Adiponectin and infarction size in subjects with and without cerebrovascular disease

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ABSTRACT

الأهداف: التحقق ما إذا كان انخفاض أديبونيكتين موجود لدى المرضى المصابين بجلطة نقص التروية.

الطريقة: أجريت هذه الدراسة قسم الكيمياء الحيوية – جامعة زيودين خلال الفترة ما بين 2007م حتى 2008م. تم قياس تركيز بلازما أديبونيكتين في هذه الدراسة عن طريق الانزيم المرتبط بالمناعة لدى الأشخاص المصابين وغير المصابين بمرض وعائي قلبي افتقاري (CVD).

النتائج: تمت دراسة اجمالي عدد 80 شخصاً، 40 مريضاً يعانون من (CVD) و40 لا يعانون من ذلك كمجموعة تحكم. كان متوسط مستوى أديبونيكتين لدى المرضى الأربعين الذين يعانون من (CVD) أقل بشكل ملحوظ من الأربعين الأخرين في مجموعة التحكم الذين لا يعانون من (CVD) (4.36±0.21µg/mL) مقابل (6.97±0.241µg/mL): نسبة الخطأ (p=0.000). كان انخفاض تركيزات أديبونيكتين متصل بشكل سلبي مع حجم الاحتشاء لدى المرضى المصابين بمرض وعائي دماغي افتقاري (CVD).

خاتمة: تظهر هذه البيانات أن هنالك مستويات منخفضة بشكل ملحوظ من بلازما أديبونيكتين لدى المرضى المصابين بمرض وعائي دماغي افتقاري (CVD). المزيد من ذلك أن أديبونيكتين متصل بشكل سلبي مع حجم الاحتشاء لدى هؤلاء المرضى ويقترح أن لديه دور محتمل في المرض الوعائي الدماغي.

Objectives: To investigate whether hypo-adiponectinemia is present in ischemic stroke patients.

Methods: This comparative study was carried out in the Biochemistry Department, Ziauddin University, Karachi, Pakistan in 2008. In this study, plasma adiponectin concentration was measured by an enzyme-linked immunosorbent assay in subjects with and without ischemic cerebrovascular disease (CVD).

Results: A total of 80 subjects were studied (40 patients with CVD, and 40 without CVD as controls). The mean plasma level of adiponectin of the 40 patients with ischemic CVD was significantly lower than that of the 40 subjects without CVD (4.36±0.21 µg/mL versus 6.97±0.241 µg/mL; *p*=0.000). Decreasing concentrations of adiponectin were negatively correlated with infarction size in ischemic CVD patients.

Conclusion: These data show that there are significantly lower levels of plasma adiponectin in patients with ischemic CVD. Moreover, adiponectin is negatively correlated with infarction size in these patients suggesting the possible role of adiponectin in cerebrovascular disease.

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Adiponectin is secreted from adipocytes and has a possible role in inflammation. Adiponectin is a 30 KDa plasma protein (adipocytokine) secreted from the adipocytes, which have insulin sensitizing, anti-atherogenic, and anti-inflammatory properties.[1] Several studies shows decreased adiponectin levels in insulin resistance, obesity, type 2 diabetes, hypertension, and coronary artery disease (CAD).[2-4] Adiponectin levels are significantly lower in patients with CAD than in

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matched controls, suggesting a possible association of reduced adiponectin in vasculopathic states.[5,6] The anti-inflammatory effects of adiponectin include suppression of leukocyte colony formation, reduction of phagocytic activity, and reduction of tumor necrosis factor (TNF)-α secretion from macrophages.[7,8] In tissue cultures, adiponectin attenuates monocyte attachment to endothelial cells by reducing the expression of adhesion molecules on endothelial cells.[9] 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 found in various animal studies suggesting the possible role of adiponectin in inflammation.[10,11] Although these findings indicate that adiponectin plays a crucial role in the development of atherosclerosis, limited studies have been carried out on plasma adiponectin and ischemic cerebrovascular disease (CVD). Because ischemic CVD is the second most common cause of death in developing countries, more common than cardiovascular disease,[12] and the second leading cause of mortality worldwide,[13] a study of whether adiponectin is involved in CVD is important. In this study, we investigate whether hypoadiponectinemia is present in ischemic CVD patients and relate it to infarction size.

