

Comparison of Adiponectin in Ischemic Heart Disease Versus Ischemic Stroke in Diabetic Patients

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Abstract: Atherosclerotic cardiovascular and cerebrovascular disease are the major causes of disability in western as well as developing countries. Even though adiponectin is associated with many traditional cardiovascular risk factors, studies comparing serum adiponectin levels in cardiovascular and cerebrovascular disease (CVD) are scarce. To compare the serum adiponectin levels in patients with ischemic heart disease versus ischemic stroke. Experimental Study. 120 subjects (40 controls, 40 patients with ischemic stroke and 40 patients with ischemic heart disease (IHD) age, sex and waist hip ratio matched) were selected according to inclusion criteria from Ziauddin University Hospital. Baseline characteristics were determined and serum adiponectin was done on ELISA. Blood glucose and lipid profile was done by kit methods. Serum Adiponectin levels decreases significantly ($P < 0.001$) in ischemic heart disease (IHD) compared with ischemic stroke patients (IS). Blood lipid levels also increases significantly ($P < 0.05$) in IHD patients compared with IS patients and controls. **Conclusion:** Adiponectin has significant role in cardiovascular disease compared with cerebrovascular disease.

Key words: Adiponectin · ischemia · stroke · lipid profile

INTRODUCTION

Type 2 diabetes is considered the sixth leading cause of death in the United States, with most of these deaths attributed to cardiovascular disease [1]. Patients with type 2 diabetes mellitus have a significantly higher risk for cardiovascular disease than those without diabetes. Stroke is the second leading cause of death worldwide and a major cause of long-term disability [2]. Inflammation plays an important role in ischemic cardiovascular and cerebrovascular diseases.

Adiponectin, an adipose tissue-specific plasma protein plays an important role in regulating energy homeostasis, glucose and lipid metabolism and antiinflammatory responses in the vascular system [3-5]. This novel molecule has been implicated in the development of atherosclerotic cardiovascular (CV) disease. Low plasma concentrations of adiponectin have been associated with type 2 diabetes, myocardial Infarction (MI) and ischemic stroke [6-9]. Data from animal and human studies suggest that this adipocytokine has insulin-sensitizing, antiatherogenic and antiinflammatory

properties [2]. Potential mechanisms of adiponectin include its inhibition of smooth muscle cell proliferation, monocyte adhesion to endothelium and macrophage uptake by LDL [9].

Limited studies have been carried out to compare serum adiponectin levels in ischemic cardiovascular and ischemic cerebrovascular disease. The objective of this study is to determine and compare the levels of adiponectin and lipid profile in ischemic cardiovascular disease and ischemic cerebrovascular disease in diabetic patients.

MATERIALS AND METHODS

The study included 120 subjects between the age of 50 and 70 years, who were selected from Ziauddin Hospital, Karachi. Informed consent was obtained from all subjects themselves or by relatives as legally required prior to participation in the study following approval of the study by the Ethical Committee Ziauddin University.

120 subjects were divided into three groups. Each group comprised of forty subjects. First group comprised

of forty controls, second group was of patients suffering from ischemic stroke and third group comprised of patients suffering from ischemic heart disease. Known diabetic patients were included in second and third group. Cases and controls were matched for age, sex and waist hip ratio. Waist and hip circumference was measured in duplicate using a measuring tape. On the basis of waist / hip ratio, women are classified as obese if the ratio is greater than 0.95 and men if greater than 0.85. Diagnosed cases of angina and myocardial infarction were taken on the basis of chest pain, ECG changes i.e. ST elevation and Q wave inversion and biochemical markers i.e. raised levels of Troponin T, CKMB, AST and LDH.

Excluded were patients with cardiovascular disease other than MI and angina, history of recent (within 2 weeks before admission) infection, concurrent 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, 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, IHD and stroke. Women on hormone replacement therapy, smoking or alcohol consumers were also excluded from the study.

