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Serum Leptin Level and The Risk of Ischemic Stroke

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ABSTRACT

Background: Acute ischemic stroke (AIS) is an acute neurological dysfunction due to focal cerebral ischemia, which lasts >60 minutes with radiological evidence of infarction. Leptin is an adipokine hormone, and it resembles pro-inflammatory cytokines in structure and function. Researchers suggested the role of leptin in developing cardiovascular disease. Nevertheless, its effect as a stroke risk factor is controversial. Therefore, we conducted this study to evaluate the association between serum leptin level and the risk of AIS. **Patients and Methods:** We performed this study between September 2018 and September 2019 . A total of 60 patients (19 male and 41 female)presented with a clinical diagnosis of first-ever AIS were enrolled in the our study . All patients were assessed for risk factors, including hypertension (HTN), diabetes mellitus (DM), body mass index (BMI), smoking, cardiac diseases, hyperuricemia, and dyslipidemia. Thorough general and neurological examination .serum leptin levels and routine laboratory tests were assessed. Neuro-imaging including CT brain were done for all patients. We used the SPSS software e version 24.0 for statistical analysis. **Results**The median leptin serum level was 16.6 mg/l (IQR= 12.5-20). Of the included AIS patients, 80% had HTN, 66.7% were diabetic, 51.7% were obese, 43.3% had cardiac disease, 31.7% were smokers, 33.3% had dyslipidemia, 33.3% had hyperuricemia. 40% had early signs of middle cerebral artery infarction, while81.70% had an anterior circulation infarction,51.70% of patients had a left-sided brain lesion. The large-sized infarctions were more frequent (63.30%), followed by medium-sized infarctions (25%).There was a statistically significant association between serum leptin level and DM, HTN, obesity, cardiac disease (all $p<0.001$), as well as large-sized infarction (p-value= 0.02).**Conclusion**:Our study findings propose that high levels of serum leptin level may be associated with first-ever stroke risk. Further multicenter clinical trials are required to comprehensively assess leptin's role in AIS and confirm this association.

Keywords : Leptin; Ischemic stroke; risk factor; vascular

Introduction

Stroke is the second most prevalent cause of mortality and the third most common cause of long-term disability worldwide (1). The Global Burden of Disease Study findings showed that the stroke incidence rate is reduced in high-income countries and increased in low-income countries. Moreover, the stroke-related mortality rate is reduced in both low-income and high-income countries (2).

Stroke is classified into two major types: ischemic stroke and hemorrhagic stroke. Acute ischemic stroke (AIS) is an acute neurological dysfunction due to focal cerebral ischemia, which lasts >60 minutes with radiological evidence of infarction, while the hemorrhagic stroke occurs as a result of intracerebral hemorrhage or subarachnoid hemorrhage (3).

Previous literature has assessed the risk factors that would affect the incidence of AIS; these factors included old age, hypertension (HTN), diabetes mellitus (DM), smoking, body mass index (BMI), high blood cholesterol levels, previous history of transient ischemic attack, heart diseases (4–6).

Leptin is an adipokine hormone produced by adipocytes to regulate weight control, and it resembles pro-inflammatory cytokines in structure and function. Leptin has a significant role in neuroendocrine function as well as metabolic processes. Moreover, it has been studied as a contributing factor for atherosclerosis in obesity (7,8). Increased prevalence of numerous vascular risk factors such as HTN, DM, and hyperlipidemia have been associated with high circulating leptin levels (9). Researchers have assessed leptin's effect on blood pressure, platelet aggregation, arterial thrombosis, and inflammatory vascular responses; this effect suggests the role of leptin in developing cardiovascular disease (10–12). Nevertheless, its effect as a stroke risk factor is controversial (7). Because leptin regulates both proatherogenic and antiatherogenic processes, the assessment of serum leptin levels can serve as a risk biomarker for inflammation and atherosclerosis in stroke patients (13). Therefore, we conducted this study to evaluate the association between serum leptin level and AIS risk.

