Plasma adrenomedullin in acute ischemic stroke

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Adrenomedullin (AM) was originally identified as a potent vasodilator peptide from human pheochromocytoma. It consists of 52-amino acids, and has approximately 20% similarity with the calcitonin gene related peptide (CGRP). Besides its vasodilator effect, AM has been reported to have various functions, such as suppression of endothelin production from vascular smooth muscle cells (VSMCs) and chemo-attractant release from macrophages and inhibition of the growth and migration of VSMCs. Therefore, AM may suppress the progression of atherogenesis. Adrenomedullin is synthesized by many mammalian tissues including the adrenal medulla, endothelial and vascular smooth muscle cells, myocardium, and central nervous system. Plasma levels of AM have now been shown to be increased in a number of states including congestive heart failure, acute myocardial infarction, essential hypertension, sepsis, and renal diseases. In the brain, it has been reported that ischemic injury up-regulated AM expression in the cerebral cortex and it is thought to play a role in exacerbating the damage or acting as a neuroprotective factor. Adrenomedullin and its receptors are expressed in the brain with the highest level found in the thalamus, hypothalamus, and adeno-and neurohypophysis. After the discovery of AM's functions in the brain, its effects on stroke, and atherosclerosis have been studied. It was detected in macrophages within the atherosclerotic plaque. Plasma AM is increased in patients with chronic ischemic cerebrovascular diseases and correlates with the extent of carotid artery atherosclerosis. In theory, AM could inhibit atherogenesis due to its inhibitory effect on migration and proliferation of VSMCs, inhibition of endothelial cell apoptosis and anti-inflammatory activity.[1,2] In this study, we assessed the relation of AM levels with risk factors, clinical and etiological subgroups, and neurological recovery in acute cerebral ischemia.

Seventy consecutive male and female patients admitted to the Neurology Department of Haydarpasa Numune Educational and Research Hospital (HNERH), Istanbul, Turkey between May and December 2003 with the diagnosis of acute ischemic stroke were included in this study, and they were compared with 32 age-matched control subjects. The modifiable risk factors were noted. Excluded from the study were the following: Patients with ischemic stroke admitted after the first 72 hours; those with intracerebral and subarachnoid hemorrhage as the reason for stroke; those with brain tumor or systemic malignancy; patients who have had recent or concurrent heart failure and myocardial infarction; those with renal dysfunction; those with symptomatic peripheral arterial disease; those concurrently diagnosed with sepsis. Infection or inflammation was excluded clinically or on laboratory signs. The subtype of ischemic stroke was classified according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) categories. The clinical subgroups were classified according to the Oxfordshire classification method. Stroke severity on admission was assessed by the National Institutes of Health Stroke Scale (NIHSS), ranging from 0 (asymptomatic) to 42 points (maximum score). The modified Rankin Scale (mRS) has proved to be valid and reliable for defining outcome in stroke patients. The NIHSS score was assessed at days 1 and 10, and mRS at day 30. Blood samples were taken from the antecubital vein of the patients and the controls in the supine position within 72 hours following stroke. The 7 ml blood sample was immediately taken into Lavender Vacutainer tubes containing ethylenediamine tetraacetic acid and then transferred into tubes containing 0.6 U/ml aprotinin (proteinase activity inhibitor) and finally centrifuged at +4 degrees at 1.600xg for 15 minutes to separate the serum. Plasma samples were frozen and stored at -80^{0}C until assayed. The peptide was extracted by passing the plasma samples through C-18 SEP-COLUMN at the Department of Biochemistry at Istanbul University, Cerrahpaşa Medical Faculty. Then by applying the kit protocol (Phoenix Pharmaceuticals Inc, Harbor Boulevard, Belmond, California 94002) the AM level was measured with the enzyme linked immunoassay method. The statistical analyses in this study were performed with SPSS version 13. In addition to using descriptive statistical methods in the assessment of data, the independent t-test was used in the comparison of dual groups and the chi-square and Fisher's exact test were used in the comparison of quantitative data. In the comparison of the means of the patient and control groups, the one-sample t test from student's t-tests was applied. The data were assessed with the Mann Whitney U test and the Spearman correlation test. The results were evaluated at a significance level of p<0.05 and a confidence interval of 95%.

