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Original Research Article

Evaluation of serum leptin levels in diabetic and hypertensive subjects resident in Port Harcourt

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ABSTRACT

Background: Diabetes and hypertension which are metabolic conditions are becoming more common and prevalent in the world. Prolonged rise of blood sugar levels, is a hallmark of diabetes, a metabolic condition caused by problems with insulin production, insulin resistance or both while hypertension is the persistent high blood pressure in the arteries. Leptin is a hormone that inhibits appetite, reduces fat storage in adipocytes and aids in the regulation of energy balance hence implicated in diabetes and insulin resistance. Thus, can metabolic state affects its level in the serum.
Methods: The study population is two hundred and forty (240) subjects that are residents of Port Harcourt aged between 30-70 years. Sixty subjects were used as the control, sixty subjects were diabetes, the other sixty subjects were hypertensive while the remaining sixty were subjects with both hypertension and diabetic mellitus. An enzyme-linked immunosorbent assay (ELISA) method was used to quantitatively measure leptin levels in the serum sample, glycated haemoglobin were determined quantitatively using sandwich immunodetection and blood pressure was measured using mercury sphygmomanometer.
Results: The data generated were statistically analysed using Graph Pad Prism version 9.0.2. The results showed no significant difference in leptin levels in diabetics, hypertensive and subjects having both diabetes and hypertension when compared with the control subjects having none of these metabolic disorders (p =0.4166).
Conclusions: Our results shows that leptin levels in the population were relatively within the reference ranges both for males (0.5-12ng/ml) and females (0.5-15ng/ml). It clearly shows that metabolic conditions (diabetes or hypertension) does not affect leptin levels in serum.

Keywords: Diabetes, Hypertensive, Metabolic state, Serum leptin

INTRODUCTION

Diabetes and hypertension are among the cluster of metabolic abnormalities that results to metabolic syndrome.[1] More than 250 million are proposed to be diabetics globally by 2030.This figure are projected to rise to more than 400 million. Given that diabetes is linked to a higher risk of cardiovascular disease, as well as both macro- and micro-vascular disorders like blindness, amputation and kidney failure which are serious health danger flags.[2,3]

Hyperglycemia, or prolonged rise of blood sugar levels, is a hallmark of diabetes, a metabolic condition caused by problems with insulin production, insulin resistance or both. It is divided into two main groups.[4] Type 1 diabetes,

which affects between 5 and 10 percent of people with the disease, is an autoimmune condition that kills out pancreatic beta cells, leaving the body completely insulin deficient. The other, more prevalent type of diabetes, type 2, affects >90% of people with the disease and is brought on by a concomitant insulin resistance and relative, but not total insulin insufficiency. Diabetes is a long-term metabolic condition that can seriously harm the heart, blood vessels, eyes, kidneys and nerves.[5] In Nigeria, the prevalence of diabetes mellitus is 3.7% with about 3.6 Million adults suffering from this disease.[6]

Hypertension, which is also known as high, elevated or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. This blood pressure is created by the force of blood pushing against the walls of the blood vessels (arteries) as the heart pumps. The higher the pressure, the harder the heart has to pump. Hypertension is a serious medical condition that significantly increases the risks of heart, brain, visual impairment, kidney and other diseases.[5] An estimated 1.28 billion adults aged 30-79 years worldwide have hypertension, most (two-thirds) living in low- and middle-income countries. Hypertension is a major cause of premature death worldwide. One of the global targets for non-communicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030.[7]

Type 2 diabetes mellitus and hypertension frequently coexist and because they share some common risk factors such as endothelial dysfunction, vascular inflammation, atherosclerosis, dyslipidaemia and obesity among others, they are said to be strongly related to one another.[8]

The central nervous system (CNS) appears to be crucial in the regulation of glucose metabolism and hypertension. In connection to this, Leptin, an adiposity hormone has been found to cross pathways with hypertension and glucose metabolic mechanisms thereby affecting the risk of cardiovascular diseases and also helping in regulating glucose metabolism hence being able to play a role in the development of diabetes if its metabolism and activities are disrupted.[9]