1. Patients and Methods

We conducted this cross-sectional descriptive studybetween September 2018 and September 2019.Sixty patients presented with a clinical diagnosis of first-ever AIS were enrolled. study patients were collected from the Neurology Critical Care Unit and Neurology Stroke Unit, Zagazig University Hospitals, Zagazig, Egypt. The ethics committee of Zagazig University approved the study (IRB:5070).The study's aim was explained to every participant, and informed written consent was obtained from patients or the nearest relatives before enrolling in our study. All patients admitted received treatment according to our Intensive Care Unit and stroke unit protocol in the Neurology Department. All patients were monitored for blood pressure, temperature, blood glucose level, and blood gases on the first day after stroke.

Eligibility criteria

We included patients who met the following criteria: both genders >18 years old, patients presenting with first-ever AIS as diagnosed by WHOcriteria of stroke(14) and presented within 48 hours from the onset of the insult.

We excluded patients with hemorrhagic stroke, patients with CT scan findings of old or venous infarctions, patients with head injury or surgery,patients with central nervous system infections or systemic sepsis, patients with brain tumors or other systemic malignancies and patients with metabolic emergencies.

Clinical and neurological assessment

All patients were subjected to detailed medical history with an emphasis on past medical history to detect risk factors including HTN, DM, obesity defined as BMI ≥ 30 kg/m2 (15), smoking >10 cigarettes per day for more than one year before the stroke, cardiac diseases such as atrial fibrillation, ischemic heart disease, rheumatic heart diseases, cardiac myxoma, prosthetic valve, and congenital cardiac disorders, hyperuricemia, and dyslipidemia defined as cholesterol > 200mg/dl and or triglycerides >150 mg/dl (16). All patients underwent full general and neurological examinations as well as routine laboratory investigations (complete blood count, liver and kidney function tests, blood glucose levels, coagulation profile, and lipid profile).

Radiological assessment

All patients underwent a plain CT scan of the brain to confirm the diagnosis of AIS using Philips scanner (Tomoscan 350) with scanning time= 4.8, matrix size= 512 x 512, and slice thickness= 9 mm. All axial scans were performed in supine positioning. The CT brain was evaluated for the presence of any early infarction signs, as defined by Wardlaw and Mielke (2005) (17). In early AIS cases orsuspected brain stem lesions, magnetic Resonance Imaging (MRI) of the brain was performed. A second plain CT scan or MRI of the brain was performed within 2-3 days for volumetric analysis to detect the infarct site and size. The infarct size on the CT scan was estimated according to the rules used by Smith et al. (*Size = 0.5* x *a* x *b* x *c* x number *of slices), where a* and *b* represent the largest perpendicular diametersdetected by the CT scan and *c* represents the slice thickness which measures 9 mm) (18). According to Alemam et al., (19), ischemic stroke lesion size is classified into: small (size< 1.5 cm^3), medium (size ranged from 1.5 cm^3 to 3 cm^3), and large (size>3 cm^3).

Serum leptin concentrations

We collected 3ml peripheral venous blood samples from the participants on plain vacutainer tubes, then centrifuged at 1000×g for 10 min. Then, we collected and stored the serum samples at -80 ∘C until the time of leptin analysis. The concentration of serum leptin was measured using the enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Data were entered in a self-structured proforma and were later transferred to SPSS for analysis. We tested the data for normal distribution using the Shapiro Walk test. Dichotomous variables were represented as frequencies and relative percentages. Continuous variables were expressed as mean and standard deviation (SD) or median and inter-quartile range (IQR) for parametric and non-parametric data. We used the t-test or the Mann Whitney test to calculate the difference between continuous variables in two groups for parametric and non-parametric variables, respectively. We used the Statistical Package for Social Sciences (SPSS) 24.0 software (SPSS, Inc., Chicago, IL, USA) for statistical analysis. An alpha level below 0.05 was considered for statistical significance.

