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Adipocytokines in Subjects with and without Ischemic Cerebrovascular Disease

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Abstract

Objective of the Study: To investigate adipocytokines in patients with ischemic cerebrovascular disease and to develop an association between them

Methods: In this study plasma adiponectin, leptin and Interleukin 6 (IL 6) concentration were measured by ELISA. Blood glucose and lipid profile was done by standard kit methods.

Results: A total of 80 subjects with and without CVD were studied. The mean plasma level of IL6 of the forty patients with ischemic CVD was significantly higher than that of the forty subjects without CVD (41.64 + 2.50 versus 22.76 + 0.76 pg/mL; P < 0.001). The mean plasma level of adiponectin was significantly lower in patients with ischemic CVD than that of subjects without CVD (4.36 ± 0.21 µg/mL versus 6.97±0.241 µg/mL; P < 0.001). Serum Leptin concentrations were significantly higher (p < 0.001) in stroke patients (51.61 + 1.39) as compared with controls (37.76 + 1.207). Leptin levels were significantly negatively correlated with adiponectin (P < 0.01) and significantly positively correlated (P < 0.01) with interleukin 6 in stroke patients.

Conclusion: Present report provides additional support to the evidence of involvement of cytokines in inflammatory immune response of patients with cerebrovascular disease.

Key Words: Adiponectin, Leptin, Interleukin 6, Ischemia, cerbrovascular disease.

Introduction

Adipocytes secretes adipocytokines such as adiponectin, leptin and interleukin 6 (IL 6) which may have possible role in inflammation. Adiponectin is 30 KDa plasma protein (adipocytokine) and has antiatherogenic and anti-inflammatory properties (1). Suppression of leukocyte colony formation, reduction of phagocytic activity and reduction of TNF secretion from macrophages are some of the anti inflammatory effects of adiponectin (2, 3). Adiponectin also suppresses lipid accumulation in monocyte-derived macrophages through the suppression of macrophage scavenger receptor expression. An association between circulating adiponectin levels and endothelial function has been demonstrated in animal and human models suggesting the role of adiponectin in inflammation (4). Although the role of the adipocytokine leptin in human obesity and insulin resistance has yet to be fully clarified, recent studies have provided evidence that leptin also has significant effects on vascular development and repair. Leptin acts as endogenous mediator of neuroprotection during cerebral ischemia. Zang *et al*., 2008 reported that leptin receptors are abundantly present in the hypothalamus and its neuroprotective effect is mediated through these receptors (5-7).

Human Interleukin 6 (IL6) is a single polypeptide chain of 185 amino acids which has several endocrine and metabolic actions. Major sites of secretion and action of IL6 are adipose tissues and skeletal muscles (8). The liver is also one of the target organs (9). Elevated levels of circulating interleukin 6 levels have been seen in severe inflammatory, traumatic and infectious diseases. Ionic imbalances and free calcium accumulation are associated with ischemic brain injury. As a result of injury proinflammatory metabolites are released which are believed to promote expression and release of proinflammatory cytokine cascade i.e. release of IL1 and TNF alpha initially followed by Il 6 and IL 8 secretion (10, 11).

Limited studies have been carried out on the role of adipocytokines in ischemic cerebrovascular disease patients and the possible association between them.

Objective of the study

To investigate the role of adipocytokines (interleukin 6, adiponectin and leptin) in patients with is-

chemic cerebrovascular disease and to develop relationship between them

Material and Methods

Eighty subjects between the age of 50 and 70 years (forty each with and without CVD according to inclusion criteria) were recruited in the study. The controls were matched for age, sex and waist hip ratio selected from Ziauddin and Liaquat National Hospitals, Karachi.

Informed consent was obtained from all the subjects either personally or via relatives as legally required prior to participation in the study following approval of the study by the Ethical Committee Ziauddin University. Convenient sampling was done to recruit the subjects. Subjects were included from the same socioeconomic status to avoid variations in lifestyle and/or physical exercise. On the basis of waist/hip ratio, women were classified as obese if the ratio is greater than 0.8 and men if greater than 0.9 (12).

