Original Article

Role of Leptin in Acute Ischemic Stroke

Bindu Menon, Ramalingam Krishnan

Department of Neurology, Apollo Speciality Hospitals, 1Department of Biochemistry, Narayana Medical College, Nellore, Andhra Pradesh, India

Abstract

Purpose: Leptin has been implicated as a pathogenetic contributor to atherosclerosis. We aimed to investigate the association of leptin level with ischemic stroke. Materials and Methods: We prospectively enrolled 52 patients with acute ischemic stroke and measured leptin levels and compared with age- and sex-matched healthy controls. Risk factors, body mass index (BMI), biochemical parameters, intima–media thickness (IMT) on carotid vertebral Doppler and neuroimaging was done. Data were entered into MS-Excel and appropriate statistical analysis was done using SPSS software version 21.0. P = 0.05 was considered statistically significant. Results: Serum leptin was significantly elevated in stroke patients (6598.1 ± 1035.1) compared to controls (3090.7 ± 698.86) (P < 0.01). Patients had higher BMI (26.9 ± 1.7) than controls (26.9 ± 1.7) (P < 0.00). BMI, total cholesterol, low-density lipoprotein (LDL) cholesterol, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C reactive protein (CRP) were significantly elevated in stroke patients than controls. Correlation analysis among patient group showed that serum leptin positively correlated with CRP (r - 0.41, P - <0.05), WBCs (r - 0.28, P - <0.05), ESR (r - 0.429, P - <0.01) total cholesterol (r - 0.31, P - <0.05), LDL-cholesterol (r - 0.19, P - <0.05), and IMT (r - 0.714, P - <0.001). Conclusion: Our study showed high leptin levels in patients with stroke. Stroke patients with high leptin had higher BMI and inflammatory markers. The results of our study indicate that leptin may have a role in atherosclerosis mediated through inflammation. Future research should be directed toward understanding the role of leptin in the pathogenesis of cerebrovascular diseases and its potential role in preventive treatment of ischemic stroke.

Keywords: Leptin, risk factors, stroke

Introduction

Stroke is a leading cause of morbidity and mortality worldwide affecting millions of people every year. Stroke is a major health problem in India.[1] According to the India stroke factsheet updated in 2012, the estimated age-adjusted prevalence rate for stroke ranges between 84/100,000 and 262/100,000 in rural and between 334/100,000 and 424/100,000 in urban areas.[2] Worldwide in 2010, roughly 10% of the 52,769,700 death and about 4% of the 2,490,385,000 disability-adjusted life years were due to stroke.[3] Interstroke study showed that hypertension, current smoking, waist-to-hip ratio, diet risk score, regular physical activity, diabetes mellitus, alcohol intake, psychosocial stress, cardiac causes, and ratio of apolipoproteins B to A1 accounted for 88.1% of the population-attributable risks for all stroke.[4] Apart from the conventional risk factors, leptin has been studied as a risk factor for obesity-associated atherosclerosis.[5,6] Leptin is also known as the satiety hormone regulating the body weight by suppressing hunger. Leptin, a product of the ob gene (obese gene), is a 16-kDa nonglycosylated peptide hormone, synthesized mainly in adipose cells to regulate weight

Access this article online

Quick Response Code: Website: www.ruralneuropractice.com DOI: 10.4103/jnrp.jnrp_5_18

Address for correspondence: Prof. Bindu Menon, Department of Neurology, Apollo Specialty Hospitals, Nellore - 524 002, Andhra Pradesh, India. E-mail: bneuro_5@rediffmail.com

How to cite this article: Menon B, Krishnan R. Role of leptin in acute ischemic stroke. J Neurosci Rural Pract 2018;9:376-80.
control via its cognate receptor in hypothalamus centrally.\(^\text{[7]}\) Leptin has been classified as cytokines due to its structural similarities to the long-chain helical cytokine, which include interleukin-2 (IL-2), IL-12, and growth hormone.\(^\text{[5]}\) Leptin is also identified to stimulate the immune system by enhancing the proinflammatory cytokine production and phagocytosis by macrophages. Leptin regulates the immune system and its role in mediating inflammation is the cause of atherosclerosis.\(^\text{[8]}\) In addition, leptin affects the pituitary hormonal axes, indirectly influencing secretion of glucocorticoids, thyroid hormones, androgens, and catecholamines.\(^\text{[9]}\)

Leptin has also been shown to raise serum C-reactive protein (CRP) concentration, which is not only a potential inflammatory marker but also a direct cause of CVD.\(^\text{[10]}\) Effect of leptin on blood pressure, sympathetic activation, insulin resistance, platelet aggregation, arterial thrombosis, angiogenesis, and inflammatory vascular responses suggests leptin’s role in the development of cardiovascular disease and stroke.\(^\text{[11-14]}\) A study from India suggested a link between high leptin to obesity and diastolic blood pressure in male patients with myocardial infarction.\(^\text{[15]}\) However, its role as a risk factor for stroke is still debatable.\(^\text{[16]}\) The present study aimed to demonstrate the relationship of leptin with ischemic stroke in our region.

