C-Reactive Proteins and Cardiovascular Risk Indices in Hypertensive Nigerians.

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Abstract

C-reactive protein (CRP), a biomarker of inflammation, has been found to play a role in the pathogenesis of cardiovascular disease and its determination has been proposed as one method of improving the prediction of the risk of cardiovascular events. CRP was determined in 150 hypertensive patients aged 30-59 years and 30 apparently healthy normotensive individuals matched for age and socioeconomic status by ELISA technique. Atherogenic index (LDL-C/HDL-C) and coronary heart disease risk (HDL-C/TC) were also calculated from the lipid profile. Among the hypertensive patients, only 1 (1.2%) female had a dangerous coronary heart disease risk, while 14 (9.3%) (6 males and 8 females) were at high risk of CHD and only 16 (10.7%) (11 males and 5 females) had probable protection against CHD. Hypertensive patients were significantly ($p < 0.05$) heavier than the normotensive patients ($28.34 \pm 4.40 kg/m^2$ vs. $25.79 \pm 2.91 kg/m^2$), with significantly higher atherogenic indices and CRP. Among the hypertensive patients, CRP positively correlated with atherogenic index ($r = 0.551$, $p < 0.05$) and CHD risk ($r = 0.589$, $p < 0.05$). However, in normotensive patients, CRP was positively correlated with atherogenic index ($r = 0.492$, $p < 0.01$) but negatively correlated with CHD risk ($r = -0.475$, $p < 0.01$). In conclusion, hypertensive Nigerians have significantly higher CRP than their normotensive counterparts, which correlates with CHD risk.

INTRODUCTION

The role of inflammation in the pathogenesis of atherosclerosis is well established [12]. The increasing recognition of inflammation as an important component of atherogenesis provides the plausibility for the potential use of inflammation markers such as c-reactive protein (CRP), interleukin-6 (IL_{-6}) and serum amyloid A (SAA) as indicators for atherogenesis or as a predictor of atherosclerosis/ or coronary heart disease complications [3456]. Although these inflammation markers are not specific as they may arise from other systemic inflammation, such as with connective tissue disease, local infection like gingivitis or prostatitis , studies have shown a relationship between high levels of these markers and high incidence of CHD and sudden death [278]. For instance, it has been found that the risk for heart attack in people in the upper third of high sensitivity C-reactive protein (hs-CRP) levels (< 0.3mg/dl) is twice that of those whose hs-CRP is in the lower third [9]. It has been reported that CRP is useful in predicting the risk of heart disease and stroke. CRP as an index of inflammation is believed to promote directly all stages of atherosclerosis, including plaque rupture and its measurement has been found to provide a clinical tool for cardiovascular risk assessment. Additionally, CRP has been found to independently predict recurrent events in patients with known CAD [10]. Paucity of data on CRP in hypertensive Nigerians prompted us to conduct this research. The objective is to provide scientific information that may have clinical relevance in the management of hypertension in Nigeria.

SUBJECTS AND METHODS

Subjects: This study was conducted in the Department of Chemical Pathology in conjunction with the Department of Internal Medicine at the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. The protocol for the study was approved by the Research and Ethics Committee of the University of Benin Teaching Hospital. On obtaining consent, hypertensive patients (diagnosed by a Consultant Physician in the Department of Internal Medicine of the University of Benin Teaching Hospital based on World Health Organisation-International Society of Hypertension Guideline of blood pressure ≥ 140/90mmHg) aged 30-59 years were recruited. Inclusion criteria included being hypertensive for ≥ one year, use of neutral antihypertensive agents such as calcium channel blockers, angiotensin converting enzyme inhibitors, and angiotensin II

receptor blockers. Excluded from the study were patients with diabetes mellitus, taking oral contraceptives, taking thiazide and/or beta-blockers, taking lipid lowering drugs and patients with systemic inflammation or systemic infections. Socio-demographic data were obtained by semi-structural questionnaire administered by one of the authors (JOI). One hundred and fifty (150) hypertensive patients were recruited while thirty age and socio-economically matched apparently healthy normotensive subjects served as the control. Height and weight were measured with the subject in light clothes without shoes, and BMI (Kg/m2) was calculated. Six millilitres (6.0ml) of venous blood samples were collected between 08.00-10.00 hours after 8-12 hours overnight fasting of which 3ml were dispensed into dry plain bottle and 3ml into EDTA bottle respectively. Serum and plasma were extracted from clotted and retracted blood in the dry plain bottle and the EDTA anticoagulated blood respectively after centrifugation at 2000g for 5 minutes.