White adipose tissue (WAT) produces and secretes leptin, a polypeptide hormone that regulates food intake, energy expenditure, and autonomic function. Leptin is a hormone primarily produced by enterocytes and adipocytes in the small intestine. It inhibits appetite, which in turn reduces fat storage in adipocytes and aids in the regulation of energy balance.[10] Leptin circulates in proportion to body fat mass, enters the central nervous system (CNS) in proportion to its plasma level, and interacts with its receptor, which is expressed in key brain areas. Leptin deficiency promotes hyperphagia and weight gain, whereas leptin treatment results in decreased food intake, increased energy expenditure, and weight loss. This strong body of evidence implies that leptin is essential for the control of energy balance.[11] While the effect of leptin to reduce food intake and body adiposity can improve insulin sensitivity in peripheral tissues via indirect mechanisms, several observations suggest that leptin can directly affect glucose metabolism independent of its effects on energy balance. However, recent evidence implicates leptin not only in the regulation of energy balance but glucose homeostasis as well.[12]

Any disruption in the physiological mechanism surrounding the sympathetic activation of the heart and kidney can cause a rise in blood pressure. This is experienced when the renin angiotensin system is activated leading to sodium retention, circulatory expansion, vaso constriction thereby causing an elevated blood pressure.[13] Leptin activates the sympathetic nervous system in the hypothalamus thereby increasing blood pressure.[14] It may also affect blood pressure and contribute to hypertension through sympathetic activation in the vascular and renal system.[15] High levels of circulating leptin are believed to be responsible for the increase in renal sympathetic tone seen in overweight people.[16] Leptin can be used as a biomarker for metabolic syndrome diagnosis and early detection linking to hypertension.[17] This study therefore looks at these two category of subjects if their metabolic state has any influence on the leptin levels in their serum sample.

METHODS

This was an analytical cross sectional study where samples were collected and compared with control subjects. This study was carried out among patients attending University of Port. Harcourt Teaching Hospital in Port Harcourt which is the current capital of Rivers State, Nigeria within the period of three months (September, 2022 to November 2022). The study population consisted of two hundred and forty subjects. Sixty (60) subjects were used as the control (non diabetic/non hypertensive), sixty (60) subjects were diabetic, sixty (60) were hypertensive while the other sixty (60) were subjects with both hypertension and diabetes.

Inclusion criteria

Subjects who gave their informed consent to participate in the study and were residents of Port Harcourt metropolis for at least 5 years were included in this study.

Exclusion criteria

Subjects who were non residents in Port Harcout and those whose body mass index were classified as obese after checking their weights and heights were excluded.

Sample collection

The Subjects fasted overnight prior to sample collection. Using a 10ml syringe, blood sample was collected from the subjects by venipuncture into well labelled EDTA

and plain bottles. The samples were promptly transported to the Department of Chemical pathology laboratory, University of Port Harcourt Teaching Hospital for immediate processing. Samples were centrifuged at 3000 rpm for 5 minutes and separated into plain sample bottles and stored at -20˚C until analyzed. Serum samples were analyzed for the quantitative detection of leptin using an enzyme linked immunosorbent assay (ELISA) kit from Elisa Phoenix Pharmaceutical, USA. The test was carried out and interpreted according to the manufacturer's instructions while the sample collected in EDTA bottle was immediately used for the estimation of glycated haemoglobin.

Estimation of HBAIc: HBA1C concentration was measured using a Finecare™ HbA1c rapid quantitative test, China within 24 hours after sample collection. ID Chip was inserted into the instrument, 10 µL of whole blood Sample were drawn with a transfer pipette and added into the buffer tube. The specimens with buffer were mixed well for 1 minute by inverting the tube several times. The test cartridge was loaded with 75 µL of sample mixture and test cartridge inserted into the cartridge holder and the test button was clicked to display the results after 5 minutes. The manufacturer's instruction were strictly followed. A diagnosis of T2DM was confirmed when a patient had a glycated haemoglobin (HbA1C) value of 48 mmol/mol (6.5%) or higher as reported.[18]

Blood pressure measurement: The subject's blood pressure was determined using a mercury sphygmomanometer. Subjects were made to seat on a chair beside a table with both legs on the floor; the subject's arm was made to rest on a table surface that is at level with their arm. Using a stethoscope with a proper-sized cuffs, the cuffs was properly wrapped around the arm with the stethoscope over the brachial artery (in the bend of the elbow) and then it was inflated. Gradually, the pressure in the cuff was deflated while listening to hear the sound of the heart beat (Korotkoff). The first Korotkoff sound (systolic pressure) was noted and the diastolic pressure also was noted, which is the last Korokoff sound before the sounds go silent.