Patients with major cardiac, renal, hepatic and cancerous diseases, stroke due to aneurismal rupture, arteriovenous malformation, moyamoya disease and other vascular malformations, recent (within 1 month) history of head trauma, transient ischemic attack, intracerebral haemorrhage, CT/MRI results that were inconclusive for the lesion location, coronary artery disease(CAD) or collagen disease were excluded from the study. Women on hormone replacement therapy, smokers and/or alcohol consumers were also excluded from the study.

Patient Grouping

Forty subjects with ischemic stroke were classified into five groups according to TOAST classification i.e (1) Large Artery Atherosclerosis (LAA) (2) Cardioembolism (3) Small Artery Disease (SAD) (4) Para-atherosclerosis (5) No determined cause despite extensive evaluation. Large artery atherosclerosis was again dived into extracranial and intracranial.

Study Protocol

Fasting plasma samples were obtained and stored at –80°C for subsequent assay within 48 hours of stroke onset. The Adiponectin (13), leptin (14) and Interleukin 6 (IL 6) were determined by chemiluminescent enzyme immunoassay (CLETIA) (Fujireb io inc, Tokyo, Japan) (12). Fasting and random blood glucose was done by glucose oxidase method using kit obtained from Merck (15). Triglycerides (16), cholesterol, LDL cholesterol, HDL cholesterol (17) were also done by kits obtained from Merck.

Statistical Analysis

Data is shown as mean and standard error of mean. Analysis was performed using the statistical package for the Social Sciences (SPSS ver.12). P value was determined by Students t test. Pearson correlation analysis was used to evaluate the bivariate relationship between adiponectin , leptin and plasma interleukin 6 concentrations. $P < 0.05$ was considered statistically significant.

Results

Of forty stroke patients who were included in the study, intracranial Large Artery Atherosclerosis (LAA) was found in them. Middle Cerebral Artery occlusion was found in 25 (63.5%) and Basilar Artery occlusion was found in 15 (36.50%) patients (Table 1). Altered consciousness was found in 15 (37.50%), Slurred speech in 10 (25%) and weakness in 15 (37.50%) patients (Table 1). The clinical characteristics of our subjects are shown in Table 2. The mean plasma level of Interleukin 6 (IL 6) was significantly higher in patients with ischemic CVD than that of subjects without CVD (41.64 + 2.50 versus 22.76 + 0.76pg/mL; $P < 0.001$ (Fig. 1). The mean plasma level of adiponectin was significantly lower in patients with ischemic CVD than that of subjects without CVD (4.36 ± 0.21 μg/mL versus 6.97 ± 0.241 μg/mL; $P < 0.001$) (Fig. 1). Serum Leptin concentrations increased significantly ($p < 0.001$) in stroke patients (51.61 + 1.39) compared with controls (37.76 + 1.207) (Fig. 1). Leptin levels were significantly negatively correlated with adiponectin

Table I

Clinical and Imaging Features of the Stroke patients according to TOAST Classification

Clinical Features	**No. of Subjects (%) Total No = 40**
Altered Consciousness	15 (37.5%)
Slurred Speech	10 (25%)
Weakness	15 (37.5%)
Imaging	**Large Artery Atherosclerosis (LAA)**
Middle Cerebral Artery	35 (63.50%)
Basilar Artery	15 (36.50%)

No. of Subjects are shown and percentage is given in parenthesis

Table 2

Characteristics of subjects with and without CVD
Values are expressed as mean and standard error of mean (SEM). Number of cases is shown in parenthesis. Student's t test is applied to obtain significance.

