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Assay of Leptin and Adiponectin Levels in Adult Obese Patients in Port Harcourt, Nigeria

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ABSTRACT

Background: Low-grade inflammation, characterized by increase of pro-inflammatory cytokines and decrease of anti-inflammatory cytokines, has been implicated in the pathogenesis of obesity and its associated complications. The study determined the levels of Adipokines (Leptin and Adiponectin) in obese and non-obese subjects with or without hypertension in a Nigerian population, in comparison with healthy (control) subjects.
Method: This cross-sectional study involved 60 physician diagnosed obese subjects attending the Endocrine and cardiology Clinics of the University of Port Harcourt Teaching Hospital, and 60 sex matched non-obese subjects recruited from the blood donor centre. Obese subjects were divided into two groups: subjects with (30) and without (30) hypertension based on Physician assessment. Controls were also divided into two groups: controls with (30) and without (30) hypertension based on Physician assessment. Serum levels of leptin and adiponectin were evaluated using commercial Enzyme linked immunosorbent assay (ELISA) technique.
Result: The results showed that the mean serum level of leptin was significantly ($P<0.05$) higher in the subjects (1598.65+151.1; 2833.35+297.1) compared to controls (1408.28+503.4;1269.85+511.3). Also, the mean serum level of adiponectin was significantly ($P<0.05$) lower in the subjects (6.64+4.0;7.12+1.3) compared to controls (8.34+5.4;18.25+8.2).
Conclusion: The results suggest that serum levels of leptin and adiponectin were altered, and that obesity and hypertension are associated with elevated and decreased serum concentration of leptin and adiponectin, respectively in the Nigerian population studied, which greatly affects the physiology of the immune cells and hereby generating a pathogenic environment in obesity and hypertension.

Keywords: Obesity, Hypertension, Adipokines, Leptin, Adiponectin.

INTRODUCTION

The understanding of obesity-induced changes in adipose tissue microenvironment better explains the impact of obesity on the metabolic function and immunological processes.[1,2] Obesity initiated by the intake of excessive calories,[3,4] leads to adipocyte hypertrophy altering the cellular composition of the adipose tissue and modulating the activity and population of other cells within the adipose tissue.[1]

Adipose tissue from obese organisms when compared to adipose tissue from lean organisms is infiltrated with a large number of macrophages associated with systemic inflammation and insulin resistance.[5,6] In addition, the macrophage phenotype is also altered and is atypically associated with inflammation and tissue destruction, producing pro-inflammatory cytokines such as tumor necrosis factor, inducible nitric oxide synthase, reactive oxygen and nitrogen intermediate.[7]

Obesity also impacts on the subsets of T-cells present in adipose tissue.[1]In obese conditions, the generation of Th1 signal by the accumulation of CD4+ Th1 cells initiate cell mediated immune response and synthesis of pro-inflammatory cytokines associated with obesity related dysfunctions.[8,9] Similarly, adipose tissue from obese organisms is infiltrated with mast cells, neutrophils and eosinophils, accompanied by obesity-associated metabolic dysfunction,[10] adipose tissue dysfunction,[11,12] alternative activation of macrophages and glucose intolerance, respectively.[13]

The impact of obesity and metabolic parameters on immunity and pathogen defence include the disruption of lymphoid tissue integrity, alterations in leukocyte development, phenotypes and activity and coordination of innate and adaptive immune response. These changes impact negatively on chronic disease progression, immunity from infection and vaccine efficacy.[14]

With the complexity of obesity-related disease and various disease mechanisms, there is the need to augment information obtained from clinical parameters. In this search, adipokines are considered a promising future.[15]

There have been various research findings on adipose tissue regarding energy storage and its regulation of complex metabolic and endocrine function. Adipocyte products (Leptin and adiponectin) have been reported to be involved in the control of energy expenditure, lipid and carbohydrate metabolism as well as regulation of immune responses.[2] Also, in relation to the degree of adiposity, leptin and adiponectin have been shown to be counter-regulated in vivo.[16]

However, among blacks, especially Africans, there are no reports on the levels of inflammatory adipokines in obese hypertensive patients as previous studies on obesity and hypertension in Nigeria for example, have mainly focused on the prevalence rate and anthropometric markers. Hence, it was deemed important to have data unique to an African population.

Also, the relationship between adipokines, leptin and adiponectin in obesity and hypertension is unspecified as well as their role as modulators of the immune system in obese hypertensive Nigerian adults.

This study was aimed at assessing the serum concentration of adipokines in patients with obesity-related hypertension as well as the socio-demographic patterns of adult Nigerian patients with Obesity and Hypertension. Data collated from the study, was used to evaluate the effect of adipokines (adiponectin and leptin) on the regulation of immunological responses in obesity and metabolic disorders.

MATERIALS & METHODS

The study was conducted between March 2019 and July 2019. Simple random sampling approach was utilized to ensure that all subjects had equal chance of being selected. The study population consisted of men and women presenting with central obesity and hypertension at the Cardiology and Endocrine Clinics of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, as well as apparently healthy individuals at the hospital.