Of the 70 patients with ischemic stroke, 37.1% were female, and 62.8% were male, and of the control subjects 50% were female, and 50% were male. The mean age of the patient group was within the range of 64.9±13.1, and the mean age of the control group was within the range of 62.1±11.4. As for the distribution of risk factors, hypertension (77.1%) was found to have the highest rate followed by smoking, IHD, HLP, history of stroke, DM and familial history of stroke. The mean plasma AM level was significantly higher in the stroke group (141.6±68.06) than in the

control group (90.9±30.6) (p=0.000). No statistically significant difference was found between AM values with respect to age and gender in the patient group. We investigated the relation of risk factors to plasma AM levels in the present subjects, and AM was not altered by the absence or presence of each of the 6 major risk groups. In the clinical subgroup distribution, the mean AM in the Total Anterior Circulation Infarcts (TACI) group was significantly higher than in those not in this group (t=2.949, df=68, p=0.004), while in the Lacunar Infarcts (LI) group, the mean AM was significantly lower than those not in this group (t=-3.275, df=68, p=0.002). As for the etiological subgroups, while mean plasma AM was significantly higher in cases of ischemic stroke due to large-artery disease (LAD) than in those without LAD (t=4.364, df=68, p=0.000), but not in cardioembolic or other etiologic subtypes of ischemic stroke. In the Cardioemboli (CE) group, it was significantly lower than in those without CE (t=-3.699, df=68, p=0.000) (**Table 1**). The mean NIHSS scores of day 1 and day 10 were 9.05 ± 4.8 and 9.2 ± 9.3, and there was no statistical significance between them. The plasma AM levels of the 17 patients with an increase of 3 or more points in the values on day 1 and day 10 was found to be 147.9±62.3. However, no progression was detected in the NIHSS scores of 53 patients and the AM level of this group was 139.6 ± 70.2, and no statistically significant difference was found between the patients groups with and without progression. Upon examination of the mRS scores in detecting independence and evaluating functional outcomes; there were 22 patients with a score of 2 or less (independent group) (31.4%), and 48 patients with a score of 3 or more (dependent group) 48 (68.6%). On day 30, the mean AM of patients with a score of 2 or less was 108.4 ± 58.7, and the mean AM of those with a score of 3 or more was 156.9 ± 67.1 and when the 2 groups were compared, there was a significant elevation in the dependent patient group (p=0.005). There was a significant difference, especially for patients with a score level of 4-5 (mean AM 188.05±80.3). A positive correlation was detected between the plasma AM level and those with LAD among the etiological subgroups (r=0.510 p=0.000) and patients with TACI according to lesion localization and size (r=0.325, p=0.006). A negative correlation was detected between the plasma AM level and those included in the cardioembolic group among the etiological subgroups (r=-0.612 p=0.000) and patients with LI according to lesion localization and size (r=-0.365, p=0.002). No relation was detected among the other parameters.

In the present study, the plasma AM level was significantly higher in the stroke group than in the control group. Our study subjects had a high percentage of risk factors. However, there was no alteration in the plasma AM level in the presence or absence of each of

Table 1 - Adrenomedullin levels according to risk factors, clinical, and etiological subgroups.

Risk Factors	n (%)	Adrenomedullin mean±SD	t	*P-value*
Hypertension	54 (77.1)	149.8±69.5	1.886	0.064
Diabetes mellitus (DM)	20 (28.6)	149.3±64.5	0.560	0.557
Hyperlipidemia (HLP)	23 (32.9)	136.2±55.5	-0.466	0.642
Ischemic heart disease (IHD)	24 (34.3)	133.3±71.9	-0.738	0.463
Smoking	27 (38.6)	142.3±71.1	0.068	0.946
History of strokes	21 (30.0)	142.2±65.03	0.045	0.964
Familial history of stroke	6 (8.6)	150±72.7	0.311	0.756
Clinical subgroups				
TACI	10 (14.3)	197.4±82.4	2.949	0.004*
PACI	28 (40.0)	148.1±56.9	0.643	0.522
PCI	18 (25.7)	139.5±65.8	-0.156	0.877
LI	14 (20.0)	91.8±48.2	-3.275	0.002*
Etiologic subgroups				
LAD	42 (60.0)	167.4±62.8	4.364	0.000*
CE	14 (20.0)	86.3±41.6	-3.699	0.000*
SAD	8 (11.4)	118.7±70.1	-1.012	0.315
OC	6 (8.6)	120.6±67.9	-0.788	0.433