	Normal Controls (40)	Stroke Patients (40)	P Value
Age (Years)	58.02 + 0.890	58.15 + 0.865	0.920
Waist Hip Ratio	0.82 + 0.005	0.83 + 0.005	0.843
Pulse / minute	75.40 + 0.487	89.00 + 2.767	0.000*
Temperature C	98.35 + 0.075	98.16 + 0.063	0.053
Blood Pressure Systolic (mm Hg)	125.10 + 1.285	130.88 + 2.020	0.018
Blood Pressure Diastolic (mm Hg)	86.63 + 1.166	89.95 + 1.534	0.088
FBS (mmol/L)	5.33 + 0.115	5.58 + 0.208	0.300
RBS (mmol/L)	8.92 + 0.151	9.39 + 0.40	0.275
Triglycerides (mmol/L)	1.84 + 0.045	1.92 + 0.09	0.468
Cholesterol (mmol/L)	4.69 + 0.066	4.82 + 0.17	0.474
LDL (mmol/L)	2.57 + 0.106	3.11 + 0.148	0.004*
HDL (mmol/L)	1.72 + 0.055	1.17 + 0.057	0.000*

P value < 0.05 is considered significant
FBS: Fasting Blood Sugar RBS: Random Blood Sugar
HDL: Digh Density Cholesterol: LDL Low Density Cholesterol

(r = -0.91, P < 0.01) and significantly positively correlated (r = 0.85, P < 0.01) with interleukin 6 in stroke patients. Adiponectin was negatively correlated with interleukin 6 (-0.89, P < 0.01) (Fig. 2). Of the 80 subjects with and without ischemic CVD, no significant difference was found in the 2 groups' WHR, fasting blood glucose, random blood glucose, systolic BP and diastolic BP. However significant increased LDL (3.11 + 0.148 versus 2.57 + 0.106) and decreased HDL levels (1.17 + 0.057 versus 1.72 + 0.055) were found in subjects with CVD versus those without CVD (Table 1).

Discussion

Subjects with Ischemic CVD included in our study show intracranial Large Artery atherosclerosis (LAA). The study by Bogousslavsky *et al*., 1986 (18) demonstrates that atherosclerotic involvement of the intracranial vessels occurs more frequently in Asians than in white individuals. Our present data demonstrates that leptin and interleukin 6 (IL 6) concentrations were significantly increased and adiponectin decreased in subjects with ischemic CVD than without CVD after adjustment for age sex and waist hip ratio. Moreover positive correlation was found between interleukin 6 and leptin but negative correlation was found between interleukin 6 and adiponectin. Leptin also shows negative correlation with adiponectin in these subjects.

Fig. 1

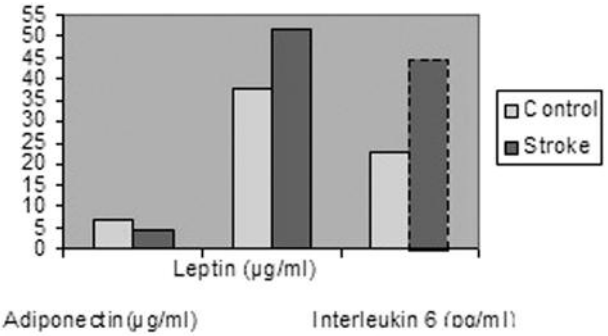
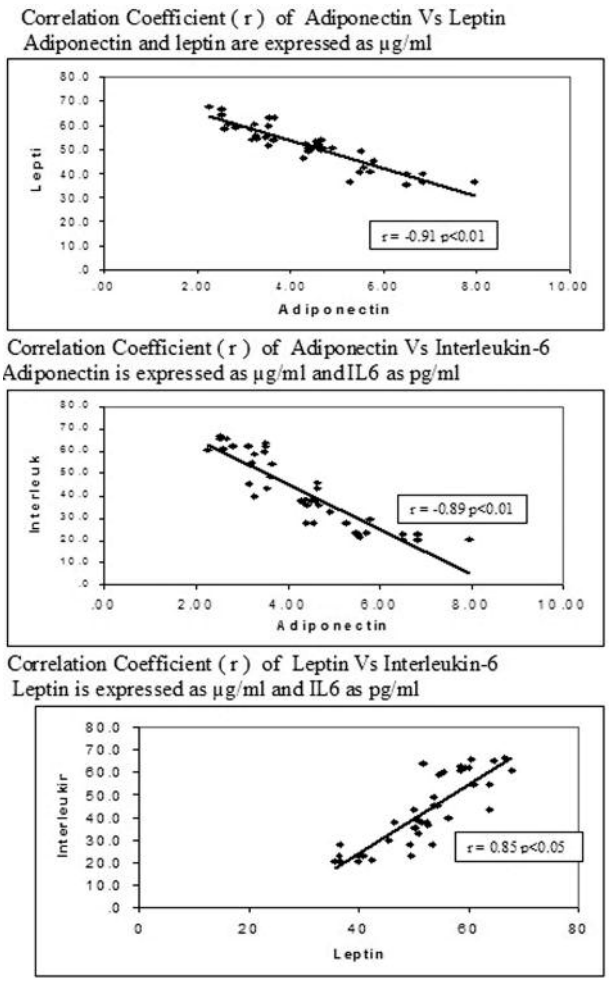