Leptin estimation: The frozen serum samples were thawed and concentration of leptin in serum were determined using sandwich type enzyme-linked immunosorbent assay. Wells for diluted standard, blank and samples were determined. 7 wells for standard and 1 well 100microliter each of working standard solution, samples were added into appropriate wells which were covered with plate sealer and incubated for 80minutes at 37°C. The liquid in each well was removed and the wells washed with 200microliter of 1× wash solution for 3 times. The remaining liquid were completely removed by snapping the plate onto absorbent paper. 100microliter of biotinylated antibody solution was added to each well and then covered with plate sealer and incubated for 50minutes at 37°C. The aspiration and wash process in step 2 was repeated 3times. 100microliter of streptavidin-HRP working solution was added to each well, the wells were covered with plate sealer and incubated for 50minutes at 37°C. The aspiration and wash process was repeated 5times. 90microliter of TMB substrate solution was added to each well and covered with new plate sealer and incubated for 20minutes at 37°C. Finally, 50microliter of stop reagent was added to each well. Measurements were taken using a microplate reader at 450nm wavelength.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism version 9.0.2 (San Diego, California, USA). Results were presented as Mean ± Standard deviation (SD). Inferential statistics using Tukey multiple comparison tool was employed to compare differences between the values among the different groups. Statistical significance was set at $P<0.05$.

RESULTS

Table 1 shows a demographic picture of the study population with the different percentages of sexes involved in the various categories of subjects and their mean leptin levels, while Table 2 shows the serum leptin levels in the different classification of subjects.

Table 1: Demographic details of participants.

Subjects	Sex	Frequency (%)	Leptin levels (ng/ml±SD)
Apparently healthy (control) subjects	Male	33 (55.0)	4.85±0.08
	Female	27 (45.0)	5.10±0.10
Diabetic subjects	Male	30 (50.0)	4.82±0.12
	Female	30 (50.0)	8.20±1.10
Hypertensive subjects	Male	24 (40.0)	3.66±0.04
	Female	36 (60.0)	7.52±1.18
Diabetic and Hypertensive Subjects	Male	30 (50.0)	3.84±0.06
	Female	30 (50.0)	4.28±0.08

Table 2: Comparison of serum leptin levels in diabetic, hypertensive and subjects with DM and HBP comorbidity.

Groups	Leptin levels (ng/ml±SD)
Control (n-60)	4.74 ± 0.08
Hypertensive (n-60)	5.62 ± 0.10
Diabetes (n-60)	6.76 ± 0.16
Hypertensive/diabetes (n-60)	3.98 ± 0.06
F value	0.9578
P value	0.4166
Remark	Non significant

DISCUSSION

Leptin has been implicated with blood pressure through the renal sympathetic activation in a study by Bravo.[19] Also a study by Mohiti and Doudi shows that leptin regulates blood sugar either by controlling appetite and fat or instructing the liver what to with its stored glucose hence the essence of knowing if these metabolic syndromes, diabetics and hypertension has any significant influence on serum leptin levels.[20] However, the results in this study shows no significant differences between diabetics, hypertensives and subjects having both diabetes and hypertension when compared with control subjects having none of these metabolic disorders. It was observed that the leptin levels in the study population were relatively within the reference ranges both for males (0.5-12ng/ml) and females (0.5-15ng/ml).