BIOCHEMICAL ASSAYS

Lipid profile: Serum total cholesterol and plasma triglyceride concentrations were determined by enzymatic colorimetric assay as described previously [1112]. Plasma HDL-cholesterol was determined enzymatically after precipitation of other lipoprotein as described by [13], while LDL-cholesterol was calculated using Friedewald equation [14] Atherogenic index (LDL-C/HDL-C) and coronary heart disease risk (HDL-C/TC) were calculated. All samples were analysed within 24 hours after collection.

CRP ASSAY

Principe: Serum C-reactive protein was analysed by enzyme-linked immunosorbent assay technique [15]. High sensitivity-CRP ELISA method is based on the principle of solid phase ELISA [16] in which the CRP in the sample is sandwiched between immobilised monoclonal antibodies and anti-CRP antibodies in the enzyme conjugate solution. The washing off of the unbound labelled antibodies and reaction with tetramethylbenzidine (TMB) reagent lead to the development of colour, which intensity is proportional to the concentration of CRP in the sample.

Procedure: Briefly, 10µl of appropriately diluted CRP standard, samples and controls were dispensed into appropriately labelled microtitre wells (that have been brought to room temperature i.e. 20-25OC) after which 100µl of enzyme conjugate reagent was added, thoroughly mixed for 30 seconds and incubated at 20-25OC for 45 minutes. The wells were later washed for 5 times with distilled water and properly dried by striking sharply on absorbent paper. 100µl of tetremethylbenzidine solution was then added to each well, gently mixed for 5 seconds and incubated at 20-25OC for 20 minutes. Thereafter, 100µl of 1N hydrochloric acid (stop solution) was added to each well, gently mixed for 30 seconds to stop the reaction and for the development of a yellow colour, the absorbance which was read with a microtitre well reader at 450 nm within 15 minutes. The concentration of CRP in milligram per decilitre (mg/dl) was calculated thus:

Figure 1

$$\text{CRP (mg/dl)} = \frac{A_s}{A_{std}} \times \text{Cst}$$

Where; A_s is the absorbance for the samples or controls, A_{std} is the absorbance for the standard and C_{std} is the concentration for the standard. The assay was done in duplicates and the mean CRP calculated for each sample and control.

STATISTICAL ANALYSIS

Statistical analyses were performed with Statistical Package for Social Science (SPSS) 7.5. Data were analyzed for mean and standard deviation. Proportions were expressed as percentage while comparison of mean plasma lipids were done with one-way analysis of variance (ANOVA) and association between CRP and atherogenic indices was determined by Pearson correlation analysis with significant level set at $p < 0.05$.

RESULTS

Figure 2

Table 1: Characteristics of Hypertensive and Normotensive Patients (percentage in parenthesis)

Patients characteristics	Hypertensive n = 150		Normotensive N = 30	
Age (years)	46.8 ± 8.2		38.8 ± 13.2	
Occupation				
Artisan	13 (8.7)		5 (16.7)	
Civil servants	43 (28.7)		7 (23.3)	
Business/trading	53 (35.3)		6 (20)	
High skilled	22 (14.7)		10 (33.3)	
Professionals	7 (4.7)		1 (3.3)	
Clergy	12 (8)		1 (3.3)	
Farming				
Marital Status	138 (92)		20 (66.7)	
Married	9 (6)		10 (33.3)	
Single	2 (1.3)		-	
Widowed	1 (0.7)		-	
Divorced				
Educational level	13 (8.7)		-	
Nil	36 (24)		4 (13.3)	
Primary	52 (34.7)		10 (33.3)	
Secondary	49 (32.7)		16 (53.3)	
Tertiary				

