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ASSESSMENT OF HIGH SENSITIVITY C-REACTIVE PROTEIN (HS-CRP) AND TOTAL ANTIOXIDANT STATUS IN PATIENTS WITH CHRONIC KIDNEY DISEASE, DIABETES AND HYPERTENSION

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Abstract
The levels of high sensitivity C-reactive protein (hs-CRP) and total antioxidant capacity (TAC) and its relationship with other risk factors like blood pressure and anthropometric measurements were assessed. A total of 180 patients (90 controls, 90 patients (45 males; 45 females respectively) aged 18-76years old, diagnosed as having chronic kidney disease, diabetes and hypertension, but clinically stable were recruited from University of Benin Teaching Hospital. The controls were apparently healthy individuals. Anthropometric measurements were carried out (weight and height) to calculate the Body mass index (BMI). Serum samples were collected for hs- CRP and TAC assays. The BMI was significantly higher $P < (0.05)$ in hypertensive and diabetic patients but was normal in CKD (Chronic kidney disease) patients. Blood pressure was increased in CKD patients. Biochemical results revealed that CRP levels were significantly higher $P < (0.05)$ while TAC levels were reduced in all the patients compared with controls. There was no correlation $P \geq 0.05$ between CRP and BMI but TAC was negatively correlated with CRP in CKD patients. However, in diabetic patients, CRP correlated positively with BMI and blood pressure while TAC correlated negatively with CRP. In conclusion, we recommend these biomarkers as adjuvant in the management of these diseases.

INTRODUCTION

Chronic kidney disease (CKD), diabetes and hypertension are major causes of mortality and morbidity in most countries, inspite of the availability of effective primary and secondary therapies[1,2].The risk factors associated with these diseases include obesity, hyperlipidemia, age, sex, smoking and positive family history[3].

Low grade inflammation is often reflected by increased plasma levels of several biomarkers of inflammation such as C-reactive protein (CRP) and products of lipid peroxidation.[4] The total antioxidant capacity (TAC) is a new concept that has been proposed to assess the combined effects of multiple antioxidant in a biological system.[5] Recent reports have identified oxidative stress and inflammation as significant and novel risk factors in many diseases[6].

KEYWORD: *High sensitivity CRP, Total antioxidant capacity, chronic kidney disease, diabetes, hypertension*

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Oxidative stress is caused by high levels of free radicals in the blood resulting in a decline of antioxidant defences. This leads to damages in healthy cells and tissues by peroxidation of lipid membrane, oxidation of proteins, DNA and possible disruption of cytokine function. It has been suggested that Oxidative stress may play a role in the pathogenesis of chronic kidney disease, diabetes and hypertension[7]. CRP is a pentameric non immunoglobulin protein produced by the liver in the acute phase response to inflammation, infection and tissue damage. It assists in complement binding to foreign and damaged cells and enhances phagocytosis. CRP is a marker of inflammation and has emerged as a strong independent risk marker for evaluating vascular inflammation.[8,9]

Earlier reports show a positive association between CRP and hypertensive patients, oxidative stress, and reduction of vitamin E and C levels[10,11,12] . Scientific reports about the associations between body mass index, C-reactive protein and total antioxidant capacity in patients with chronic kidney disease, diabetes and hypertension are scarce. This study was carried out to assess the levels of CRP and TAC in patients presenting with chronic kidney disease, diabetes mellitus, hypertension and to correlate these inflammatory markers with other well established risk factors associated with these diseases.

SUBJECTS AND METHODS

Study Population

A total of 180 subjects: 90 controls, 90 test (45 males ; 45 females respectively) were used for this study. Three groups of thirty subjects each; aged 18-76years old, diagnosed as having chronic kidney disease, Diabetes mellitus and hypertension; but clinically stable were recruited from University of Benin Teaching Hospital outpatient clinics. The controls were apparently healthy individuals recruited from hospital workers and University staff. Patients who were having medical complications such as cardiac failure were excluded from the study. Patients on micro nutrients such as zinc, selenium and vitamin C and E were also excluded.

The study protocol was carefully explained to all participants before they voluntarily signed an informed consent form. A questionnaire was used to collect information from participants. The information included age, sex, smoking and drinking habit, marital status and occupation.

Methods.

