysical parameters of the hypertensive patients and control

Parameters	Case N = 50	Control N = 50	t-value	p-value
Age (Yr)	56.12 ± 11.4	59.8 ± 10.8	2.12	0.371
Weight (kg)	74.7 ± 1.4	66.94 ± 0.9	4.59	0.000
Height (m)	1.61 ± 0.01	1.60 ± 0.01	1.14	0.258
BMI (kg/m ²)	30.0 ± 0.63	26.2 ± 0.24	5.54	0.000
SBP (mmHg)	148.0 ± 1.68	110.4 ± 1.34	17.5	0.000
DBP (mmHg)	93.6 ± 0.85	72.6 ± 0.90	17.06	0.000

Table 1 above showed the biophysical parameters of the patients and controls. The level of statistical significance was considered at $p < 0.05$. There was a significant difference in the weight, BMI, SBP and DBP amongst the patients and control groups. * Represent statistically significant difference at $p < 0.05$ when compared with the control.

BMI = Body mass index SBP = systolic blood pressure, DBP = diastolic blood pressure

Table 2: Serum Biochemical parameters of the hypertensive patients and control

Parameters	Case N = 50	Control N = 50	t-value	p-value
CRP	18.98±1.32*	5.96±0.15	9.83	0.000
Total Cholesterol (mg/dl)	234.8±4.33*	154.5±1.8	17.13	0.000
HDLC (mg/dl)	36.04±0.39	50.64±1.11*	12.39	0.000
LDLC (mg/dl)	175.21±4.02*	85.22±1.43	21.07	0.000
VLDLC (mg/dl)	23.58±0.47*	18.68±0.09	10.17	0.000
Triglyceride	117.92±2.36*	93.38±0.47	10.17	0.000

AI	4.86±0.10*	1.73±0.05	27.89	0.000
CRI	6.52±0.11*	3.11±0.06	28.18	0.000

Table 2 above showed the biochemical parameters of the patients and controls. The level of statistical significance was considered at $p < 0.05$. There was a significant difference in the serum CRP, total cholesterol, HDLC, LDLC, VLDLC, triglyceride, AI, and CRI among the patients and control groups. * Represent statistically significant difference at $p < 0.05$ when compared with the control.

HDLC = High density lipoprotein, LDLC = low density lipoprotein, VLDLC = very low density lipoprotein, AI = atherogenic index, CRI = coronary risk index, and CRP = C-reactive protein

Table 3: Serum trace elements of the hypertensive patients and control

Parameters	Case N = 50	Control N = 50	t-value	p-value
Zn (µg/L)	68.66±1.04*	96.00±0.79	20.91	0.000
Mg (mg/L)	1.25±0.04*	2.12±0.03	17.48	0.000
Se (µg/L)	37.42±1.63*	62.10±1.39	11.52	0.000

Table 3 above showed the serum trace element of the patients and controls. The level of statistical significance was considered at $p < 0.05$. There was a significant difference in the serum Zn, Mg, and Se amongst the patients and control groups. * Represent statistically significant difference at $p < 0.05$ when compared with the control.

Zn = Zinc, Mg = magnesium and Se = selenium.

4.0 DISCUSSION

The etiologic role of oxidative stress and micronutrient deficiency in cardiovascular diseases are interrelated and of great importance. While oxidative stress causes endothelial dysfunction, atherosclerosis, apoptosis, increase lipid peroxidation, necrosis, and CVD; micronutrient deficiency causes autoimmunity dysfunction, accumulation of autoimmune complexes, recruitment of inflammatory cells and finally CVD, thereby acting as antioxidants, immune modulators and anti-inflammatory agent [15].

A measure of acute or chronic inflammatory activities in CVD is C-Reactive protein (CRP). CRP level is reduced by drugs (e.g. aspirin, clopidogrel, statins, celecoxib, niacin, thiazolidinedione, beta blockers, ACE inhibitors, derivatives [8, 16]; and regular exercise

163 [17]. The observed increase in serum CRP in CVD patients is a reflection of the
 164 inflammatory events associated with the condition and suggests high cardiovascular mortality
 165 and morbidity risk. CRP production starts during early stage of the inflammatory process, it
 166 thus has much clinical relevance and high prognostic role in the event of CVD. CRP reduces
 167 NO production, induces LDL oxidation, and destabilizes fibrous layer of atheroma thereby
 168 causing thrombus formation in the endothelial wall [18].

169 Abnormalities in lipid profile (dyslipidaemia) characterised by hypercholesterolemia,
 170 hypertriglyceridemia; increased LDLC, VLDLC; and reduced HDLC are risk factors for
 171 CVD. Atherogenic (AI) and coronary risk indices (CRI) had been reported to be good
 172 predictor of future cardiovascular events [19, 20] and thus could be of great importance in
 173 evaluating and screening for coronary artery disease [21]. These indices are arithmetic
 174 manipulations of serum triglyceride, total cholesterol, HDLC and LDLC. The serum values
 175 of both indices was noted to increase (AI= 4.8 and CRI = 6.5; normal values are <2.5 for AI
 176 and < 4.0 for CRI) [22] in patients with hypertensive heart disease and this constitutes a
 177 predictor of complications among hypertensive patients.

178 Micronutrients (Zn, Mg, and Se) had form integral part of antioxidant enzymes such as
 179 superoxide dismutase and glutathione peroxidase. Abnormalities of these trace elements
 180 therefore affect their free radicals scavenging power. The degree of oxidative stress and
 181 inflammatory status in CVD can be indirectly measured by considering antioxidant status and
 182 inflammatory responses of individuals with the disease condition. Selenium and Zinc acts as
 183 a biological antioxidant by scavenging free radicals thereby decreasing lipid peroxidation and
 184 stabilizes the membrane [23]. Serum level of antioxidant microelements (zinc, magnesium ,
 185 and selenium) was observed to be reduced in hypertensive heart disease. Similar reduction in
 186 serum Zn and Se had been reported in CVD [23, 24]. Though conflicting results are available
 187 on serum magnesium in coronary heart disease (CHD) and the pathway is unclear but low

serum magnesium has been implicated in cardiovascular mortality [25]. The observed hypomagnesaemia in this study also corroborates earlier finding of reduced serum magnesium level in cardiovascular diseases [25].

5.0 CONCLUSION

Based on the result from this study, CVD is associated with abnormalities in inflammatory markers, lipid profile, and micronutrients. The risk factors or markers of impending CVD in hypertensive patients demonstrated by these patients include obesity ($\text{BMI} \geq 30\text{kg/m}^2$), high systolic and diastolic blood pressure, high serum marker of inflammatory involvement, dyslipidaemia, high atherogenic and coronary risk indices, and reduced antioxidant micronutrient level. Considering the observed reduction in serum Zn, Mg and Se in hypertensive heart disease, dietary supplementation with Mg, Zn and Se will be of great impact in improving the morbidity and mortality associated with CVD.

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