**Materials and Methods**

**Study population**

Fifty-two patients with first-ever ischemic stroke admitted to the department of neurology were included in this study. Patients with hemorrhagic and recurrent stroke, thyroid disease, moderate or severe anemia, renal insufficiency, dyslipidemia, and chest or urine infection were excluded from the study. Fifty healthy age- and sex-matched individuals were included as controls.

All the individuals under the study were categorized based on body mass index (BMI), as normal weight 18.5–22.99 kg, overweight 23.0–27.49 kg, and obese >27.5 kg. BMI was calculated using the formula – weight (kg)/height (m\(^2\)). Magnetic resonance imaging and carotid vertebral Doppler study was done for all patients. Carotid intima–media thickness test (CIMT) was measured at the far wall of the distal common carotid artery (CCA) to diagnose the extent of carotid atherosclerotic vascular disease.

**Laboratory analysis**

Venous blood was collected in the fasting condition from the study participants. A portion of whole blood was used for analyzing erythrocyte sedimentation rate (ESR) using ESR analyzer from Sysmax and white blood cell (WBC) counts using Hematology Analyzer from Beckman Coulter USA. Remaining blood samples were allowed to centrifuge and stored at −80°C for further analysis. Biochemical parameters analyzed in serum were fasting glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, and CRP using Chemistry Analyzer from HUMAN, GmBh, Germany.

**Leptin analysis**

The serum samples were assayed for leptin levels using commercially available enzyme-linked immunosorbent assay kit. The assays were conducted according to the manufacturer’s instructions, and a highly sensitive ELISA kit was used to detect the leptin levels in the sample. Each plate was checked before using to ensure that the calibration curve measured leptin standards (0–1000 pg/ml) within the stated limits of the assay. The samples were run in duplicates. The kit made use of biotin conjugate and human leptin antibody. Absorbance of the substrate color reaction was read on ELISA reader using 450 and 490 nm. The total leptin was determined in picograms (pg) and the calculation of the concentration in each sample was performed by dividing the amount of leptin by the volume of sample (pg/ml).

**Statistical analysis**

SPSS 12 statistical software package was used (SPSS Inc., Chicago, IL, USA). Continuous variables were described as mean and standard deviation. Comparison of means was done by independent sample t-test. The relationship between study parameters and leptin concentration was analyzed using Spearman’s rank correlation coefficient test. Statistical significance was set at \( P < 0.05\).

**Results**

Fifty-two patients and fifty controls were recruited in the study. All parameters tested in both patient and control groups are reported in Table 1. Serum leptin was significantly elevated in stroke patients (6598.1 ± 1035.1 pg/ml) when compared to controls (3090.7 ± 698.86 pg/ml) \( P < 0.01\). Patients had higher BMI (26.9 ± 1.7) than controls (26.9 ± 1.7) \( P < 0.00\). There was no statistically significant difference between the groups with respect to age, fasting plasma glucose, HDL-cholesterol, very-low-density lipoproteins, and triglycerides. BMI, total cholesterol, LDL cholesterol, WBC, ESR, and CRP were significantly elevated in stroke patients than controls. BMI \((r = 0.074, P < 0.05)\), CRP \((r = 0.41, P < 0.05)\), WBC \((r = 0.28, P < 0.05)\), ESR \((r = 0.429, P < 0.01)\) Total Cholesterol \((r = 0.31, P < 0.05)\)
<0.05), LDL-Cholesterol ($r = 0.19$, $P < 0.05$) and IMT ($r = 0.714$, $P < 0.001$). [Figure 1a-e].

**DISCUSSION**

Our study showed high leptin levels in patients with stroke. A link between leptin, vascular diseases, and coronary artery disease has been demonstrated in previous studies. The vascular effect of leptin has been suggested to be due to atherogenic, thrombotic, and angiogenic action. Leptin is postulated to exert atherogenic effect via platelet aggregation, formation of arterial thrombosis, and inflammatory vascular response. In our study, we found that stroke patients had high leptin and BMI. Obesity is excess body fat and BMI is a universally accepted measure of obesity. Framingham data strongly suggest that most of the relationship between body weight and coronary heart disease risk is mediated through the standard, major risk factors, i.e., blood pressure, total cholesterol, HDL cholesterol,