Study protocol: 10 ml of the venous blood sample was taken after an overnight fast, 5ml was kept in sodium fluoride tubes to assess fasting blood glucose and remaining 5 ml was centrifuged to obtain serum which was stored at $-20\pm 5^{\circ}\text{C}$ to analyze lipid profile, serum adiponectin levels. Subjects were given 75 g glucose in 250 ml water and blood samples obtained to determine random blood glucose. Serum adiponectin levels were determined by Kit from Gesendet: Donnerstag (DRG instruments GmbH), Germany and samples were analyzed by ELISA [10]. Fasting and random blood glucose was done by glucose oxidase method using kit obtained from Merck [11]. Triglycerides [12], cholesterol, LDL cholesterol, HDL cholesterol [13] were also done by kits obtained from Merck.

Statistical analysis: For quantitative variables mean and standard error of mean was determined. P value was obtained by analysis of variance (ANOVA). Post hoc test was applied to compare the groups. P value < 0.05 was considered significant.

RESULTS

The study comprised of 120 subjects. Amongst them 40 controls, 40 subjects with Ischemic Heart Disease (IHD) and 40 with Ischemic Stroke (IS) were compared for serum adiponectin and blood lipid levels. Age, sex and waist hip ratio was comparable in three groups.

Serum adiponectin level decreases significantly ($P < 0.05$) in IHD patients compared with IS patients and controls. Serum cholesterol, LDL, triglycerides and fasting blood glucose increases significantly ($P < 0.05$) in IHD group compared with IS group and controls. Similarly HDL level decreases significantly in IHD group compared with IS group and controls. Serum adiponectin level decreases significantly ($P < 0.05$), fasting and random blood glucose increases significantly ($P < 0.05$) in ischemic stroke patients compared with controls.

DISCUSSION

This study focuses on serum adiponectin levels in patients with ischemic heart disease and ischemic cerebrovascular disease. Serum adiponectin levels decreases significantly in both groups compared with controls, while adiponectin levels decreases significantly in ischemic heart disease compared with ischemic cerebrovascular disease. Adiponectin is an adipocyte-derived plasma protein (adipokine) that accumulates in injured arteries and has potential antiatherogenic properties. Hypoadiponectinemia can cause endothelial dysfunction by decreasing insulin sensitivity. Plasma adiponectin level was decreased in the prediabetic insulin-resistant phase in rhesus monkeys (*Macaca mulatta*), hypoadiponectinemia might play a causative role in the development of insulin resistance [14]. Second, hypoadiponectinemia may be directly linked to early atherosclerotic vascular damage and a subsequent endothelial dysfunction. Experimentally, Ouchi *et al.* [15] showed that adiponectin inhibited TNF- α -induced expression of endothelial adhesion molecules in endothelial cells and that adiponectin reduced atherogenic transformation of macrophage to foam cells by suppressing scavenger receptor expression [16].

It is thought to be involved in suppressing the proliferation and migration of vascular smooth muscle by decreasing the effects of various growth factors. Studies have demonstrated that adiponectin levels in patients with diabetes and existing coronary artery disease are significantly lower than in patients without coronary artery disease and it has been related to insulin resistance,

Table 1: Adiponectin and blood lipid levels in ischemic heart disease and ischemic stroke

	Controls	Ischemic stroke	Ischemic heart disease	P-value
Serum adiponectin (ng/dl)	12.46±0.56	7.69±0.36*	3.98±0.27*\$	0.001
Fasting blood Glucose (mmol/l)	4.92± 0.07	7.76±0.17*	9.45±0.26*\$	0.001
Random blood Glucose (mmol/l)	7.94±0.21	12.87±0.32*	13.38±0.26*	0.001
Triglycerides (mmol/l)	1.84±0.28	1.92±0.61	4.82±1.50*\$	0.001
Cholesterol (mmol/l)	4.69±0.41	4.82±1.10	7.54±1.54*\$	0.001
LDL cholesterol (mmol/l)	2.57±0.67	3.11±0.94	4.55±1.40*\$	0.001
HDL cholesterol (mmol/l)	1.72±0.35	1.17±0.36*	0.79±0.12*\$	0.001

