Resistin levels in women with ischemic stroke

Jan Kochanowski [1], Mariusz Grudniak [1], Agnieszka Baranowska-Bik [2], Ewa Wolinska-Witort [3], Malgorzata Kalisz [3], Boguslawa Baranowska [3,4], Wojciech Bik [3]

1 Department of Neurology, Medical University of Warsaw, Warsaw, Poland
2 Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland
3 Department of Neuroendocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland
4 Department of Clinical Physiology, Centre of Postgraduate Medical Education, Warsaw, Poland

Correspondence to: Mariusz Grudniak, MD.
Department of Neurology, Second Faculty of Medicine,
Bielanski Hospital, Medical University of Warsaw,
Ceglowska 80, 01-809 Warsaw, Poland.
TEL: +4822 5690517; FAX: +4822 5690239; E-MAIL: mariusz.grudniak@wum.edu.pl

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Abstract

OBJECTIVES: Resistin may be an independent inflammatory marker of atherosclerosis. Therefore, its circulating level might be important prognostic factor of cardiovascular disease in humans. We aimed in this study to assess plasma resistin concentration in Polish women with acute ischemic stroke, who additionally suffer from chronic diseases: diabetes, hypertension and/or obesity. The changes of resistin levels after 10 days from the onset of stroke and possible associations between resistin and pro-inflammatory cytokine TNFα were also evaluated.
MATERIAL AND METHODS: Material consisted of 41women with ischemic stroke (aged 60–85 years) and 64 controls (aged 60–85 years). Circulating resistin and TNFα concentrations were measured using ELISA. Blood was taken twice in the stroke group, in the first and tenth day from the onset of clinical symptoms, and only once in the controls. Clinical and biochemical data (blood pressure, weight, height, glucose, insulin, lipid profile) were collected.
RESULTS: Higher concentrations of resistin and TNFα were observed in ischemic stroke patients at the first day comparing to the controls. Second evaluation after 10 days in comparison with the first measurement revealed significantly higher TNFα levels and non-significant lower values of resistin. Resistin positively correlated with TNFα and stroke severity.
CONCLUSIONS: Changes in resistin and TNFα concentrations were observed in the course of stroke. Further investigations are required to assess the implication of these findings. Higher resistin concentration might be associated with worse neurological deficits.

INTRODUCTION

It has been reported that second most common cause of death worldwide is cardiovascular disease (CVD), that includes also ischemic stroke (Mortality Data Geneva 2011). Stroke is a life-threatening condition that is caused by occlusion of the vessels supplying the blood to the brain. There are some identified risk factors that can lead to this disease. Among them are: hypertension, diabetes, several kinds of heart diseases and obesity (Sacco 1995). All those disorders could be treated properly if they are diagnosed on time. However, there are also some untreatable and unchangeable risk factors including age, race or sex. In addition, it has been estimated that in twenty to thirty percent of

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ischemic stroke cases the risk factors are undetermined, despite the fact that many studies concerning the prevention of brain infarct have been conducted.

A close correlation between obesity and cardiovascular disease has been established. Obesity was recognized as one of the most important risk factors of cardiovascular events, in particular ischemic stroke (Kuklina *et al.* 2012). In general, obesity is associated with increased amount of fat tissue deposits. Moreover, fat tissue apart from its fat storing function is now regarded as an endocrine organ as it is able to produce and to secrete many biologically active substances (Galic *et al.* 2010). It releases, among others, peptides named adipokines. The discovery of leptin in 1994 confirmed endocrine function of the adipose tissue (Zhang *et al.* 1994). Other adipokines like adiponectin and resistin also belong to the family of fat-derived hormones (Kershaw & Flier 2004). Despite the intensive researches being carried out, the exact and detailed function of them is still unknown. However, it is known that the main role of these peptides is to regulate metabolism.