| Variables                      | Patients ($n=52$) | Controls ($n=50$) | P   |
|--------------------------------|------------------|------------------|-----|
| Age (years)                    | 53±11.8          | 55.1±5.5         | NS  |
| Sex (male/female)              | 3:1              | 2:1              | NS  |
| BMI (kg/m²)                    | 26.9±1.7         | 22.35±4.05       | 0.00|
| Glucose (mg/dl)                | 108.5±11.2       | 106±11.6         | NS  |
| Total cholesterol (mg/dl)      | 190.71±33.9      | 160.7±33.3       | 0.00|
| HDL-cholesterol (mg/dl)        | 44.23±16.3       | 43.1±8.8         | NS  |
| LDL-cholesterol (mg/dl)        | 122.06±34.4      | 94.3±24.2        | 0.00|
| Triglycerides (mg/dl)          | 147.8±47         | 142±42           | NS  |
| VLDL (mg/dl)                   | 28.46±9.5        | 25.5±8           | NS  |
| CRP (mg/L)                     | 14.2±6.1         | 5.0±1.2          | 0.00|
| WBC (cu/mm)                    | 9671.9±1189      | 7070.4±1532      | 0.00|
| ESR                            | 37.7±9.4         | 14.1±1.5         | 0.00|
| Leptin (pg/ml)                 | 6598.1±1035.1    | 3090.7±698.86    | 0.01|

NS: Not significant, BMI: Body mass index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoproteins, CRP: C-reactive protein, WBC: White blood cell, ESR: Erythrocyte sedimentation rate

**Figure 1:** Correlation between serum leptin levels and others variables. Correlation between the serum leptin levels and body mass index (a), erythrocyte sedimentation rate (b), C reactive protein (c), white blood cell (d), intima–media thickness (e)
and diabetes.[17] Studies have demonstrated a positive correlation of leptin with BMI.[18] Obesity is a known risk factor for the development of atherosclerosis and is considered a health burden. The central action of leptin is to regulate hunger and satiety. Our study showed that patients with stroke had higher leptin and BMI, probably depicting its central action. Correlation analysis showed that BMI positively correlated with leptin, implicating leptin’s role in stroke via visceral adiposity. Our study showed that patients had higher levels of cholesterol and markers of inflammation such as CRP, WBC count, and ESR. Various studies have shown that stroke is associated with increase in the classic markers of the inflammatory response, such as CRP,[19,20] ESR,[20] and WBC count.[21] This relationship might be more than casual in our study as there was positive correlation between leptin and markers of inflammation. Inflammation is increasingly being recognized to play a central role in atherosclerosis. The peripheral action of leptin is on the vascular system by inducing atherosclerosis by inflammatory response and endothelial dysfunction. We now understand obesity too as a chronic inflammatory disease. With the given findings of high leptin levels in patients with stroke with high BMI and inflammatory markers, it indicates that leptin’s role in atherosclerosis could be through inflammation.

We compared the CIMT in patients and controls. We demonstrated that leptin positively correlated with the IMT of CCA. CIMT has been recognized as a noninvasive diagnostic tool for the identification of atherosclerosis. Several studies have documented that the IMT of the CCA increases progressively with age and is associated with several conventional risk factors. Studies revealed that leptin-induced local inflammation in vascular endothelium is likely to be involved in the development of advanced atherosclerotic lesions.[22] An earlier study investigating the role of leptin with IMT of the CCA concluded that leptin was independently associated with IMT; however, the association was dependent on obesity which is in accordance with our study too.[23] A direct atherogenic pathway of leptin on the carotid artery wall for development of stroke has also been suggested.[24] Understanding the role of leptin in stroke has therapeutic implications. Monocyte chemoattractant protein-1 (MCP-1) is one of the critical factors attracting macrophages to adipocytes. Leptin has been shown to enhance MCP-1 production, hence promoting the first step of atherosclerosis.[25] Peroxisome proliferator-activated receptor gamma (PPAR-γ) controls the conversion of monocyte-derived macrophages into lipid loaded foam cells.[26] Indeed, treatment with leptin has been seen to accelerate the development of atherosclerotic lesions by decreasing the expression of PPAR-γ in macrophages and macrophage-derived foam cells.[27]

Limitation of our study was a small sample size and hence we could not categorize patients into different stroke subtypes for evaluation. A subgroup classification by age and gender was also not done due to small sample size. However, this study paves the way for further larger sample size study for more evidence.