Methods. This comparative study was carried out in the Biochemistry Department, Ziauddin University, Karachi, Pakistan in 2008. The study included 80 subjects between the ages of 50-70 years (40 with, and 40 without CVD according to inclusion criteria), age, gender, and waist-hip ratio matched selected from Ziauddin and Liaquat National Hospitals, Karachi, Pakistan. Amongst them, 21 were males, and 19 females. Informed consent was obtained from all the subjects themselves or by their relatives as legally required prior to participation in the study following approval of the study by the Ethical Committee of Ziauddin University. Convenient sampling was carried out to recruit the subjects. Subjects were included from the same socioeconomic status to avoid variations in lifestyle and physical exercise, while smokers and alcohol consumers were excluded from the study as they can affect the adiponectin levels. Subjects suffering from an acute or sudden focal neurological defect lasting >24 hours, and positive brain image lesions by CT, MRI, or MRA examination were characterized as ischemic CVD patients.[4] Patients were admitted to the neurological wards and attended to by specialized medical and nursing staff. Blood pressure and temperature measurements, blood chemistry, and nonenhanced brain CT scan were performed for all patients on hospital arrival. The waist and hip circumference were measured in duplicate using a measuring tape. 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.[14,15] Excluded were patients with a history of recent (within 2 weeks before admission) infection, concurrent major cardiac, renal, hepatic, and cancerous diseases, stroke due to aneurysmal rupture, arteriovenous malformation, moyamoya disease, and other vascular malformations, recent (within one month) history of head trauma, transient ischemic attack, intracerebral hemorrhage, CT/MRI results that were inconclusive for the lesion location, coronary artery disease (CAD), collagen disease, or acute viral infections because such conditions could increase the levels of inflammatory markers, potentially modifying the relationships between inflammatory markers and CVD. Women on hormone replacement therapy, smoking or alcohol consumers were also excluded from the study.

Study protocol. Within 48 hours of onset of CVD, fasting plasma samples were obtained and stored at -80°C for subsequent assay. The plasma concentration of adiponectin was determined by a commercially available sandwich ELISA (human ADPN ELISA; from Gesendet: Donnerstag (DRG instruments GmbH, Marburg, Germany) with a detection limit of 0.2 µg/mL and intra-assay and interassay coefficients of variation 6.4% and 7.3%.[16] All assays for both standards and samples were performed in duplicate on 96 well plates according to indications and suggestions from the manufacturers, and standard curves of the relationship between optical density and molecule concentrations calculated accordingly. Values of biomarkers were expressed µg/ml (adiponectin). Fasting and random blood glucose was carried out by glucose oxidase method using a kit obtained from Merck, (Gesendet, Germany).[17] All the CT examinations were performed on a CT Systec 3000 plus (GEC, Tokyo, Japan) scanner with a 512 x 512-matrix display. A cerebral CT was performed on all patients on admission; a second CT was completed between the fourth and seventh day after the patient's inclusion in the study. In the second examination, we determined infarct volume using the appropriate software. Infarct volume was assessed in cubic centimeters according to the formula 0.5 x a x b x c, where a and b represented the largest perpendicular diameters measured on CT scan, and c the slice thickness.[18]

Statistical analysis. Data are shown as mean and standard error of mean. Analysis was performed using the statistical package for the Social Sciences (SPSS version 12). A *p*-value was determined by Students t test. Pearson correlation analysis was used to evaluate the bivariate relationship between infarction size with plasma adiponectin. $P<0.05$ was considered statistically significant.

Table 1 - Characteristics of subjects with and without cerebrovascular disease.

Characteristic	Normal controls (n=40)	Stroke patients (n=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
Systolic blood pressure (mm Hg)	125.10±1.285	130.88±2.020	0.018
Diastolic blood pressure (mm Hg)	86.63±1.166	89.95±1.534	0.088
Fasting blood glucose (mmol/L)	5.33±0.115	5.58±0.208	0.300
Random blood glucose (mmol/L)	8.92±0.151	9.39±0.40	0.275
Adiponectin (µg/ml)	6.97±0.24	4.36±0.21	0.000

Values are expressed as mean and standard error of mean (SEM). Student's t test is applied to obtain significance

Table 2 - Correlation of adiponectin in subjects with and without cerebrovascular disease.