Resistin is believed to be involved in the mechanism of insulin resistance. In humans resistin is secreted in the great amount by macrophages and monocytes of the blood and in smaller amounts by fat cells in contrast to the mouse model in which it has been found that mouse resistin is mainly secreted by adipocytes (Nagaev & Smith 2001). Resistin is a small protein consists of 114 aminoacids (12.5 kDa). The resistin gene in humans is located on chromosome 19 (Steppan & Lazar 2004) and its expression is modulated by glucose and insulin levels, and also by several substances including growth hormone, glicocorticoids, beta-receptor agonists and drugs deriving from thiazolidinedione (Karbowska *et al.* 2009). Furthermore, some other factors involved in the inflammatory processes like cytokines, e.g. interleukin 6 (IL-6) or tumor necrosis factor α (TNFα), may also influence on resistin secretion. Our current knowledge about receptors of resistin is limited, so further investigations should be performed. It is known that resistin may stimulate endothelial cells. Resistin may modulate indirectly the process of expression of adhesion molecules, especially VCAM-1 that plays an important role in the early stages of atherosclerosis (Calabro *et al.* 2004). Moreover, resistin might be an independent inflammatory marker of atherosclerosis and thus its peripheral level might be the important prognostic factor in CVD in humans (Burnett *et al.* 2005).

Finally, it has been reported that adipose tissue is also able to secrete inflammatory cytokines including interleukin 6 (IL-6) and tumor necrosis factor α (TNF α) (You & Nicklas 2006). As there is a connection between atherosclerosis and inflammatory processes, it could be speculated that resistin and other proteins secreted by adipose tissue are the links in this association (Cho *et al.* 2011).

We aimed to assess any possible correlations between circulating resistin concentrations and TNFα levels as well as clinical and biochemical parameters in women with ischemic stroke.

MATERIAL AND METHODS

Subjects

The investigated population consisted of 105 Caucasian, Polish descent women: 41 with the first ever ischemic stroke episode (aged 60–85 years) and 64 controls without stroke in their medical history (aged 60–85 years) matched for age and body mass index (BMI). The stroke patients were hospitalized in the Neurology Department, Second Faculty of Medicine, Medical University of Warsaw, Poland. Control individuals were recruited from the Outpatient Clinic as volunteers. Inclusion criteria for all participants included diagnoses of one of the following diseases: hypertension, type 2 diabetes or lipid disturbances, established on their medical history. All subjects were under appropriate treatment.

The diagnosis of ischemic stroke was determined with WHO criteria. Stroke severity was assessed according to the National Institute of Health Stroke Scale (NIHSS) criteria (Lyden *et al.* 1994). Every stroke patient had a CT brain scan. The existence of tumor, hemorrhage or other abnormalities in the central nervous system excluded the subject from the investigation.

Hypertension was defined according to *European Society of Hypertension* (ESH) and *European Society of Cardiology* (ESC) criteria.

The exclusion criteria were as follows: (1) chronic circulatory failure, (2) history of neoplasm, (3) acute inflammatory process, (4) acute renal or hepatic dysfunction, (5) history of excessive alcohol consumption.

The study protocol was approved by the Local Ethics Committee in the Centre of Postgraduate Medical Education in Warsaw. All subjects and/or their relatives were informed of the purpose of the study and written consent was obtained.

Medical examination and anthropometric measurements

On the day of blood collection a medical examination was performed to assess the health status of the subjects and to collect clinical data including blood pressure. Anthropometric measurements (weight, height) were performed and body mass index (BMI) was calculated. Clinical data from two groups under study were presented in Table 1.

Analytical methods

Blood samples were taken from all participants in the morning after 12 hours of fasting. In the stroke patients blood was taken in the first 24 hours after the onset of symptoms, and for the second time after 10 days. The controls had only one blood sample being taken. The specimens were centrifuged immediately at 4 °C and plasma was frozen at –70 °C for further analysis.

Resistin level was measured using ELISA method (Bio Vendor Laboratory Medicine, Czech Republic).

Tumor Necrosis Factor α concentration was measured with ELISA (Thermo Scientific, USA). Insulin concentration was measured using IRMA methods (Immunotech, Czech Republic). The intra- and inter-assay coefficients of variation were <10% for all investigated parameters.

Lipid and glucose profiles were measured using standard laboratory tests.

Insulin resistance was calculated using a homeostasis model assessment of insulin resistance (HOMA-IR) according to the formula: fasting plasma glucose (mmol/l) × fasting plasma insulin concentration (μIU/ml)/22.5 and defined as HOMA-IR >2.5.