Blood pressure was recorded in the sitting position after five minutes of rest, weight and height measurements were taken[13] and Body mass index (BMI) calculated.

Determination of Total Antioxidant capacity (TAC).

Blood samples were obtained once from the subjects by venipuncture. Five millimetres of blood samples were collected into sterile centrifuge tubes and centrifuged at 4000rpm for 10minutes. The sera were collected and stored in the deep freezer at -20^{o}C. The TAC assays were carried out using commercial kits from Randox Laboratories (Randox Laboratories Ltd, Diamond Road, Crumlin, Antrim, Ireland)[14]. The assay was calibrated using 6-hydroxy-2, 5, 8- tetra-methylchroman-2-carboxylic acid (trolox). Briefly, 500 µl of Reagent 1 containing potassium phosphate and copper sulphate (12: 1) was added to 30µl of the sample or standard in a cell. The initial absorbance was read at 660nm. Thereafter, 75µl of Reagent 2 containning 4,4 dicarboxy-2-2 biquinoline was added to the cell and incubated for 10minutes at room temperature. The absorbance was read finally. The total antioxidant capacity

was then calculated. The results were expressed as mMol/L of trolox equivalent.

High sensitive C-reactive protein (HS - CRP) high sensitive-CRP was assessed in sera using the enzyme linked immunosorbent assay (ELISA) technique. The kit was produced by DRG International Inc, 1167 U.S Highway, 22 East Mountainside, NJ 07092, USA. Lot NO: RN- 39692. A semi automated microtitre well reader (Chemwell incorporated, USA) was used.[15] Briefly, the test and control serum samples were diluted 100 fold. Standard CRP (100 µL), diluted test and control samples were dispensed in the appropriate wells respectively. Thereafter, 100µL of CRP enzyme conjugate reagent was dispensed into each well. The specimen was thoroughly mixed for 30 seconds and Incubated at room temperature (18-25) for about 45 minutes. The incubated mixture was flicked and 100 µL of tetramethylbenzidine (TMB) solution was dispensed into each well, mixed gently for five seconds and incubated at room temperature for twenty minutes. The reaction was stopped by adding 100 µL of 1N HCl to each well, mixed gently for 30 seconds and the Absorbance read at 450nm with a microtitre reader within 15minutes.

Ethical Consideration: Ethical approval was obtained from the Research and Ethics Committee of the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

STATISTICAL ANALYSIS

The statistical software SPSS (version 15) was used for the data analysis. The results are expressed as mean standard deviation (S.D). The student t-test was used to determine if there were statistically significant differences between the control and patients. P values < 0.05 were considered statistically significant. The relationship between anthropometric parameters, hsCRP, TAC, and blood pressure were also analysed using pearson correlation.

Results

The characteristics of the study population are shown in Table 1. BMI was significantly higher P < (0.05) in hypertensive and diabetic patients when compared with the control. Blood pressure was higher in CKD patients. The diabetic patients had normal Blood Pressure values while it was uncontrolled in the hypertensive. The results revealed that hs-CRP levels were significantly increased P < (0.05) while TAC levels were decreased in all the patients recruited for the study (Table 2). The patients with chronic kidney disease had significantly higher values of CRP (20.96 mg/L) than the diabetic and hypertensive patients with values of (7.66mg/L and 9.07mg/L) respectively.

The pearson's correlation analysis of hs-CRP and TAC with other risk factors blood pressure and BMI are presented in Table 3 and 4. CKD patients showed positive correlations of TAC with BMI, systolic and diastolic blood pressure. In diabetics, BMI was weakly correlated(r = 0.146) with TAC while blood pressure was negatively correlated .The hypertensive patients showed positive correlation with BMI. Associations of CRP with other cardiovascular disease risk factors revealed that in CKD, BMI has no correlation (r = 0, p $\geq$ 0.05), while systolic and diastolic blood pressure was negatively correlated (r = -0.27,-0.32, respectively; p $\geq$ 0.05). In diabetic patients, the risk factors were positively correlated while they were negJatively correlated in the hypertensives.

