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Plasma adrenomedullin levels in patients with migraine during naturel attack and attack free period

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Abstract

Objective: The role of Calcitonin gene related peptide (CGRP) in migraine has been demonsrated. The aim of this study was to examine the role of Adrenomedullin (AM) which is a member of the calcitonin/CGRP/amylin family in migraine patients during naturel attack and attack free period.

Material and Method: Twenty-six migraine patients (11 with aura, 15 without aura) and 26 healty participants were involved. Blood samples were obtained from each patient in attack and attack free period, then compared with each other and control group.

Results: Mean plasma AM levels were 19 pmol/l during migraine attacks, 25.23 pmol/l between attacks, and 33.01 pmol/l in the control group. AM levels of migraine patients were significantly lower than controls during non-attack periods (p=0.001) and more interestingly, it further decreased during attack periods (p=0.001). A comparison of the mean plasma AM levels of migraine with and without aura cases revealed the same statistically significant difference (p=0.001).

Conclusion: The persistently low AM levels in migraine patients gave the impression that in physiological conditions there may be a balance between CGRP and AM and this may be changed towards to the site of CGRP in migraine pathophysiology while causing a decline in AM levels as we had found. Further studies regarding on AM involvement in migraine pathophysiology are needed to confirm these results.

Keywords: Adrenomedullin, calcitonin/CGRP/amylin family, headache, migraine, pathophysiology

Introduction

Pivotal role of Calcitonin gene related peptide (CGRP) in migraine mechanisms has been recognized long ago (1-4). Although studies have demonstrated that CGRP infusion might trigger and CGRP antagonists effectively limit the migraine attacks in migraine sufferers (5,6). About 20 years ago, Goadsby et al. (1) reported higher serum CGRP levels in the internal jugular vein blood during migraine attacks, but another study couldn't verify these results (7). Due to these conflicting results it is still uncertain whether serum CGRP levels change during the natural course of migraine attack (8). Adrenomedullin (AM) that was originally isolated from human pheochromocytoma cells belongs to CGRP super family (9). Two of the major sources for AM are endothelial and vascular smooth muscle cells. The vasodilator effect of AM may be important in the maintenance of resting vascular tone and regulation of specific blood-brain barrier properties (2,10). Because of higher concentration of AM, it may be especially important in the cerebral circulation (11). Due to its similarities to CGRP, AM was also suggested to have a possible role in migraine pathophysiology (12). Animal studies have all shown the vasodilator effect of AM and increase in cerebral blood flow (CBF) (10,13). However in Petersen et al. (14) study intravenous AM is not a mediator of migraine headache and does not affect CBF and mean blood flow velocity in the middle cerebral artery (VMCA). Therefore

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in the present study we aimed to show the relationship between the AM during nature attack, without attack in migraine patients and the control group.

Material and Methods

Study population

Twenty-six patients, aged 18-50 years, diagnosed with migraine, according to International classification of headache disorders-2 criteria (15) and age, gender matched 26 healthy subjects were enrolled to study. Local ethics committee approved the study and written informed consents have been obtained from all included cases before the enrolment. Migraine patients who reported having chronic migraine or headache due to drug overuse were not included in the present study. Exclusion criteria were; presence of high arterial blood pressure (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg), body mass index (BMI) <18 and ≥30 kg/m^2, history of renal functional disorders, endocrinological disorders, rheumatological diseases, peripheral vascular disease, inflammatory conditions, active cancer, diabetes mellitus (hunger plasma glucose ≥126 mg/dL), hypercholesterolemia (>200 mg/dL), pregnancy or lactation and regular use of vasoactive drugs. The medical information and physical and neurological examination findings of the study patients and the control group were recorded on a previously structured form by an experienced neurologist. Routine laboratory studies of the patients included routine blood tests; serum electrolytes, serum creatinine, blood urea nitrogen and fasting blood glucose levels; liver function tests; cranial tomography or cranial magnetic resonance imaging were performed in all patients. The glomerular filtration rate (GFR) was calculated by modification of diet in renal disease (16).