**Conclusion**

In this sample study, our results revealed the presence of high serum leptin levels in the patients with stroke. Moreover, there was a positive association between elevated leptin levels, obesity, inflammation, and CIMT in acute ischemic stroke indicating the role of leptin in atherogenesis possibly via inflammation. There is a need to further evaluate leptin’s role in the pathogenesis of cerebrovascular diseases and its potential role in preventive treatment of ischemic stroke.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Poungvarin N. Stroke in the developing world. Lancet 1998;352 Suppl 3:III19-22.
2. Garraway WM, Wishnant JP, Drury I. Stroke fact sheet India. Available from: http://www.sancd.org/Updated%20Stroke%20Fact%20sheet%202012.pdf. [Last accessed on 2013 Jul 21].
3. Murray CJ, Volz T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. Lancet 2012;380:2197-223.
4. O’Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): A case-control study. Lancet 2010;376:112-23.
5. Beltowski J. Leptin and atherosclerosis. Atherosclerosis 2006;189:47-60.
6. Payne GA, Tune JD, Knudson JD. Leptin-induced endothelial dysfunction: A target for therapeutic interventions. Curr Pharm Des 2014;20:603-8.
7. Sánchez-Margalet V, Martín-Romero C, Santos-Alvarez J, Gómez R, Najib S, González-Yanes C, et al. Role of leptin as an immunomodulator of blood mononuclear cells: Mechanisms of action. Clin Exp Immunol 2003;133:11-9.
8. Fernández-Riejos P, Najib S, Santos-Alvarez J, Martín-Romero C, Pérez-Pérez A, González-Yanes C, et al. Role of leptin in the activation of immune cells. Mediators Inflamm 2010;2010:568343.
9. Arnalich F, López J, Codoco R, Jim nez M, Madero R, Montiel C, et al. Relationship of plasma leptin to plasma cytokines and human survivalin sepsis and septic shock. J Infect Dis 1999;180:908-11.
10. Dubey L, Hesong Z. Role of leptin in atherogenesis. Exp Clin Cardiol 2006;11:269-75.
11. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. J Clin Invest 2001;108:1533-40.
12. Bodary PF, Westrick RJ, Wickenheiser KJ, Shen Y, Eitzman DT. Effect of leptin on arterial thrombosis following vascular injury in mice. JAMA 2002;287:1706-9.
13. Söderberg S, Ahrén B, Stegmayr B, Johnson O, Wiklund PG, Weinhejl L, et al. Leptin is a risk marker for first-ever hemorrhagic stroke in a population-based cohort. Stroke 1999;30:328-37.
14. Sattar N, Wannamethee G, Sarwar N, Chernova J, Lawlor DA, Kelly A, et al. Leptin and coronary heart disease: Prospective study and systematic review. J Am Coll Cardiol 2009;53:167-75.
15. Ekmen N, Helvaci A, Gunaldi M, Sasani H, Yildirmak ST. Leptin as an important link between obesity and cardiovascular risk factors in men with acute myocardial infarction. Indian Heart J 2016;68:132-7.
16. Bourjana Z, Tzimouloz K, Goulas A, Hatzitolios AI. The role of adipokines in ischemic stroke risk stratification. J Int Stroke 2016;11:389-98.
17. Wilson PW, D’Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB, et al. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-47.
18. Wiesner G, Vaz M, Collier G, Seals D, Kaye D, Jennings G, et al. Leptin is released from the human brain: Influence of adiposity and gender. J Clin Endocrinol Metab 1999;84:2270-4.
19. Muir K, Wei C, Alwan W, Squire IB, Lees KR. C-reactive protein and outcome after ischemic stroke. Stroke 1999;30:981-5.
20. Vila N, Filella X, Deulofeu R, Ascaso C, Abellana R, Chamorro A, et al. Cytokine-induced inflammation and long-term stroke functional outcome. J Neurol Sci 1999;162:185-8.
21. Pozzilli C, Lenzi GL, Argentino C, Bozzao L, Rasura M, Giubilei F, et al. Peripheral white blood cell count in cerebral ischemic infarction. Acta Neurol Scand 1985;71:396-400.
22. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. FASEB J 1999;13:1223-8.
23. Ciccone M, Vettor R, Pannacciuli N, Minenna A, Bellacciacco M, Rizzon P, et al. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. Int J Obes Relat Metab Disord 2001;25:805-10.
24. Wannamethee SG, Shaper AG, Whincup PH, Lennon L, Sattar N. Adiposity, adipokines, and risk of incident stroke in older men. Stroke 2013;44:3-8.
25. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzmán M, Brownlee M, et al. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 2001;276:25096-100.
26. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001;7:53-8.
27. Cabrero A, Cabero M, Llaverias G, Alegret M, Sánchez R, Laguna JC, et al. Leptin down-regulates peroxisome proliferator-activated receptor gamma (PPAR-gamma) mRNA levels in primary human monocyte-derived macrophages. Mol Cell Biochem 2005;275:173-9.