LAD - large artery disease, CE - cardioemboli, SAD - small artery disease, OC - other causes, LI - lacunar infarcts, TACI - total anterior circulation infarcts, PACI - partial anterior circulation infarcts, PCI - Posterior circulation infarcts, *significant

the risk factors. Risk factors for atherosclerosis have also been reported to contribute to the elevation of plasma AM concentration, with a positive correlation to the levels of markers of endothelial injury. In chronic cerebrovascular diseases, the relation between AM and atherosclerosis-related risk factors has been investigated. Kuwasako et al[3] stated that AM is related to the number of risk factors for atherosclerosis, and that AM level is a good indicator to reveal the degree of endothelial damage in atherosclerotic disease. Moreover, Shinomiya et al[4] have recently shown in patients with chronic ischemic stroke, that an increased plasma AM level is associated with carotid atherosclerosis, independent of the blood pressure level or presence of risk factors. The positive association between the AM concentration and the degree of atherosclerosis may be explained by the notion that AM is secreted from the vascular wall to exert antiatherosclerotic actions as a compensatory mechanism.[3,4] Inflammation is also a powerful inductive factor of AM production, because many kinds of inflammation related cytokines, such as interleukin and tumor necrosis factor, stimulate AM production and secretion from vascular and cardiac cells. Since little is known on the impact of AM production and its pathophysiological role on vascular atherosclerosis, various studies have been conducted on this issue. Suzuki et al[2] reported that compared to the inflammation markers of CRP and IL-6, AM is a more sensitive marker for coronary and peripheral arterial disease. Although the reason for this is not clear, they have attributed it to the production of AM in various tissues and organs, and that the main source of the AM, especially in the circulation is the blood vessels and the vascular endothelial cells, in particular. In our study, there is a strong, graded association between increasing plasma AM and ischemic stroke caused by large artery atherosclerosis and TACI. Effects and mechanisms of action of AM in cerebral vessels are poorly defined. It produces substantial dilatation of cerebral arterioles, and responses are mediated by activation of CGRP receptors. Activation of both adenosine triphosphate-sensitive and calcium-dependent K+ channels appears to be important in mediating microvascular responses to AM. The potency of this novel peptide, as well as recent reports describing increased expression after cerebral ischemia, implies the potential for an important role of AM in cerebrovascular physiology or pathophysiology.[5] In our opinion, this suggests that AM might be influenced by additional inflammation markers, and that its neuroprotective and antiatherosclerotic effect is crucial in acute stroke. It is known that the AM secreted from and synthesized in the vascular endothelial cells and SMCs, exerts an anti-atherosclerotic effect by inhibiting the proliferation and migration of in vitro SMCs. Studies have been conducted indicating that AM can play an important role in the formation of angiogenesis and inhibition of apoptosis. It has been suggested that AM infusion can exert an additive or synergistic effect on mesenchymal stem cell transplantation, and this in turn impacts on neurological functional recovery. It has been suggested that AM enhanced the therapeutic potency of MSC, including neurological improvement, possibly through inhibition of MSC apoptosis and induction of angiogenesis. When we performed the NIHSS assessment to comprehend the effect of AM on neurological deficit, upon examination of the mean AM values, especially in the patients with progression in NIHSS scores, we did not detect a statistically significant difference between the patient groups with and without progression. However, we observed that it was an influential indicator of functional outcomes assessed with mRS. There was a significant elevation in the dependent patient group.

In conclusion, with this study we have shown that after acute cerebral ischemia, AM is found at a significantly high ratio independent of risk factors. This increase showed a correlation with LAD and TACI. No relation was established between the severity of neurological deficit and the level of AM, but a significant correlation was found between AM elevation and functional outcomes. Studies on stroke that attempt to explain the role of AM in the pathophysiology of acute stroke, and how it may be involved in treatment objectives are scarce, therefore, more comprehensive studies that especially include comparisons with other inflammatory markers and use longitudinal criteria to determine its role in atherosclerosis are warranted.

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