Collection of the plasma samples

Ten mL blood samples were taken from the cubital vein of migraine patients during attacks (when they still had pain and before they took any medicine) and also during non-attack periods when they had no complaints at least for 48 hours and from healthy control group patients on any day. The samples were placed in tubes containing 0.6 trypsin inhibitor unit/mL aprotinin. The tubes were gently shaken shortly and following a 15 minutes rest they were centrifuged for 10 min at 1.600 g/min. The obtained plasma samples were stored in deep-freeze at -20 ˚C until the examination time.

Adrenomedullin study technique

The residual erythrocytes in the plasma were removed from the bottom by filtration and washed with isotonic (9.0 g/L NaCL) for 10 times. Red blood cell sediment was destroyed with adding ice-cold deionized water. Harmless hemolysate was obtained through centrifugation at 10.000 xg for 5 min. Plasma AM level was measured with high performance liquid chromatography (HPLC) in picomole/l. The obtained fluid was applied to Super Coil C18 columns (Cecil 1000 HPLC, Super co, Cambridge UK). The applied material was mixed with 60% acetonitrile in 0.1% trifluoro acetic acid. Rat AM (1-50 pm/mL) (Phoenix Pharmaceuticals Mountain View CA, USA) (Figure 1) was used to determine standard AM levels (14). The investigators who quantified the AM plasma levels were blind to attack, non-attack and control samples.

Statistical analysis

The socio demographic and clinical characteristics of the patients and controls were given as a simple distribution. Chi-square and Fisher's exact tests were used to compare the socio demographic and clinical characteristics of the patients and controls. Student t-test was performed to examine the relationship of quantity variables such as age range between the patients and controls. To compare the two-implementation groups, Student's t tests for normally distributed continuous variables and Mann Whitney U test for non-normal variables, and Anova test was performed to compare groups according to continuous variables. And least significant difference test was used to detect subgroup differences. Spearman correlation analysis was performed to examine the relationship between the laboratory results and the AM levels. The p values lower than 0.05 was accepted as significant. Mean and Standard deviations and percentages were given as descriptive statistics. Package for the Social Sciences for Windows (SPSS, version 20.0, Chicago, IL, USA) software was used for statistical evaluation.

Results

The clinical characteristics of the control group and migraine patients are summarized in Table 1. The patient and control groups were similar in terms of age and gender. No significant differences were found in the mean arterial pressure and

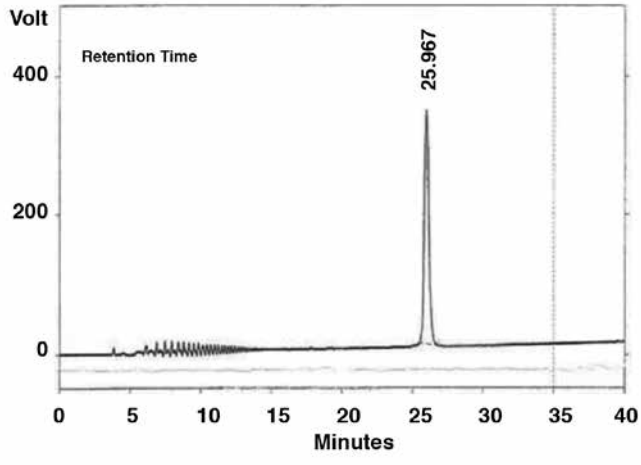

Figure 1. Standart human adrenomedullin retention time

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biochemical laboratory tests (Table 1). Of a total of 26 individuals in the migraine group, 11 had migraine with aura (42.3%) and 15 had migraine without aura (57.7%). Mean plasma AM levels were 19.00±4.25 pmol/l (in a range of 14.65 pmol/l and 25.48 pmol/l) during migraine attacks, 25.23±5.2 pmol/l (in a range of 20.08 pmol/l and 30.98 pmol/l) between attacks, and 33.01±6.8 pmol/l (in a range of 22.47 pmol/l and 41.09 pmol/l) in the control group (Figure 2). The mean serum AM level in attack period was significantly lower than that of the non-attack period samples in migraine cases (p=0.001). There were statistically significant relations between migraine groups and control group (p=0.001). A comparison of the mean plasma AM levels of migraine with aura during attack and attack free period (respectively; 18,62± 5.87 pmol/L and 25.23±4.3 pmol/L, p=0.001) revealed statistically significant difference. The result was the same in migraine patients without aura (respectively; 18.95±3.8 pmol/l / 26.01±4.3 pmol/l, p=0.001). When we compared with the aura group between the group without aura during attack there was no statistically significant difference (p=0.646). The result was the same in with the aura group between the group without aura between attack (p=0.606). The associations between the plasma AM concentration and the BMI, mean arterial pressure (MAP) and GFR in the control and patient groups are summarized in Table 2. When we seperate the groups by gender there were no statistically significant difference in attack period, between attack period and in the control group (Table 3).

