Original Article

Role of Leptin in Acute Ischemic Stroke

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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 Leptin, 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

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control via its cognate receptor in hypothalamus centrally.[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.[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.[8] In addition, leptin affects the pituitary hormonal axes, indirectly influencing secretion of glucocorticoids, thyroid hormones, androgens, and catecholamines.[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.[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.[11-14] A study from India suggested a link between high leptin to obesity and diastolic blood pressure in male patients with myocardial infarction.[15] However, its role as a risk factor for stroke is still debatable.[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), 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,

Table 1: Study parameters in patients and controls

Variables	Patients (*n*=52)	Controls (*n*=50)	*P*
Age (years)	53±11.8	55.1±5.5	NS
Sex (male/female)	3:1	2.5:1	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	142.2±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
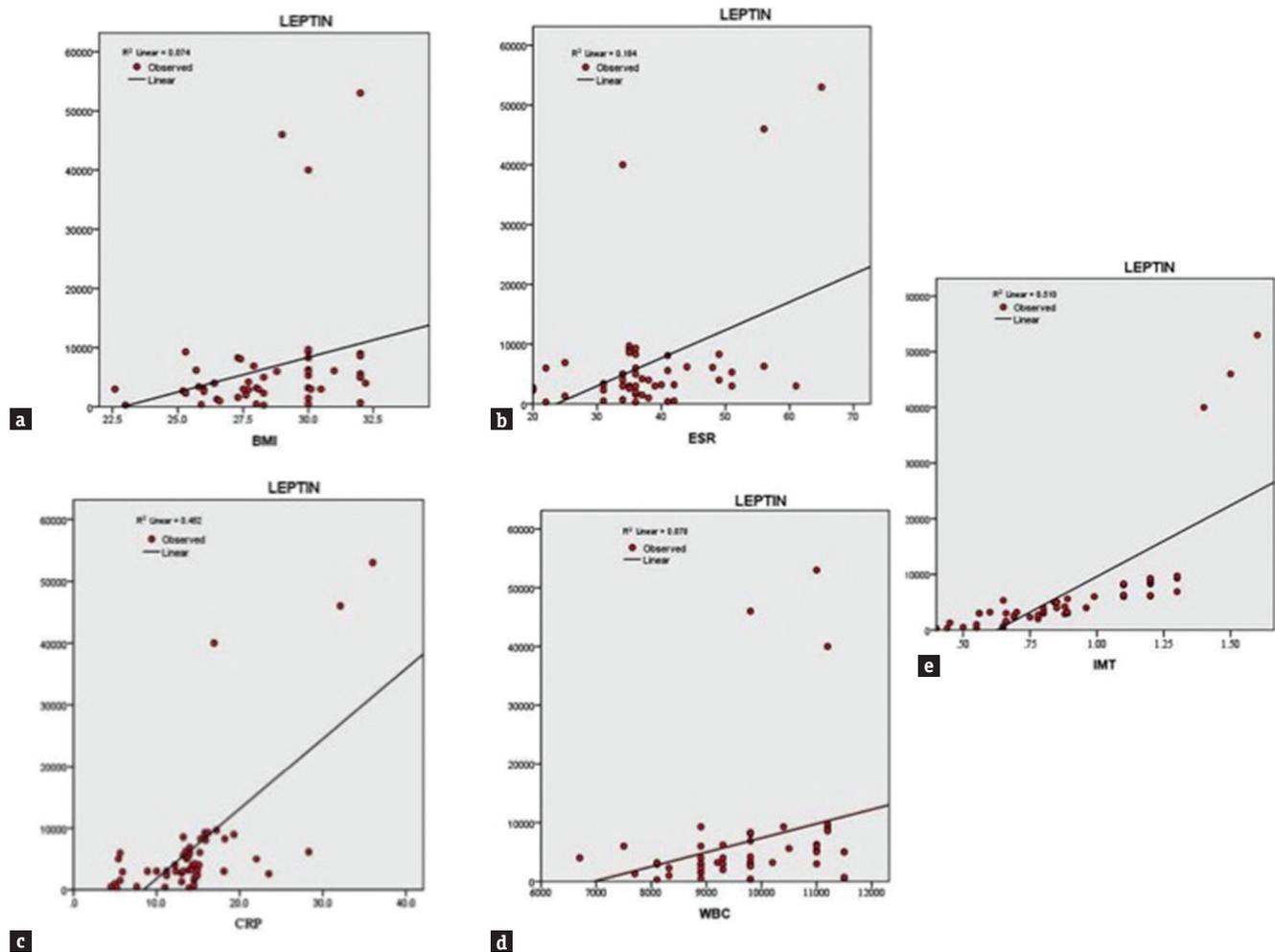

Figure 1: Correlation between serum leptin levels and others variables. Correlation between the serum leptin levels and body mass index (a), erythrocyte sedimentation rate (b), C reactive protein (c), white blood cell (d), intima–media thickness (e)

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.

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Conflicts of interest
There are no conflicts of interest.

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