Results

Our study included 60 patients; 41 patients (68.3%) were females and 19 patients (31.7%) were males. Their age ranged between 33 and 90 years, with a mean age of 65 (SD= 8). The median leptin serum level of the studied patients at admission was 16.6 mg/l (IQR= 12.5-20). Of the included patients, 80% had HTN, 66.7% were diabetic, 51.7% were obese, 43.3% had cardiac disease, 31.7% were smokers, 33.3% had dyslipidemia, and 33.3% had hyperuricemia. Twenty-five patients (41.70%) had leukoaraiosis. The demographic characteristics and the ischemic stroke riskfactorsamong the studied patients are shown in **Table 1.** Twenty-four patients (40%) had early signs of middle cerebral artery infarction. The majority of the ischemic stroke patients (81.70%) had an anterior circulation infarction. 51.70% of the included patients had a left-sided brain lesion. The large-sized infarctions were prevalent (63.30%), followed by medium-sized infarctions, as shown in **Figure 1**.

We detected a statistically significant association between serum leptin level and DM, HTN, obesity, and cardiac disease (all $p<0.001$). Moreover, we detected no statistically significant association with gender ($p= 0.685$), smoking ($p= 0.336$),hyperuricemia ($p= 0.367$), and dyslipidemia ($p= 0.384$), **Table 2**.

There was no statistically significant association between serum leptin level and brain imaging findings (all $p>0.05$), except for large-sized infarction (p-value= 0.02), **Table 3**.

Table 1: The baseline characteristics and risk factors distribution in 60 studied ischemic stroke patients

Age, years	Mean (SD)	65 (8)	
Leptin serum level, mg/l	Median (IQR)	16.6 (12.5-20)	
		Frequency	Percentage
Sex, n (%)	Males	19	31.70%
	Females	41	68.30%
Diabetes Mellitus, n (%)	Yes	40	66.70%
	No	20	33.30%
Hypertension, n (%)	Yes	48	80%
	No	12	20%
Smoking, n (%)	Yes	19	31.70%
	No	41	68.30%
Cardiac diseases*, n (%)	Yes	26	43.30%
	No	34	56.70%
Obesity, n (%)	Yes	31	51.70%
	No	29	48.30%
Hyperuricemia, n (%)	Yes	20	33.30%
	No	40	66.70%
Dyslipidemia, n (%)	Yes	20	33.30%
	No	40	66.70%

Leukoaraiosis, n (%)	Yes	25	41.70%
	No	35	58.30%

*Ischemic heart diseases, Valvular heart diseases, Cardiomyopathy.