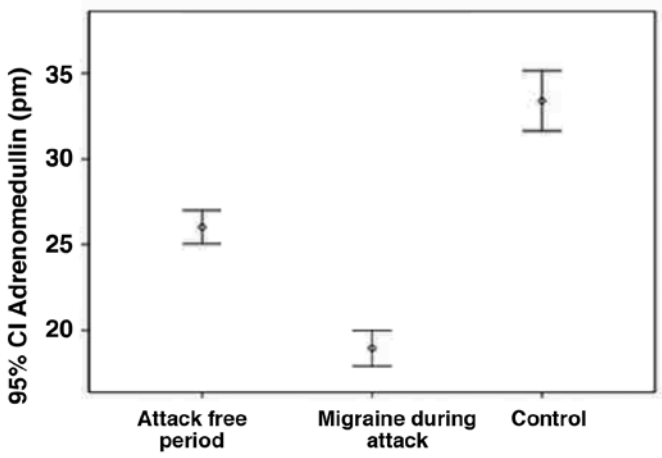

Figure 2. Plasma adrenomedullin levels in migraine patients with and without attacks and in controls

CI: Confidence interval

Discussion

In the present study, the plasma AM levels in migraine patients were significantly lower than that of control cases in attack-free period and it decreased to much lower levels during the

Table 1: Characteristics of the study subjects

	Control, n=26	Patients between attack n=26	Patients during attack n=26	p
Age (years)	28.12±5.89 (18-48)	28.65±5.87 (19-47)		0.349
Male:female ratio (n=n)	17:9	22:4		0.472
BMI	25±2.1	26±2.8		0.285
MAP (mmHg)	50±8	55±6	52±8	0.398
BUN (mg/dL)	20.03±3.36	20.95±4.46		0.416
Scr (mg/dL)	1.3±0.25	1.2±0.3		0.428
GFR (mL/min)	101±9	98±7		0.105
Plasma glucose (mg/dL)	95±8.2	86±6.8		0.286
CRP (mg/dL)	3.4±1.8	2.9±0.9		0.102

BMI: Body mass index, MAP: Mean arterial pressure, BUN: Blood urea nitrogen, Scr: Serum creatinine, GFR: Glomerular filtration rate, CRP: C-reactive protein

Table 2: Correlation coefficients (r) of simple regression analysis for relationships between body mass index, mean blood pressure, glomerular filtration rate, blood urea nitrogen, serum creatinine and plasma levels of adrenomedullinin patients and control groups

	BMI Corr.r/p value	MAP (mm/Hg) Corr.r/p value	BUN (mg/dL) Corr.r/p value	Scr (mg/dL) Corr.r/p value	GFR (mL/min) Corr.r/p value
Plasma levels of AM in patients	0.117/0.17	0.243/0.24	0.34/0.07	0.16/0.09	-0.39/0.068
Plasma levels of AM in control group	0.212/0.19	0.32/0.21	0.46/0.06	0.22/0.08	-0.42/0.06

BMI: Body mass index, AM: Adrenomedullin, MAP: Mean blood pressure, BUN: Blood urea nitrogen, Scr: Serum creatinine, GFR: Glomerular filtration rate

attacks. When we divided the patient group migraine with aura and without aura the results were similar. Low plasma AM levels in migraine cases may suggest a biological interaction and/or a possible variation in AM synthesis and release in migraine patients. Anyway, we can state that migraine cases have persistently low AM plasma levels. It is now well known that CGRP has an important role in migraine pathophysiology. Due to its similarities to CGRP, AM was also suggested to have a possible role in migraine pathophysiology (12). Their physiological properties are similar in some aspects. Additionally, calcitonin receptor-like receptors (CL) function both as CGRP and AM receptor, depending on receptor activity modifying protein (RAMP) function that determines which ligand will be bound by CL (17). RAMP1 transforms CL into a CGRP-binding form, while RAMP2 and RAMP3 give it an AM-binding property.