* Significant compared with controls

\$ Significant compared with Ischemic Stroke

•The values are shown as mean and standard error of mean. P = 0.001 value was obtained for all parameters using ANOVA. Post hoc test was then applied to compare the groups. P<0.05 was considered significant

with levels significantly lower in patients with than without diabetes. It also has increased insulin sensitivity and suppressed hepatic glucose production [17]. Study conducted by Engeli, [18] found out that in type 2 diabetic patients who are susceptible to atherosclerosis plasma concentration of adiponectin are decreased. Similar study by Diez *et al.* [19] decreased serum adiponectin level was observed in patients with coronary artery disease when compared with normal controls. Another study carried out by Kojima *et al.* [20] in which serum adiponectin levels were measured in patients with acute myocardial infarction on admission. It was observed that level of adiponectin decreased significantly in these patients compared with controls. Controversial reports on whether adiponectin levels are independently associated with stroke or brain infarction are being published. The use of adiponectin as a cerebrovascular disease predictor may be premature [21] (published online).

The results of present study also confirms that triglycerides and cholesterol levels were significantly higher (P<0.05) in diabetic patients with ischemic heart disease as compared to ischemic stroke patients and controls (Table 1). LDL cholesterol increases and HDL cholesterol decreases in diabetic patients with ischemic heart disease. Blood cholesterol and triglycerides levels reflect plasma free fatty acid levels in any subjects. The free fatty acid induced insulin resistance is

physiologically important during starvation by preventing carbohydrates for oxidation in nervous system and during pregnancy. This well recognized accelerated starvation pattern provides carbohydrates for the growing fetus. Low Density Lipoproteins (LDL) and high density lipoproteins (HDL) are major determinants of cholesterol transporters in human plasma. The pre- atherogenic role of LDL-cholesterol and HDL cholesterol are high biological predictors of cardiovascular disease [22, 23]. HDL-C is considered as the single major risk factor for predicting the risk of atherosclerosis and coronary artery disease (CHD) [24]. Rise in HDL cholesterol and decrease sp in LDL cholesterol reduce the incidence of coronary artery disease.

CONCLUSION

This study supports that adiponectin has more significant role in ischemic cardiovascular disease compared with ischemic cerebrovascular disease.

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REFERENCES

- Hurst, R. and R. Lee, 2003. Increased incidence of coronary atherosclerosis in type 2 diabetes mellitus: mechanisms and management. *Ann. Intern. Med.*, 139: 824-834.
- Chamorro, A., 2004. Role of inflammation in stroke and atherothrombosis. *Cerebrovasc Dis.*, 17 (suppl 3): 1-5.
- Diez, J.J. and P. Iglesias, 2003. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur. J. Endocrinol.*, 148: 293-300.
- Goldstein, B.J. and R. Scalia, 2004. Adiponectin: A novel adipokine linking adipocytes and vascular function. *J. Clin. Endocrinol. Metab.*, 89: 2563-2568.
- Ouchi, N., S. Kihara, T. Funahashi, Y. Matsuzawa and K. Walsh, 2003. Obesity, adiponectin and vascular inflammatory disease. *Curr. Opin. Lipidol.*, 14:561-566.
- Weyer, C., T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R.E. Pratley *et al.*, 2001. Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. *J. Clin. Endocrinol. Metab.*, 86: 1930-1935.