Groups	WHR	Pulse	Temp	Systolic BP	Diastolic BP	FBS	RBS
Subjects without CVD							
Adiponectin (r-value)	0.24	-0.14	0.05	-0.08	0.19	-0.10	-0.10
Significance (*p*-value)	0.13	0.37	0.72	0.62	0.90	0.50	0.51
Subjects with CVD							
Adiponectin (r-value)	-0.01	-0.21	0.07	-0.15	0.10	0.16	0.16
Significance (*p*-value)	0.91	0.19	0.64	0.33	0.53	0.31	0.30

WHR - waist-hip ratio, Temp - temperature, BP - blood pressure, FBS - fasting blood sugar, RBS - random blood sugar

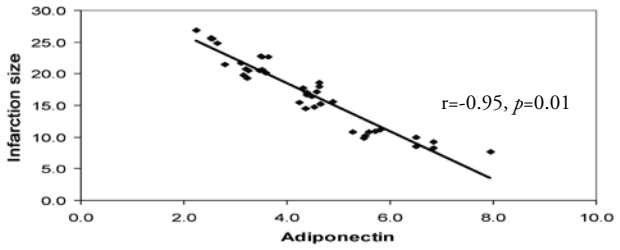

Figure 1 - Correlation coefficient of adiponectin versus infarction size (adiponectin is expressed as µg/ml and infarction size as cm^3).

Results. The clinical characteristics of our subjects are shown in **Table 1**. The mean plasma level of adiponectin was significantly lower in patients with ischemic CVD than that of subjects without CVD. Of the 80 subjects with and without ischemic CVD, no significant difference was found in the 2 groups' waist-hip ratio, fasting blood glucose, random blood glucose, systolic and diastolic blood pressure. A significant negative correlation (r=-0.95, p=0.01) exists between adiponectin, and infarction size in patients with CVD (**Figure 1**). However, no significant correlation exists between adiponectin and blood pressure (systolic and diastolic), pulse, temperature, fasting and random blood glucose in subjects with and without CVD (**Table 2**).

Discussion. The present study shows that plasma adiponectin concentrations were significantly lower in patients with ischemic CVD than without CVD when matched for age, gender, and waist-hip ratio. Plasma adiponectin has been involved in the development of atherosclerotic disease and appears to have both anti-inflammatory and antiatherogenic properties. Efstathiou et al,[6] reported a highly significant inverse relation between adiponectin levels and subsequent 5-year mortality in stroke patients. They confirmed an 8-fold increase in risk for individuals with low adiponectin levels compared with those with the highest tertile of adiponectin levels. Results of a study by Nishimura et al,[19] also support our study, as when adiponectin-deficient (APN-KO) and wild-type (WT) mice were subjected to one 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 compared with WT mice. On the contrary, a study by Matsumoto et al,[20] showed that no significant difference in the odds of stroke between the lowest and highest adiponectin quartiles. Chen et al,[4] studied subjects with and without CVD and found significantly lower levels of plasma adiponectin in patients with ischemic CVD.[4] Similarly a study by Bang et al[21] shows hypoadiponectinemia in patients

with intracranial atherosclerosis. The role of adiponectin in CVD is still under debate. Hypoadiponectinemia may cause the patients to lose the anti-inflammatory capability required to antagonize the actions of TNF -α that stimulates the release of cytokines, such as interleukin 6.[22,23] Patients with hypoadiponectinemia may, therefore, have less anti-inflammatory ability and be more vulnerable to the development of ischemic vascular disease.

In the current study, adiponectin appears to be both potentially a marker of the extent of underlying neurologic injury and a marker of persistent inflammatory response. That adiponectin is partly a marker for the extent of neurologic injury is supported by the negative correlation between initial infarct volume and adiponectin. Studies by Clark[24,25] report a negative correlation of adiponectin with infarction size.

In conclusion, the present report shows that plasma adiponectin concentration is low in patients with ischemic CVD, and it finds a possible close relationship between adiponectin and infarct size. Further studies may reveal whether serum adiponectin concentrations hold prognostic information on risk of ischemic stroke also in a prospective setting. The study has certain limitations. We are unable to distinguish between particular forms of adiponectin as it is not easy in the human population and moreover, the ELISA technique available to us is unable to distinguish between lower weight and high molecular weight complexes of adiponectin. The adiponectin levels can be estimated in cerebrovascular disease patients at the time of admission and again after every 3-6 months for 2 years, which may reveal the prognostic importance of the adipocytokine.