Table 1 shows the sociodemographic data of the subjects. While majority of both the normotensive and hypertensive patients were married, the latter were significantly ($p < 0.05$) older (46.8 ± 8.2 vs. 38.8 ± 13.2) and mainly businessmen. Of the 150 hypertensive patients, only 1 (1.2%) female had dangerous coronary heart disease risk while 14 (9.3%), made up of 6 males and 8 females, were at high risk of CHD. Only 16 (10.7%), 11 males and 5 females, had probable protection against CHD (table 2).

Figure 3

Table 2: Sex Related Distribution of Coronary Heart Disease Risk among Hypertensive and Normotensive Nigerians (percentage in parenthesis)

	Hypertensive			Normotensive		
	Male	Female	Total	Male	Female	Total
Dangerous	-	1 (1.2)	1 (0.7)	-	-	-
High	6 (8.7)	8 (9.9)	14 (9.3)	-	-	-
Average	23 (33.3)	29 (35.8)	52 (34.7)	2 (12.5	4 (28.6)	6 (20)
Below average	29 (42)	38 (46.9)	67 (44.7)	9 (56.3)	6 (42.9)	15 (50)
Probable protection	11 (15.9)	5 (6.2)	16 (10.7)	5 (31.3)	4 (28.6)	9 (30)
Total	**69 (100)**	**81 (100)**	**150 (100)**	**16 (100)**	**14 (100)**	**30 (100)**

From table 3, the hypertensive patients were significantly ($p < 0.05$) heavier than the normotensive patients (BMI = 28.34 ± 4.40kg/m2 vs. 25.79 ± 2.91kg/m2) with significantly higher atherogenic indices (atherogenic index and coronary heart disease risk) and CRP.

Figure 4

Table 3: Comparison of BMI, CRP and Atherogenic Indices of Hypertensive and Normotensive Nigerians (mean ± S.D)

Parameters	Hypertensive n = 150	Non-hypertensive n = 30	p-value
BMI (kg/m²)	28.34 ± 4.40	25.79 ± 2.91	0.003*
Atherogenic index (LDL-C/HDL-C)	2.28 ± 1.28	1.55 ± 0.7	0.003+*
Coronary heart disease risk (HDL-C/TC)	0.28 ± 0.09	0.34 ± 0.09	0.002*
C-Reactive Protein (mg/dl)	0.18 ± 0.1	0.08 ± 0.04	< 0.0001*

Legend: **BMI:** Body mass index; **HDL-C**: High density lipoprotein cholesterol; **LDL-C:** Low density lipoprotein cholesterol, **TC:** Total cholesterol
*p-value < 0.05 (significant).

Although the female hypertensive patients were significantly heavier than their male counterparts (BMI = 29.29 ± 4.79kg/m2 vs. 27.24 ± 3.62kg/m2), there was no significant difference in the atherogenic indices and CRP between the two sexes (table 4).

Figure 5

Table 4; Sex related comparison of BMI, Atherogenic indices and CRP in Hypertensive Nigerians (mean ± S.D)

Parameters	Male (n = 69)	Female (n = 81)	p-values
BMI (kg/m²)	27.24 ± 3.62	29.29 ± 4.79	S*
Atherogenic index (LDL-C/HDL-C)	2.35 ± 1.40	2.22 ± 1.18	NS
Coronary heart disease risk (HDL-C/TC)	0.28 ± 0.09	0.29 ± 0.09	NS
C-Reactive Protein (mg/dl)	0.17 ± 0.10	0.20 ± 0.09	NS

Legend: **BMI:** Body mass index; **HDL-C**: High density lipoprotein cholesterol; **LDL-C:** Low density lipoprotein cholesterol, **TC**: Total cholesterol

***S** – Significant (p value < 0.05). **NS**- Non-significant (p > 0.05).

Pearson correlation analyses showed that among the hypertensive patients, CRP positively correlated with atherogenic index ($r = 0.551$, $p < 0.05$) and CHD risk ($r = 0.589$, $p < 0.05$). However, in normotensive patients, CRP was positively correlated with atherogenic index ($r = 0.492$, $p < 0.01$), but negatively correlated with CHD risk ($r = -0.475$, $p < 0.01$).