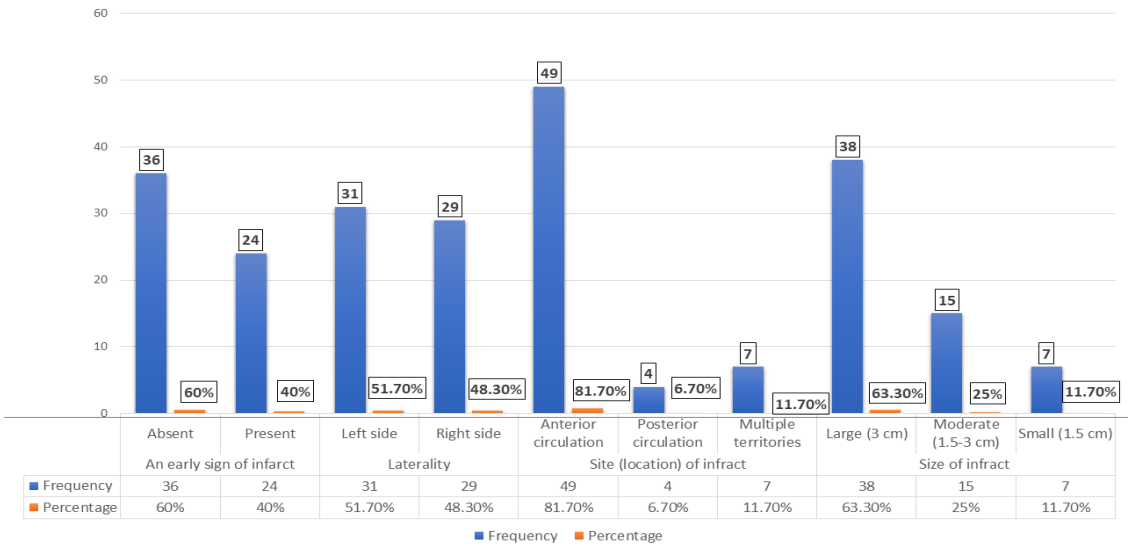

Figure 1. The radiological findings of brain imaging among ischemic stroke patients

Table 2. The association between serum leptin level and ischemic stroke risk factors among the included patients

		Serum Leptin, ng/mL.		Test	P-value
		Median	IQR		
Gender	Female	15.14	(12.34-17.21)	-0.405 *	0.685
	Male	13.68	(11.26-18.1)		
Diabetes mellitus	No	12.34	10.46-13.23	-6.456 *	**<0.001¶**
	Yes	17.23	16.2-18.8		
Hypertension	No	11.26	10.34-12.45	-6.645 *	**<0.001¶**
	Yes	16.38	15.24-18.36		
Smoking	No	15.46	(12.37-17.23)	-0.961 *	0.336
	Yes	13.54	(11.26-16.78)		
Cardiac diseases	No	12.31	10.46-12.56	-6.553 *	**<0.001¶**
	Yes	17.21	15.53-18.8		
Obesity	No	11.27	10.36-12.5	-6.457 *	**<0.001¶**
	Yes	16.47	15.46-18.38		
Hyperuricemia	No	15.19	(12.475-17.355)	-0.902 *	0.367
	Yes	12.9	(11.77-16.625)		
Dyslipidemia	No	13.96	(11.36-16.995)	-0.870 *	0.384
	Yes	15.39	(12.42-17.92)		

* Compared using Mann Whitney test; ¶ = Significant

Table 3. The association between serum leptin level and brain imaging findings among the included patients

		Serum Leptin, ng/mL.		Test	P-value
		Median	IQR		
Early signs of middle cerebral artery infarction	Absent	16.36	(12.5-21.35)	-0.287 *	0.774
	Present	16.99	(12.5-19.8)		
Laterality (side)	Left side	18.27	(13.27-21.79)	-1.3 *	0.19
	Right side	15.51	(11.88-18.86)		
Site (location) of infract	Anterior circulation	14.23	(12.34-17.23)	1.50 €	0.472
	Posterior circulation	13.35	(10.785-15.48)		
	Multiple territories	15.53	(12.56-18.1)		
Size of infract	Large	17.88	(13.98-22.79)	7.80 €	**0.02¶**
	Moderate	16.25	(14.65-18.38)		
	Small	12.54	(10.46-13.68)		

* Compared Using Mann Whitney test; € Compared Using Kruskal-Wallis test; ¶ = Significant

Discussion

Our study included 60 first-ever AIS patients, with a mean age of 65 (±8) years. The mean age of the included patients is comparable to Laskowitz et al., who reported a mean age of 60 (±16.7) (20). The median leptin serum level was 16.6 ng/ml with (IQR= 12.5 to 20) at admission. The normal range is 2.5 to 21.8 mg/l (21). Thus,leptin was elevated in the included AIS patients. This is in line with previous literature (22–24).