Statistical analyses

Statistical analyses were performed with STATISTICA 9.0 PL software. The normality of distribution was investigated using the Shapiro-Wilk and Kolmogorow-Smirnov tests with the Lillefors corrections. The differences between groups were calculated using the Kruskall-Wallis rank test and Mann-Whitney U-test. The Spearman test was applied to calculate the correlations between resistin levels and biochemical and anthropometric parameters. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Data concerning clinical and biochemical parameters are presented in Table 1.

We found only two significant differences between patients with stroke and the controls. In details, glucose levels and surprisingly, triglyceride concentrations differed markedly. In the stroke individuals glucose levels were higher and triglycerides levels were lower in comparison with those of the control group. Besides, HOMA and insulin concentrations showed no differences in these two groups even though measurements of glucose revealed significantly higher values in the stroke group.

Resistin levels in patients with brain infarct were significantly higher than those seen in the controls (Table 2). A significant increase in level of TNFα in the stroke group was also seen when compared to the controls. Furthermore, resistin concentration did not differ significantly in the stroke patients when comparison of measurements achieved on the first day and the tenth day after the onset of symptoms was performed (Table 3). However, a tendency to decrease in resistin concentration after 10 days was seen. In the same model TNFα levels were significantly higher in the 10th day of stroke.

Moreover, statistical analysis showed positive correlation between stroke severity measured with NIHSS and resistin level. There were also significant positive correlations between resistin and TNFα concentrations (Table 4).

Tab. 1. Clinical and biochemical data for patients with acute ischemic stroke and control subjects (C).

	AIS n=41	C n=64	*p*-value
Age (years)	74.26±5.67	73.89±4.65	ns
BMI (kg/m^2)	29.3±4.79	28.9±4.26	ns
SBP (mmHg)	145±28.2	135±11.9	ns
DBP (mmHg)	81.5±12.4	81.9±9.58	ns
NIHSS	7.05±5.1	x	
Insulin (μIU/ml)	10.71±7.64	12.64±8.63	ns
Glucose (mmol/l)	7.13±2.14	5.77±1.07	<0.05
HOMA-IR	3.29±2.24	3.36±2.65	ns
Total cholesterol (mg/dl)	200.21±48.85	213.84±60.51	ns
LDL cholesterol (mg/dl)	120±41.4	123±58.2	ns
HDL cholesterol (mg/dl)	55±17.4	56.3±10.6	ns
Triglycerides (mg/dl)	69.71±18.9	178.78±96.8	<0.001

Data are presented as mean ±SD; ns – non significant; AIS – acute ischemic stroke; C – Controls; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; NIHSS – National Institutes of Health Stroke Scale; HOMA-IR – Homeostasis model assessment of insulin resistance

Tab. 2. Plasma concentrations of TNFα and resistin in the acute ischemic stroke (1st day) patients and the controls.

	AIS 1st day n=41	C n=64	*p*-value
TNFα (pg/ml)	7.22±2.90	4.05±4.59	<0.05
Resistin (ng/ml)	8.41±4.16	6.54±5.20	<0.001

Data are presented as mean ±SD; AIS – acute ischemic stroke; C – Controls; TNFα – tumor necrosis factor alpha

Tab. 3. Comparison of TNFα and resistin concentrations between patients with acute ischemic stroke in the 1st day and after 10 days.

	AIS 1st day n=41	AIS 10th day n=41	*p*-value
TNFα (pg/ml)	7.22±2.90	8.39±3.01	<0.05
Resistin (ng/ml)	8.41±4.16	7.29±2.53	ns

Data are presented as mean ±SD; AIS – acute ischemic stroke; TNFα – tumor necrosis factor alpha

Tab. 4. Correlations of resistin with NIHSS and TNFα in the stroke subjects.

Parameter A	Parameter B	R	*p*-value
Resistin	NIHSS	0.36	<0.05
Resistin	TNFα	0.37	<0.05

NIHSS – National Institutes of Health Stroke Scale
TNFα – tumor necrosis factor alpha

DISCUSSION

The role of resistin in pathophysiology of ischemic stroke is still unclear. It could be speculated that this adipokin may exert a modulating effect of a great importance on the course of brain infarct but only few investigations concerning this problem have been published by now. There is a possibility that this peptide is a risk factor of cardiovascular incidents independently of obesity in which an overproduction of resistin was observed (Steppan *et al.* 2001). Additionally, a connection between resistin and inflammatory processes and atherogenesis was confirmed (Reilly *et al.* 2005; Sommer *et al.* 2009).