Subjects for this study were duly informed about their roles and the aim and objectives of the study. Written informed consent was obtained from all the subjects recruited into the study, and their biodata were obtained through questionnaires and from their medical notes. Blood Samples were collected using 5mL auto-disposable syringes with a 21-gauge needle. For each

participant, 3mL of venous blood was collected and transferred into a plain bottle and centrifuged for 10 minutes to separate the serum from cells. The serum was then collected into another plain bottle using a Pasteur pipette and stored in a -20ºC deep freezer. The samples were preserved in this state until utilised for cytokine analysis.

Serum Leptin and Adiponectin concentrations were quantified using capture Enzyme Linked Immunosorbent Assay (ELISA) kits (Aviva Systems Biology, San Diego, CA, USA), and according to the manufacturer's instructions. The analysis was done in the Laboratory of the Department of Chemical Pathology, with the assistance of a medical laboratory scientist.

STATISTICAL ANALYSIS

The data generated were analysed using SPSS v25 software. Summary statistics of each variable were presented as mean ± SD and as the number of subjects (percentage) as appropriate. Continuous variables were analysed by chi-square tests, while categorical variables were analysed by independent sample t-tests. The Analysis of Variance (ANOVA) was used to compare means between four groups (Control group, obese hypertensive group, obese group and hypertensive group). The Dunn's post-test was used to compare the levels in the different groups. Pearson's correlation was used to assess the correlation between the immunological parameters in the different groups. An observation was considered significant if the p value < 0.05.

RESULT

This study involved 120 participants, 60 of whom were subjects while the other 60 were controls. Participants were further divided according to the presence or absence of hypertension. Table 1 shows that equal numbers of male and female adults were involved in the study. The mean age + SD was 43.5 + 12.4. Table2 shows distribution of participants by age, with a greater number of the subjects and controls with hypertension found within 40-49 years.

Table 3 shows a statistically significant difference in the waist circumference between the groups with the obese group having a higher mean waist circumference of 108.9+ 6.8 cm.

The difference in mean leptin concentration across the different groups was statistically significant ($p<0.05$). The concentrations in both groups of obese subjects were significantly higher when compared to the control group. Similarly, values in both obese groups were significantly ($p<0.05$) higher when compared to the hypertensive group. Also, the obese group had a significantly higher mean ($p<0.05$) than the obese hypertensive group. For adiponectin, the mean concentrations values were significantly different ($p<0.05$). The values were significantly higher in the control group when compared to other groups, (Table 4), but there was no significant difference between the obese groups and the hypertensive group even when the latter had a greater mean concentration.

Table 5 shows significant and negative correlation coefficients between immunological parameters in the different study groups.

Table1: Sociodemographic information of subjects

Variables	Subjects		Control	
	S^{H+}	S^{H-}	C^{H+}	C^{H-}
Gender				
Female	30	30	30	30
Male	30	30	30	30
Age group	Frequency		Percentage	
20 - 29 years	30		12.50%	
30 - 39 years	69		28.75%	
40 - 49 years	76		31.67%	
50 - 59 years	33		13.75%	
60 - 69 years	25		10.42%	
70 - 79 years	7		2.92%	
Mean age (years)	*43.5 ± 12.4*			

(SD: Standard deviation; H^+: Group with hypertension; H^-: Group without hypertension) Values are presented as Mean ± SD for continuous variables and percentage for categorical variables.

Table 2: Distribution of age groups across the different groups

Age Groups	Subjects		Control	
	S^{H+}	S^{H-}	C^{H+}	C^{H-}
20 - 29 years	0 (0.0)	14 (23.33)	0 (0.0)	16 (26.67)
30 - 39 years	10 (16.67)	21 (35.00)	16 (26.67)	22 (33.67)
40 - 49 years	24 (40.00)	21 (35.00)	17 (28.33)	14 (23.33)
50 - 59 years	14 (23.33)	2 (3.33)	9 (15.00)	8 (13.33)
60 - 69 years	12 (20.00)	0 (0.0)	13 (21.67)	0 (0.0)
70 - 79 years	0 (0.0)	2 (3.33)	5 (8.33)	0 (0.0)
Total	60 (100.0)	60 (100.0)	60 (100.0)	60 (100.0)

Table 3: Waist Circumference (WC) in different groups

Group	Subjects		Control	
	S^{H+}	S^{H-}	C^{H+}	C^{H-}
Waist Circumference(cm)	104.9 ±13.9	108.7 ±6.8	86.2 ±11.6	75.0 ±19.2
ANOVA	0.0001*			

*Difference is statistically significant between the groups ($p < 0.05$)

DISCUSSION

The study evaluated the levels of adipokines as well as their possible role in the regulation of the immune cells in the pathogenesis of obesity related hypertension in Nigerian patients.