In terms of gender difference as a stroke risk factor, 68.3% of the included stroke patients in our study were females. In Kuwait, Al-Shammri et al., out of the 62 patients included, 32 were females (25). In a study of Somay et al, 50.4% were females(26). On the contrary, the results of Goldstein et al.,Umemura et al., and Feigin et al., found that the risk and the absolute number of stroke events were significantly greater in men than in women(27,28,29). while In Laskowitz et al., out of the 1146 patients, 611 (53%) were men (20).

In our study, HTN and DM were reported in 48 patients (80%) and 40 patients (66.7%)respectively .Fure et al. (30) and Amin et al. (31) reported HTN in (50%-58.1%) of AIS patients. also Beckett et al. reported a relationship between blood pressure and stroke risk (32). This could be attributed to the high platelet activation and thrombus formation in metabolic

syndromes such as HTN and dyslipidemia (33).A stroke risk reduction was associated with a decline of borderline HTN (systolic blood pressure= 130 to 140 mmHg and diastolic blood pressure= 85 to 89 mmHg]. The percentage of diabetic patients experiencing AIS was 18.6 % in Zhang et al. (34), 25% in El-Anwar et al. (35), 32.9% in Ani and Ovbiagele(36), 17.1% in Ntaios et al. (37), 29.4% in Kim et al. (38), 37% in Içme et al. (39), 27.7 % in Yang et al. (40). This significant variation in literature may be caused by the differences in their eligibility criteria and selection of patients, as well as the sample size difference and the diabetes definition (34). The BRAIN study reported a likelihood of AIS to mimics for each vascular risk factor as follows: odds ratio=3.38, 95% CI =2.4 to 4.8 for HTN and odds ratio=1.55, 95% CI =1.1 to 2.2 for DM (20).

In terms of cardiac diseases as risk factors, 26 patients (43.3%) had a previous history of cardiac diseases in our study. This is in agreement with the results reported by Fure et al. (30) and Faiz et al. (41); they reported that about 25%-29% of AIS patients had a previous cardiac disease.

Of the included patients, 20 patients (33.3%) had dyslipidemia. This agrees with the previous findings of dyslipidemia in 30%-69% of the AIS patients documented by Faiz et al. (41) and Král et al. (42). On the other hand, previous reports failed to find a direct association between total cholesterol and overall stroke risk (40,43–45).

In our study, 19 patients (31.7%) were smokers. O'Donnell et al. and Yang et al. documented that current smoking represented 18.9% and 21.8 % of AIS risk factorsrespectively(40,46). In a long-term cumulative risk study conducted by Giang et al., the authors found that being a current smoker was associated with an increased risk of coronary heart disease but not for AIS after adjusting of competing risk. Also, they mentioned that this result should not be interpreted to mean that smoking does not affect stroke, and they explained this finding as it could possibly be attributed to a decreasing proportion of smokers over time (45).

We found that there is a statistically significant association between serumleptin leveland DM. In line with these observations, Söderberg et al. and Liu et al. showed that higher quartiles of leptinwereassociated with increased blood sugar (49,50). These results mismatch with a previous study that reported no statistically significant correlation between leptin serum level and blood sugar (51).

In our study, there was also a significant association between serum level of leptin and HTN**.** Similarly, Romero-Corral et al., and Kim et al., reported that the patients from the group corresponding to the highest leptin levels were significantly more hypertensive (22,38). On the contrary, Xiao et al. (52) and Umemura et al. (28) found no significant correlation. This difference may be due to different cohort characteristics.

In our study, there was no significant association between serumleptin level and smoking. This is opposite to data obtained by Ning et al. (53) and Wang et al. (54), who reported that the patients from the group corresponding to the highest levels of leptin were significantly more likely to have a history of smoking. This difference may be due to our patients' female

predominance, who are less smokers than males, and because most of our patients were Goza smokers, not cigarette smokers.