The prospective studies showed a positive correlation between resistin and cardiovascular incidents. Despite the fact that Weikert at al. (Weikert *et al.* 2008) found a positive correlation between resistin concentration and myocardial infarct, an association between resistin level and brain stroke remained vague.

The exact role of resistin is still unknown. We observed an increase in resistin level in the brain infarct group in comparison to the control group of women. Additionally, we failed to find any significant differences between measurements of plasma resistin in the first and tenth day of stroke. Our results corroborate with data from the study conducted by Efstathiou *et al.* (Efstathiou *et al.* 2007). These investigators confirmed elevated resistin level in women with ischemic stroke, especially in those with atherogenic subtype of the disease. The same group of researchers noticed, what might be also very important, increased resistin concentrations in the follow-up examination after 5 years. Moreover, these authors also revealed statistically higher mortality rate in the group of patients with originally increased levels of resistin (80%) in comparison to the group of patients with low level of this peptide at the beginning of the investigation (15%). The similar observation was also made by Rajpathak *et al.* (Rajpathak *et al.* 2011) who furthermore indicated that increased resistin level is an independent, even of obesity, risk factor of ischemic stroke in postmenopausal women. Data from the 10-years longitudinal study by Prugger and colleagues confirmed that resistin was a risk factor of stroke in middle-aged men (Prugger *et al.* 2012).

Subsequently, we noticed a positive correlation between resistin and neurological deficit that was measured at the baseline using NIHSS. This finding together with data concerning influence of resistin on the mortality rate in brain infarct cases may indicate that higher levels of resistin are associated with severity of stroke course and outcome.

The research performed by Rajpathak and co-workers revealed a positive correlation of resistin with proinflamatory cytokines like IL-6 and TNFα (Rajpathak *et al.* 2011). The results of our group also confirmed the existence of positive association between resistin and TNFα, which may suggest the role of inflammatory process in ischemic stroke. We noticed that plasma levels of TNFα were significantly higher in the stroke group in comparison with those of the controls. Moreover, the highest levels of TNFα were seen in the 10^{th} day of stroke. DeGraba reported activation of cytokines during ischemic stroke, as elevated levels of TNFα as well as IL-1β, inductors of neuronal injury, were detected 1 hour from the onset of stroke symptoms (DeGraba 1998). Barone *et al.* noticed that inhibiting TNFα may contribute to diminished infarct size and, consequently, this cytokine may be a possible treatment target in ischemic stroke (Barone *et al.* 1997). Contrary to the hypothesis by Barone and colleagues, other researchers indicated that TNFα may also play a positive role by contributing to recovery after stroke by specific regulation of inflammatory processes (Hallenbeck 2002). In the recently published paper Tuttolomondo *et al.* reported different levels of TNFα depending on the stroke subtype. Higher level was associated with the cardioembolic subtype but significantly lower levels of TNFα were found in individuals with the lacunar subtype (Tuttolomondo *et al.* 2012). Moreover, Mazzotta *et al.* did not notice any significant differences between measurement of TNFα just before and after thrombolytic therapy in the brain infarct subjects. Data from study by Mazzotta and co-workers revealed also slightly higher concentrations of TNFα in the stroke patients and a positive correlation between TNFα level and neurological deficit (Mazzotta *et al.* 2004). Tuttolomondo *et al.* also noticed an association between severity of neurological deficit at admission and inflammatory factors, including TNFα (Tuttolomondo *et al.* 2012).

Besides, the possibility of age-dependent grade of inflammatory response should be also taken into consideration. The results of animal study on stroke indicated that inflammatory response to cytokines was diminished and infarct size was smaller in older mice in contrast to the younger ones (Sieber *et al.* 2011),

Our work has also some limitations and should be interpreted with caution. Firstly, we recruited a small group of patients and among them there were only women in their sixties to eighties. Secondly, only Caucasian, Polish descent individuals were included in the investigation. Finally, stroke patients were not divided into subgroups according to brain infarct subtype.

In conclusion, our data suggest that there may be a link between resistin level and pathophysiology of ischemic stroke. Resistin secretion in a course of stroke can be modulated by proinflammatory cytokines like TNFα, and may depend on the severity of stroke as well as on other inflammatory factors. The exact pathogenic mechanisms of resistin and TNFα action must be elucidated in a further intensive research.

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