The results of the study showed uneven distribution of obesity by age, with a greater number of the subjects found within 40-49 years. The datum is consistent with the report of earlier studies that have shown that the incidences of obesity and hypertension increase with age.[17–19]

Furthermore, the present study showed that the mean waist circumference was higher in the subjects, suggesting that most of the obese and obese hypertensive patients were centrally obese. This is due to the fact that obesity is a risk factor of hypertension and is known to be associated with weight gain and visceral fat has been reported to be associated with pro-inflammatory markers as well as a significant determinant of blood pressure in both men and women, independent of BMI.[20,21]

The result of the present study showed an increase in serum leptin levels which increased directly with visceral fat mass. Consistently, most studies have reported that obesity is associated with leptin resistance which manifests as hyperleptinemia with pro-inflammatory effects and possible involvement in the activation of immune cells.[14,22]

Leptin modulates the activity and function of neutrophils and eosinophils by increasing chemotaxis and secretion of oxygen radicals (superoxide anion and hydrogen peroxide).[23] In innate immunity, leptin causes increased phagocytosis by monocytes and macrophages and stimulates the secretion of pro-inflammatory mediators like TNF-α, IFN-γ and IL-6. In adaptive immune responses, leptin promotes cytotoxicity by natural killer cells and a switch towards Th-1 immune response which secrets IFN-γ and IL-2 rather than a Th-2 immune response which secretes IL-4 and IL-10.[24,25] However, leptin decreases the proliferation of Treg cells and increases the number and sensitivity of IL-17 producing Th cells (Th 17) in autoimmune and inflammatory diseases.[26,27]

Table 4: Comparison of Lectin and Adiponectin levels in the different groups

Parameters	Subjects		Control		ANOVA p-value	Multiple Comparisons (p value)					
	S^{H+}(A)	S^{H-} (B)	C^{H+} (C)	C^{H-} (D)		DvsC	DvsB	DvsA	CvsB	CvsA	BvsA
Lectin(ng/L)	1598.65 ±151.1	2833.35 ±297.1	1408.28 ±503.4	1269.85 ±511.3	0.0001*	0.1377**	0.0001*	0.0001*	0.0001*	0.0058*	0.0001*
Adiponectin(mg/L)	6.64 ±4.0	7.12 ±1.3	8.34 ±5.4	18.25 ±8.2	0.0001*	0.0001*	0.0001*	0.0001*	0.0915**	0.0524**	0.3785**

*Difference is statistically significant across the groups ($p < 0.05$)
Figures are presented in mean ± standard deviation
ANOVA, analysis of variance, was done to compare between the groups
Multiple comparisons between groups were done with Dunn's Post test.

Table 5: Correlation of Lectin and Adiponectin in the Different groups

Parameters	Subjects		Control	
	S^{H+}	S^{H-}	C^{H+}	C^{H-}
Lectin	1598.65 ±151.1	2833.35 ±297.1	1408.28 ±503.4	1269.85 ±511.3
Adiponectin	6.64 ±4.0	7.12 ±1.3	8.34 ±5.4	18.25 ±8.2
r (p-value)	-0.91 (0.005) *	-0.98 (0.0001) *	-0.45 (0.047) *	-0.03 (0.7108) **

Figures are presented in mean ± standard deviation
Figures are presented in Pearson's correlation (r) and p-values
***Correlation is statistically significant ($p < 0.05$)**
****correlation is not statistically significant ($p > 0.05$)**

Adiponectin on the other hand, was shown to be decreased in obesity. Also, unlike leptin, serum concentrations of adiponectin correlated inversely with abdominal fat mass. Adiponectin exerts immune-modulatory actions opposite to leptin. Its anti-inflammatory effects in innate and adaptive immunity inhibit the activation and phagocytic activities of monocytes and macrophages as well as the secretion of TNF-α, IFN-γ and nitric oxide.[28-30] Adiponectin has also been shown to lower the proliferation and cytokine secretion of T-cells as well as decrease the expression of MHC11 in dendritic cells. However, adiponectin stimulates the release of anti-inflammatory IL-10 and IL-1 receptor agonist in macrophages and up-regulates the proliferation of Treg cells involved in resistance to autoimmune diseases.[24,27]
Obesity and metabolic disorders induce changes in leucocyte and lymphocyte numbers and activities. It is therefore probable that changes in the serum levels of these adipokines (leptin and adiponectin) as observed in the present study may contribute to the onset and maintenance of dysfunctional immune responses present in obesity-related metabolic disorders.[31]

CONCLUSION

This study has shown a decreased serum adiponectin level and increased serum leptin level (inflammatory adipokines) in Nigerian obese and hypertensive subjects. The data from this study suggest that local inflammation in adipose tissue and altered immune response in obesity add to the development of related metabolic complications.

Declaration by Authors

Ethical Approval: This study was approved by the Research Ethics Committee of the University of Port Harcourt, Nigeria (UPH/CEREMAD/REC/MM62/017) and the University of Port Harcourt Teaching Hospital, Nigeria (UPTH/ADM/90/S.II/VOL.XI/730). The research was also carried out in line with the Helsinki Declaration.

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