A study by Kadhim in Iraq revealed the value of leptin as 10.63±2.73 ng/ml in diabetic cases compared to 4.05±1.67 ng/ml in the control cases.[21] Meanwhile from Buyukbese study, we had leptin levels to be 40.22±17.77 ng/ml in diabetes compared to 50.12±15.55 ng/ml in the control though the later cases were all obese which corresponds to the study of that states the association of a higher leptin level with obesity.[22-24] This is in contrast to this study as the leptin levels in the diabetes were not significantly different from the control which corroborates the report from Haffner which stated a non significant differences in the concentratrion of leptin in diabetes and non diabetes.[25]

This study agrees with the report of Abu-Ssayed where low levels of leptin may be attributed to lean diabetics which might be as a result of the fat distribution within the subjects since they are all on normal weight. [26,27] This study agrees with the report of Zhao which states that elevated leptin level might be a risk factor for developing diabetic mellitus not the other way round of diabetics prone to having higher leptin levels.[28] So increase in leptin can subsequently lead to the development of diabetes in future as reported by Sordeberg.[29]

Haynes et al reported an association between leptin and hypertension but in this study, there was a non significant difference between hypertensive subjects and the control.[30] However in a study by they reported a positive relationship between leptin and hypertension stating how higher levels of leptin are seen in hypertensive subjects compared to the control.[31,32]

Serum leptin concentration also has a gender dimorphism with higher serum levels in women than that in men.[33,34] This is in contrast with the presentation in this study (Table 1). Serum leptin level was higher in women than in men and this is probably owing to the adipose tissue in women being more compared to men.[35]

This study has some limitations. This study did not consider obesity and exercise as a contributory factor to the spike in leptin levels in humans. We further did not consider insulin resistance and lipid profiling in this study because study has showed its relationship with leptin and hypertension respectively.

CONCLUSION

Diabetic and hypertensive states does not cause leptin levels to increase in both males and females rather increases in leptin levels can affect the metabolic state of individuals.

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Ethical approval: The study was approved by the Rivers State University research/ethics committee with file No: RSU/CV/APU/74/VOL.XII /101 and the University of Port Harcourt Teaching Hospital with file no UPTH/ REC/2022050

REFERENCES

1. Vrdoljak J, Kumric M, Vilovic M, Martinovic D , Rogosic V, Borovac JA, et al. Can fasting curb the metabolic syndrome epidemic? Nutrients. 2022;14(3):456-60.
2. Farag YMK Gaballa MR. Diabesity: an overview of a rising epidemic. Nephrol Dial Transplant. 2011;26(1):28-35.
3. Smyth S. Heron A. Diabetes and obesity: the twin epidemics. Nat Medi. 2006;12(1):75-80.