DISCUSSION

This finding has shown that hypertensive Nigerians have significantly higher serum CRP than the normotensive individuals in the control group, which is independent of sex. This finding is consistent with previous findings by Ridker [9]. The mean value of CRP of 0.08 mg/dl in the normotensive Nigerians is in accord with the findings in the study of the clinical application of CRP for cardiovascular disease detection and prevention in which hs-CRP levels below 0.1mg/dl carry a low risk of developing heart disease as against levels above 0.3mg/dl which carry a high risk for cardiovascular disease [9]. Although plasma lipid levels were

more strongly associated with an increased risk than were inflammatory markers, elevated levels of inflammatory markers, particularly C-reactive protein, has been reported as a significant contributor to the prediction of coronary heart disease. [17].

In the present study the concentration of CRP though did not reach the value quoted for the high risk group, our subjects had significantly higher baseline value than the normotensive controls (0.18 ± 0.1 vs. 0.08 ± 0.04, $p < 0.001$). It could be argued that the lower level of CRP recorded in this study compared to quoted values for high risk group could be due to genetic variation. A multiethnic case–control study of postmenopausal women provides evidence that common genetic variants in the CRP gene are substantially associated with plasma hsCRP concentrations, suggesting ethnic variations in these associations [18]. This also corroborated earlier study among the Japanese in which the hs-CRP cut-off point for high-risk of future development of CHD was much lower than that for Western populations [19]. It has also been postulated that high-sensitivity CRP associates more closely with ischemic stroke than with CHD and that concomitant evaluation of lipid levels and hs-CRP may improve risk assessment for stroke as well as CHD [20]. Lack of significant difference in CRP levels between male and female hypertensive individuals in the present study corroborates earlier findings [321].

CRP has been described as one of the most powerful independent predictors of myocardial infarction, stroke and vascular death in a variety of settings, with prognostic value extending across various ethnic groups and in men and women in different age groups [3921]. Work by Ridker and colleagues [3] demonstrated that CRP may be a better predictor of future cardiovascular events than low-density lipoprotein (LDL) cholesterol and that baseline CRP evaluation adds prognostic value to the conventional Framingham risk assessment. In fact the view of CRP being a surrogate marker rather than mediator of atherosclerosis has recently been revisited with the findings that CRP contributes to the substrate underlying lesion formation, plaque rupture, and coronary thrombosis [20]. This is corroborated by our findings of positive correlations between CRP and atherogenic indices. CRP has been found to induce adhesion molecule expression in human endothelial cells in the presence of serum, a finding that support the hypothesis that CRP may play a direct role in promoting the inflammatory component of atherosclerosis and present a potential target for the treatment of atherosclerosis [22]. Study has also confirmed the primary role of an inflammation in unstable angina as CRP levels remained elevated 3 months after hospital discharge [23]. Evidence has shown that, even in apparently healthy subjects, there is good and consistent significant relationship (in all populations) between baseline hsCRP levels and risk of future cardiovascular events (stroke, peripheral vascular disease, sudden cardiac death and myocardial infarction) [24]. In those with existing CVD, it has been shown to predict future cardiovascular events, including recurrent ischaemia, atrial fibrillation, death, stroke and percutaneous coronary intervention [2526]. Elevated CRP also appears to correlate with softer plaques that are more prone to rupture [27] and early data suggest that it may be useful in targeting 'high-risk' patients who would most benefit from aggressive CVD prevention therapies, such as aspirin, statins and angiotensin-converting enzyme inhibitors [2829]. We therefore conclude that hypertensive Nigerians have elevated CRP which correlate with CHD risk than their normotensive counterparts, a finding that may help in the understanding and management of hypertension, which continues to be a major disease risk factor and burden on healthcare resources.

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