Table 1: Anthropometric representation of the subjects

SUBJECTS	CONTROL (n=90:45males,45 females)	TEST (n=90: 45males, 45 females)
CHRONIC KIDNEY DISEASE PATIENTS		
Age (years)	40.10 ± 3.50[a]	50.33 ± 3.04[b]
Weight (kg)	66.03 ± 1.89 [a]	67.47 ± 2.39 [a]
Height (m)	1.64 ± 0.02 [a]	1.65 ± 0.02 [a]
Body mass index (kg/m²)	24.72 ± 0.62 [a]	24.80 ± 1.04 [a]
Systolic blood pressure (mmHg)	120.00 ± 2.54 [a]	145.33 ± 4.49 [b]
Diastolic blood pressure (mmHg)	75.77 ± 1.79 [a]	88.93 ± 2.56 [b]
DIABETIC PATIENTS		
Age (years)	39.76 ± 3.39[e]	48.10 ± 2.35[f]
Weight (kg)	67.37 ± 1.69[e]	77.73 ± 3.11[f]
Height (m)	1.64 ± 0.01[e]	1.62 ± 0.01[e]
Body mass index (kg/m²)	25.07 ± 0.59[e]	29.29 ± 1.02 [f]
Systolic blood pressure (mmHg)	123.60 ± 1.99[e]	123.17 ± 1.74 [e]
Diastolic blood pressure (mmHg)	75.10 ± 1.85[e]	80.03 ± 1.61[f]
HYPERTENSIVE PATIENTS		
Age (years)	39.76 ± 3.39[c]	57.77 ± 2.78[d]
Weight (kg)	67.37 ± 1.69[c]	82.53 ± 3.02[d]
Height (m)	1.64 ± 0.01[c]	1.62 ± 0.02[c]
Body mass index (kg/m²)	25.07 ± 0.59[c]	31.61 ± 1.18[d]
Systolic blood pressure (mmHg)	123.60 ± 1.99 [c]	154.77 ± 3.42[d]
Diastolic blood pressure (mmHg)	75.10 ± 1.85[c]	98.33 ± 1.80[d]

values are mean± standard error of the means.
values with same superscript on the same row are not significantly different at $p < 0.05$

Table 2: Serum hs-CRP and Total antioxidant capacity in chronic kidney disease, diabetic and hypertensive subjects.

SUBJECTS	CONTROL (n=90:45males, 45females)	TEST (n=90:45males, 45females)
CHRONIC KIDNEY DISEASE PATIENTS		
C-Reactive protein (mg/L)	7.81 ± 3.73 [a]	20.96 ± 3.89 [b]
Total antioxidant capacity (mmol/troloxequi/L)	11.59 ± 2.98 [a]	4.67 ± 0.81 [b]
DIABETIC PATIENTS		
C-Reactive protein (mg/L)	2.64± 3.89 [b]	7.66± 3.89 [b]
Total antioxidant capacity (mmol/troloxequi/L)	12.25 ± 4.53 [b]	7.19 ± 1.08 [b]
HYPERTENSIVE PATIENTS		
C-Reactive protein (mg/L)	2.28± 3.89 [b]	9.07± 3.89 [b]
Total antioxidant capacity (mmol/troloxequi/L)	8.46± 3.89 [b]	5.69± 3.89 [b]

values are mean± standard error of the means.
values with same superscript on the same row are not significantly different at p< 0.05

Table 3. Correlation coefficient of Total antioxidant capacity (TAC) with other risk factors in chronic kidney disease, diabetes and hypertensive subjects.

Parameters of subjects	r		P value
Chronic kidney disease			
Body mass index (kg/m²)	0.183	≥	0.05
Systolic blood pressure (mmHg)	0.232	≥	0.05
Diastolic blood pressure (mmHg)	0.049	≥	0.05
C-Reactive protein (mg/L)	-0.116	≤	0.05
Diabetes			
Body mass index (kg/m²)	0.146	≥	0.05
Systolic blood pressure (mmHg)	-0.230	≤	0.05
Diastolic blood pressure (mmHg)	-0.125	≤	0.05
C-Reactive protein (mg/L)	-0.169	≤	0.05
Hypertension			
Body mass index (kg/m²)	0.017	≤	0.05
Systolic blood pressure (mmHg)	0.047	≤	0.05
Diastolic blood pressure (mmHg)	0.064	≥	0.05
C-Reactive protein (mg/L)	0.177	≥	0.05

Table 4. Correlation coefficient of High sensitivity C - reactive protein (hs-CRP) with other risk factors in chronic kidney disease, diabetes and hypertensive subjects.