4. Devendra D, Liu E, Eisenbarth GS. Type 1 diabetes: recent developments. Biomed J. 2004;328(7442):750-4.
5. Onwuli DO, Waribo HA, Anyalebechi EO, Nwafor LU. Evaluation of plasma homocysteine levels in type ii diabetes and hypertensive patients attending university of Port Harcourt teaching hospital, Nigeria. J Biosci Medi. 2023;11(5):30-9.
6. International Diabetes Federation (IDF). Diabetes Atlas. 8th ed. International Diabetes Federation, Brussels; 2017.
7. World Health Organization. Hypertension, 2023. Available at https://www.who.int/news-room/fact-sheets/detail/hypertension.accessed 25th September 2023.
8. Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Canad J Cardiol. 2018;34(5):575-84.
9. Morton GJ. Schwartz, MW. Leptin and the central nervous system control of glucose metabolism. Physiol Revi. 2013;91(2):389-411.
10. Bouret S, Levin BE, Ozanne SE. Gene-environment interactions controlling energy and glucose homeostasis and the developmental origins of obesity. Physiol Revi. 2015;95(1):47-82.
11. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 1995;269(5223):543-6.
12. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, et al. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes. 2016;45(4):531-5.
13. Lu SC, Akanji AO. Leptin, obesity, and hypertension: a review of pathogenetic mechanisms. Metab Synd Relat Dis. 2020;18(9):399-405.
14. Shibasaki K, Yamada S, Akishita M. Plasma leptin concentration and sympathetic nervous activity in older adults with physical dysfunction. J Endocr Soci. 2018;2(9):1040-9.
15. Ma D, Feitosa MF, Wilk JB, Laramie JM, Yu K, Leiendecker-Foster C, et al. Leptin is associated with blood pressure and hypertension in women from the national heart, lung, and blood institute family heart study. Hypertension. 2009;53(3):473-9.
16. da Silva AA, do Carmo JM, Hall JE. Role of leptin and central nervous system melanocortins in obesity hypertension. Curr Opini Nephrol Hypert. 2013;22:135-40.
17. Ghantous CM, Azrak Z, Hanache S. Differential role of leptin and adiponectin in cardiovascular system. Int J Endocrinol. 2015;2015:534320.
18. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nature Genet. 1994;7(2):195-200.
19. Bravo PE, Morse S, Borne DM, Aquilar EA, Reisin E. Leptin and hypertension in obesity. Vascular Health Risk Manag. 2006;2(2):163-9.
20. Mohiti J Doudi J. Relationship between leptin and Insulin in patients with type 2 Diabetes. Turk J Endocrinol Metabol. 2003;9(2):55-8.
21. Kadhim RS. Determination of leptin level with other biochemical parameters in type 2 diabetic patients world bulletin of public health. W Bullet Pub Health. 2022;13:168-71.
22. Buyukbese MA, Cetinkaya A, Kocabas R, Guven A, Tarakcioglu M. Leptin levels in obese women with and without type 2 diabetes mellitus. Mediat Inflammat. 2004;13(5-6):321-5.
23. Das P, Bhattacharjee D, Bandyopadhyay SK, Bhattacharya G, Singh R. Association of obesity and leptin with insulin resistance in type 2 diabetes mellitus in Indian population. Ind J Physiol Pharmacol. 2013;57(1):45-50.
24. Chen GC, Qin LQ, Ye JK. Leptin levels and risk of type 2 diabetes: gender-specific meta-analysis: Meta-analysis of leptin and type 2 diabetes. Obes Revi: J Int Associat Stu Obes. 2014;15(2):134-42.
25. Haffner SM, Stern MP, Miettinen H, Wei M, Gingerich RL. Leptin concentrations in diabetic and non diabetic Mexican-Americans. Diabetes. 1996;45(6):822-4.
26. Sayeed MA, Azad Khan AK, Mahtab H, Ahsan KA, Banu A, Khanam PA, et al. Leptin is reduced in lean subjects with type 2 diabetes in Bangladesh. Diab Care. 2003;26(2):547.
27. Banerji MA, Faridi N, Atluri R, Chaiken RL Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. J Clin Endocrinol Metabol. 1999;84(1):137-44.
28. Zhao S, Kusminski CM, Elmquist JK, Scherer PE. Leptin: less is more. Diabet. 2020;69(5):823-9.
29. Söderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, Alberti KG, et al. Leptin predicts the development of diabetes in Mauritian men, but not women: a population–based study. Inte J Obes. 2007;31(7):1126-33.
30. Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. Hypertension. 1997;30(3):619-23.
31. Shankar A, Xiao J. Positive relationship between plasma leptin level and hypertension. Hypertension. 2010;56(4):623-8.
32. Shanker J, Rao VS, Ravindran V, Dhanalakshmi B, Hebbagodi S, Kakkar VV. Relationship of adiponectin and leptin to coronary artery disease, classical cardiovascular risk factors and atherothrombotic biomarkers in the IARS cohort. Thromb Haemost. 2012;108(10):769-80.
33. Ahren B, Larsson H, Wilhelmsson C, Nasman B, Olsson T. Regulation of circulating leptin in humans. Endocr. 1997;7(1):1-8.
34. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, et al. Effects of gender, body composition, and menopause on plasma

concentrations of leptin. J Clin Endocrinol Metabol. 1996;81(9):3424-7.
35. Liuzzi A, Savia G, Tagliaferri M, Lucantoni R, Berselli ME, Petroni ML, et al. Serum leptin concentration in moderate and severe obesity: relationship with clinical, anthropometric and metabolic factors. Int J Obes Relat Metabol Disor. 1999;23(10):1066-73.

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