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References

1. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. ***J Clin Endocrinol Metab*** 2001; 86: 1930-1935.
2. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. ***Hypertension*** 2004; 43: 1318-1323.
3. Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. ***Clin Chim Acta*** 2004; 344: 1-12.
4. Chen MP, Tsai JC, Chung FM, Yang SS, Hsing LL, Shin SJ, et al. Hypoadiponectinemia is associated with ischemic cerebrovascular disease. ***Arterioscler Thromb Vasc Biol*** 2005; 25: 821-826.
5. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. ***Arterioscler Thromb Vasc Biol*** 2003; 23: 85-89.
6. Efstathiou SP, Tsioulos DI, Tsiakou AG, Gratsias YE, Pefanis AV, Mountokalakis TD. Plasma adiponectin levels and five-year survival after first-ever ischemic stroke. ***Stroke*** 2005; 36: 1915-1920.
7. Diez JJ, Iglesias P. The role of novel adipocyte derived hormone adiponectin in human disease. ***Eur J Endocrinol*** 2003; 148: 293-300.
8. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. ***Arterioscler Thromb Vasc Biol*** 2000; 20: 1595-1599.
9. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. ***Circulation*** 2000; 102: 1296-1301.
10. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. ***Circulation*** 2001; 103: 1057-1063.
11. Goldstein BJ, Scalia R. Adiponectin: A novel adipokine linking adipocytes and vascular function. ***J Clin Endocrinol Metab*** 2004; 89: 2563-2568.
12. Huang ZS, Chiang TL, Lee TU. Stroke prevalence in Taiwan: Findings from the 1994 National Health Interview Survey. ***Stroke*** 1997; 28: 1579-1584
13. Bonita R. Stroke prevention: a global perspective. In: Norris JW, Hachinski V, editors. Stroke prevention. New York (NY): Oxford University Press; 2001. p. 259-274.
14. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. ***Hypertension*** 2004; 43: 1318-1322.
15. Dávalos A, Castillo J, Pumar JM, Noya M. Body temperature and fibrinogen are related to early neurological deterioration in acute ischemic stroke. ***Cerebrovasc Dis*** 1997; 7: 64-69.
16. Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. ***Clin Chem*** 2004; 50: 219-221.
17. Trinder P. Enzymatic determinations of blood glucose using glucose oxidase with an alternative oxygen acceoter. ***Ann Clin Biochem*** 1969; 6: 24-31.
18. van der Worp HB, Claus SP, Bär PR, Ramos LM, Algra A, van Gijn J, et al. Reproducibility of measurements of cerebral infarct volume on CT scans. ***Stroke*** 2001; 32: 424-434.
19. Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, et al. Adiponectin prevents cerebral ischemic Injury through endothelial nitric oxide synthase dependent mechanisms. ***Circulation*** 2008; 117: 216-223.
20. Matsumoto M, Ishikawa S, Kajii E. Association of adiponectin with cerebrovascular disease: a nested case-control study. ***Stroke*** 2008; 39: 323-328.
21. Bang OY, Saver JL, Ovbiagele B, Choi YJ, Yoon SR, Lee KH. Adiponectin levels in patients with intracranial atherosclerosis. ***Neurology*** 2007; 68: 1931-1937.
22. Jaleel F, Jaleel A, Rahman MA. Relationship of adiponectin, blood lipids and glycemic control in postmenopausal diabetic women with and without complication of ischemic heart disease. ***Clin Chim Acta*** 2006: 370; 76-81.
23. Sasaki M, Kawano T, Saito T, Yuzawa M, Saito T, Ikoma A, et al. Hypoadiponectinemia in patients with cerebral infarction: comparison with other atherosclerotic disorders. ***Am J Med Sci*** 2007; 333: 140-144.
24. Clark WM. Cytokines and reperfusion injury. ***Neurology*** 1997; 49 (5 Suppl 4): S10-S14. Review.
25. Clark WM. Adiponectin: spectator or player? ***Stroke*** 2005; 36: 1919-1920.