Parameters of subjects	r		P value
Chronic kidney disease			
Body mass index (kg/m²)	0.00	≤	0.05
Systolic blood pressure (mmHg)	-0.272	≤	0.05
Diastolic blood pressure (mmHg)	-0.328	≤	0.05
Total antioxidant capacity (mmol/troloxequi/L)	-0.306	≤	0.05
Diabetes			
Body mass index (kg/m²)	0.091	≥	0.05
Systolic blood pressure (mmHg)	0.199	≤	0.05
Diastolic blood pressure (mmHg)	0.078	≥	0.05
Total antioxidant capacity (mmol/troloxequi/L)	0.079	≥	0.05
Hypertension			
Body mass index (kg/m²)	-0.119	≤	0.05
Systolic blood pressure (mmHg)	-0.237	≤	0.05
Diastolic blood pressure (mmHg)	-0.335	≤	0.05
Total antioxidant capacity (mmol/troloxequi/L)	0.074	≥	0.05

Discussion

Cardiovascular disease is often associated with diabetes and hypertension while CKD can be caused by any of these conditions. Excess body weight could be a risk factor and contribute to the prevalence of these diseases.[16, 17] Our study revealed that hypertensive and diabetic patients have higher BMI. CKD patients did not have significantly different BMI from the control subjects. The prevalence of diabetes and hypertension is often associated with overweight individuals[18] and this could lead to reduction in health-related quality of life.[19]

C-reactive protein (CRP) and total antioxidant capacity are relevant markers that were used to assess the progression of these diseases associated with the patients. The serum hs-CRP was significantly increased in diabetic, hypertensive and CKD patients. The values obtained however, were within the normal range of 0.5-10mg/L. CKD patients had CRP values higher than the normal range. CRP levels indicate chronic low-grade inflammation, with linkage to blood vessel damage and vascular disease.[20] CRP acts directly upon blood vessels to activate adhesion molecules in endothelial cells: the intercellular adhesion molecule (ICAM-1) and the vascular cell adhesion molecule (VCAM-1).[21, 22] This indicates that inflammation is associated with the pathophysiology of the diseases. Similar results have been reported.[23]

Serum hs-CRP was also positively correlated with systolic and diastolic blood pressure in diabetic and negatively correlated in hypertensive subjects. Earlier authors have established that hs-CRP correlates positively with other traditional risk factors like obesity which contribute to the pathology of these diseases.[24] The literature has conflicting reports about the association of BMI with CKD.[25,26] Our results show that in CKD, there was no association between hs-CRP and BMI.

Blood pressure has been shown to have strong association with the progression of diabetes.[27, 28] This study however showed that the blood pressure was controlled in these diabetic patients. Earlier reports showed that CKD has strong association with hypertension[29, 30.] Our findings are in agreement with theirs. The high blood pressure of the hypertensive patients observed in this study showed that the blood pressure was uncontrollable.

Total antioxidant capacities were generally lower in all the patients compared to the control. This reduction shows clearly that the patients were in oxidative stress due to the presence of high free radical scavenging species. Our results are in agreement with recent reports[31] this ultimately leads to the formation of inflammatory responses. Reactive oxygen species (ROS) are made in excess, they, can react with various molecules such as lipids, carbohydrates, proteins, and DNA altering their structure and function[32] resulting in cellular damage that leads to pathologic processes. Total antioxidant capacity was found to be negatively correlated with blood pressure in diabetic patients; this also confirms that the antioxidant capacity was compromised as blood pressure increased. There was no correlation between BMI and TAC in the patients. This confirms that there is no association between excess weight and TAC.

The use of antioxidants (vitamin A, E and C) in diet and as supplements by these patients is required to reduce the raging of these reactive species and replenish the antioxidants in the body. This may help to reduce the pathogenesis of these diseases.

Conclusions:

Serum hs-CRP was increased in chronic kidney disease, diabetes mellitus and hypertension patients but total antioxidant capacity was reduced while, hsCRP is strongly associated with raised blood pressure and BMI. TAC was negatively correlated with blood pressure in diabetic and hypertensive patients. We recommend that these biomarkers as adjuvants in the management of these diseases.

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