7. Iwashima, Y., T. Katsuya, K. Ishikawa, N. Ouchi, M. Ohishi, K. Sugimoto, *et al.* 2004. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension*, 43: 1318-1323.
8. Pischon, T., C.J. Girman, G.S. Hotamisligil, N. Rifai, F.B. Hu and E.B. Rimm, 2004. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*, 291: 1730-1737.
9. Chen, M.P., J.C. Tsai, F.M. Chung, S.S. Yang, L.L. Hsing, S.J. Shin, *et al.* 2005. Hypoadiponectinemia is associated with ischemic cerebrovascular disease. *Arterioscler Thromb. Vasc. Biol.*, 25: 821-826.
10. Suominen, P., Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. *Clin. Chem.*, 50 (1): 219-221.
11. Trinder, P., 1969. Enzymatic determinations of blood glucose using glucose oxidase with an alternative oxygen acceptor. *Am. Clin. Biochem.*, 6: 24.
12. Bucolo, G. and H. David, 1973. Quantitative determination of serum triglycerides by use of enzymes. *Clin. Chem.*, 19: 476.
13. Allain, C.C., L.S. Poon and C.S. Chan, 1974. Enzymatic determination of total serum cholesterol. *Clin Chem.*, 20: 470.
14. Hotta, K., T. Funahashi, N.L. Bodkin, H.K. Ortmeyer, Y. Arita, B.C. Hansen and Y. Matsuzawa, 2001. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes*, 50: 1126-1133.
15. Ouchi, N., S. Kihara, Y. Arita, K. Maeda, H. Kuriyama, Y. Okamoto, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, S. Yamashita, T. Funahashi and Y. Matsuzawa, 1999. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein, adiponectin. *Circulation*, 100: 2473-2476.
16. Ouchi, N., Kihara, S., Y. Arita, M. Nishida, A. Matsuyama, Y. Okamoto, M. Ishigami, H. Kuriyama, K. Kishida, H. Nishizawa, K. Hotta, M. Muraguchi, Y. Ohmoto, S. Yamashita, T. Funahashi and Y. Matsuzawa, 2001. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*, 103: 1057-1063.
17. Iwaki, M., M. Matsuda, N. Maeda, *et al.* 2003. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes*, 52: 1655-1663.
18. Engeli, S., M. Feldpausch, K. Gorzeiniak, F. Hartwig, U. Heintze, J. Janke, M. Mohlig, F.H. Pfeiffer, F.C. Luft and A.M. Sharma, 2003. Association between Adiponectin and mediators of inflammation in obese women. *Diabetes*, 52: 942-947.
19. Diez, J.J., P. IglesiasMaría, J. Fernández-Reyes, A. Aguilera, M.A. Bajo, P.A. Fidalgo, Codoceo and R. Selgas, 2005. Serum concentrations of leptin, adiponectin and resistin and their relationship with cardiovascular disease in patients with end-stage renal disease. *Clinical Endocrinology*, 62: 242-247.
20. Kojima, S., T. Funahashi, T. Sakamoto, S. Miyamoto, H. Soejima, J. Hokamaki, I. Kajiwara, S. Sugiyama, M. Yoshimura, K. Fujimoto, Y. Miyao, A. Kitagawa, N. Ouchi, S. Khhara, Y. Matsuzawa and H. Ogawa, 2003. The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. *Curr. Diab. Rep.*, 3: 151-158.
21. Association of Adiponectin With Cerebrovascular Disease. A Nested Case-Control Study. *Stroke*, 2007 Published Online.
22. Benalian, P., C. Cansier, G. Hennache, O. Khallour, P. Bayer and F. Duron, 2000. Comparison of new method for the direct and simultaneous assessment of LDL and HDL cholesterol with ultracentrifugation and established methods. *Clin. Chem.*, 46: 493-505.
23. Grover, S.A., C.S. Palmer and L. Coupal, 1994. Serum lipid screening to identify high risk individuals for coronary death. The result of lipid research clinic prevalence cohort. *Archives of Internal Medicine*, 154: 679-684.
24. Muhammad, S.B., A.A. Zamir and S. Muhammad, 2001. Lipid profile in obesity. *JAMC*, 13: 31-33.