Regarding dyslipidemia, we found no statistically significant association between leptin serum leveland history of dyslipidemia. In line with these observations, Bouziana S et al. (7) also found no association between leptin and triglyceride, total cholesterol, high-density lipoprotein, verylowdensity lipoproteins; however, it was highly significant with low-density lipoprotein serum levels. In contrast, GuoyiLiu et al. (47) showed that higher quartiles of leptin are associated with an increase in triglycerides serum level and high-density lipoprotein with no significance for others.

We found statistically significant associations between leptin serum levelsand increased BMI as well as with a previous history of cardiac diseases. Obesity has been established as a risk factor for atherosclerosis. Our study agrees with previous studies that documented that AIS patients had increased leptin levels and BMI, most likely representing its central action (46,55,56). BMI was positively linked to leptin, suggesting that leptin plays a significant role in stroke by visceral adiposity. On the other hand, Kim et al. (38) showed no correlation between leptin level and obesity.

Regarding cardiac diseases as risk factors for AIS, we observed that serum leptin level was highly significant among patients with a history of cardiac diseases ($p<0.001$). Similar to our results, two earlier studies documented that serum level of leptin was an independent risk factor to predict recurrent vascular adverse events (such as cardiovascular-related mortality and ischemic stroke) (57,58). A recent study suggested that independently of established risk factors, a high level of serum leptin could predict cardiac-related death as well as stroke in coronary artery disease patients (24). A recent systematic review and meta-analysis showed no significant association between high levels of leptin and coronary heart disease risks as well as stroke (10). Another study documented no direct relationship between leptin levels and the incident stroke risk. This difference can be justified by the different eligibility criteria for inclusion, the number of participants, and physical activity in these studies (59).

In the current study, 41.7% of the AIS patients had early signs of middle cerebral artery infarction, and 81.7% had an ischemic stroke of anterior circulation. These results are consistent with the previous studies of Yang et al. who reported that among the studied AIS patients, 11.4% had total anterior circulation syndrome, 39.1% had partial anterior circulation syndrome, and 30% had posterior circulation syndrome(60–64).Moreover, Huang et al. found that among patients with AIS, 62% of patients suffered anterior circulation stroke, 23% of them had posterior circulation stroke, while 15% multiple infarctions, including anterior and posterior circulation stroke (40,65).

As regards the side of weakness ,In our study, left-sided weakness was more frequent than right-sided weakness. Similarly, Pan et al. and Danthala&Lakshmaiahreported 53.1% and 50% of AIS patients had left-sided weakness (62,66). In contrast, another study reported that right-sided weakness appears to be more frequent (67). Regarding the size of infarction, we found that large-sized infarctions were the most prevalent (63.3%), followed by medium-sized lesions

(25%) and small-sized lesions (11.7%). A previous study found that 44% of their patients had small size infarction, 27.9 % had medium-sized infarction, and 27.6% had large-sized infarction. This variation could be attributed to differences in the sample size and the imaging method, as they used the conventional MRI (68)**.**

In the present study , the association between serum leptin level and brain imaging findings was assessed . We detected a statistically significant association between serum leptin level and the size of infarction. Leptin serum level was higher with larger size of infarction (p-value= 0.02). A finding which was in agreement with previous studies, which documented that patients with higher serum leptin levels had a larger infarction size (10,69). Also, Liu et al. (47) reported that higher leptin level was consistently associated with a larger white matter lesion volume.

In our study, few limitations should be taken into consideration. This is a single-arm study from a single-center experience which limits the generalization of these findings, and further prospective multicenter clinical trials recruiting large sample sizes are warranted to assess leptin's role on the risk of AIS. We could not assess the expression of the leptin gene.

In **conclusion**, our findings suggest that high levels of serum leptin may be linked with first-ever stroke risk. Further multicenter clinical trials are required to comprehensively assess leptin's role in AIS and confirm this association,

Conflict of Interest: None to declare.

List of abbreviations

Acute ischemic stroke	AIS
Hypertension	HTN
Diabetes mellitus	DM
Body mass index	BMI
Computed tomography	CT
Magnetic Resonance Imaging	MRI

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