Fig. 2



Study by Terao *et al*. (19) reported exacerbated vascular injury that was associated with enhanced leucocyte and platelet trafficking in microvessels. Leukocyte trafficking into adipose tissue is promoted by leptin which causes eight fold increase in mRNA interleukin 6 levels. Administration of leptin resulted in decrease in circulating serum adiponectin levels in the study, which is primarily secreted from adipose tissues. This study shows that leptin stimulates expression of proinflammatory mediators while inhibiting the production of a potentially beneficial hormone i.e. adiponectin. Norata *et al*., 2007 (20) and Ukkola *et al*., 2008 (21) also supports leptin as an independent predictor of early atherosclerotic events such as intima media thickness. Exogenous leptin administration protects against ischemic neuronal injury in vitro and in vivo in a c-Rel-dependent manner in study by Valerio *et al*., 2009 (22). The neuroprotective role is mediated by binding of leptin to the receptors and subsequent activation of the associated Jansus and tyrosine kinase2 leading to phosphorylation of the tyrosine residue of the receptors. The receptors then activates the subsequent signaling effects. Inhibition of these pathways has been shown to reduce the protective effects of leptin (6). Leptin promotes angiogenesis and activates inflammatory cells and the leptin receptor (ob gene-encoded receptor), ObR, is expressed in advanced atherosclerotic lesions (23). Study be Sodenberg *et al*., 2009 (24) reports leptin to be more responsible for cerebrovascular disease than adiponectin . Study by Nishimura *et al*., 2008 (25), shows as when adiponectin-deficient (APN-KO) and wild-type (WT) mice were subjected to 1 hour of middle cerebral artery occlusion followed by 23 hours of reperfusion, APN-KO mice exhibited enlarged brain infarction and increased neurological deficits after ischemia-reperfusion as compared to with WT mice Study by Matsumoto *et al*., 2008 (26) reveals that adiponectin levels are not independently associated with stroke and its use as a predictor may be premature. Isoforms of adiponectin receptor are found in endothelial cells but adiponectin cannot cross the blood brain barrier. Instead it tends to suppress cytokine release such as IL6, which in turn is influenced by proinflammatory events (27). Acalovsci *et al*. (10) confirmed the elevation of serum IL6 levels in clinical stroke. Inverse correlation between pro-inflammatory cytokine IL6 and anti-inflammatory cytokine IL10 in control subjects is shown in study by Perini *et al*., 2001 (28). Welsch *et al*., 2008 (29) in a nested case control study demonstrated that IL-6 and TNF-alpha, but not IL-18, were associated with risk of recurrent ischemic stroke independently of conventional risk markers High lepin concentrations causes hypoadiponectinemia , which may result in loss of anti-inflammatory capability required to antagonize the actions of TNF alpha that stimulates the release of cytokines like IL6. Patients with hypo-adiponectinemia may, therefore, have less anti-inflammatory ability and be more vulnerable to the development of ischemic vascular disease.

Conclusion

Present report provides additional support to the evidence of involvement of cytokines in inflammatory immune response of patients with cerebrovascular disease. It shows that positive correlation was found between interleukin 6 and leptin but negative correlation was found between interleukin 6 and adiponectin. Leptin also shows negative correlation with adiponectin in these subjects.

Further studies are needed to link of cytokines with ischemic cerebrovascular disease. Immune modulation at the level of cytokine can be directed toward therapeutic correction for the prevention and treatment of stroke.

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