Kis et al. (2) summarized the similarities and differences of these two peptides in terms of migraine pathophysiology.

AM and CGRP are effective on the same receptors (17), yet there are differences in some aspects. For example, AM receptors are expressed from both the endothelial layer and smooth muscle layer of brain vessels, while CGRP receptors are released from perivasculer sensory nerve endings (11,18). Furthermore, CGRP is not expressed in cerebral endothelial cells; whereas, AM is abundantly secreted in brain vessel endothelium (11,19,20). Additionally, CGRP does not have a significant role in regulating the resting muscle tone of cerebral vessels, while AM may play an important role in regulating the resting muscle tone of cerebral vessels (21,22). In fact, although they belong to the same peptide family and bind to the same receptor, we still have insufficient data whether they have an interbalance, mutual effects and interaction in physiological and pathological conditions in human being. An experimental study investigated the behavior of endothelial endo CL receptors in microvascular endothelial cell culture when AM, CGRP and their antagonists are introduced into the environment (23). Akiyama et al., (24) demonstrated that endo CL internalization as a result of AM introduction could be blocked by both AM and CGRP antagonists (AM22-52 and CGRP8-37) and that desensitized receptor due to binding to AM was also desensitized against CGRP. Akiyama et al. (25) suggested that, the receptor was desensitized against both of these two peptides, regardless of with which it encounters. Also previous studies revealed that AM presynaptically inhibits the neurotransmission of rat mesenteric resistance arteries in perivascular CGRPergic nerves, possibly by reducing CGRP release. The results of these studies raise the question whether an imbalance of CGRP and AM functions may cause physiological or pathological consequence in humans.

A recent study in 12 migraine patients (14) demonstrated that AM infusion didn't alter CBF and VMCA which contrasts to animal studies (10) and didn't trigger migraine attacks in migraine patients. Based on these findings the conclusion of this study was made as AM might not have a role in migraine attacks. However, according to our results, lack of AM may be associated with migraine attacks. In physiological conditions there may be a balance between CGRP and AM and this may be changed towards to the site of CGRP in migraine pathophysiology while causing a decline in AM levels as we had found.

Some studies showed that several factors influence plasma AM levels, e.g. age and eGFR (26,27). Gender was one of the significant factors for plasma AM levels (28). Kawano et al. (28) also showed that plasma AM levels were correlated with BMI and waist circumference in women, but not in men. In our study When we seperate the groups by gender there were no statistically significant difference in attack period, between attack period and in the control group.and there were no significant associations between the plasma AM concentration and the BMI, MAP, GFR in the control and patient groups. However the clinical characteristics of the control group and migraine patients are similar.

In this study, the blood samples were drawn from the cubital vein in all of the included cases and the reported AM levels were from the peripheral circulation. This is one of the limitations of the present study. We would assess the results much accurately if we could obtain blood samples from more central veins. However, permission could not be obtained from our local ethics committee due to the lack of sufficient data on the role of AM in migraine pathophysiology.

Simultaneous CGRP levels were not examined in this study that may be considered as another limitation. However, in the extent

Table 3: Comparison of plasma levels of adrenomedullinin levels (ng/mL) in the patient and control groups

	Female (AM ng/mL)	**Male (AM ng/mL)**	**p**
Migraine patients during attack	18.95±2.3 (n=22)	20.02±1.6 (n=4)	0.215
Migraine patients between attack	26.01±4.2 (n=22)	26.78±2.1 (n=4)	0.416
Control group	33.38±4.8 (n=17)	33.38±5.1 (n=9)	0.698

AM: Adrenomedullin

of our knowledge, this is the first study reporting low plasma AM levels in the natural course of migraine patients.

Conclusions

Our results indicate that low plasma AM levels may be important in migraine pathophysiology. Studying AM together with CGRP and/or other pain modulators in peripheral and maybe also in cerebral circulation may provide new information about migraine pathophysiology. Notifying the potency of CGRP antagonists in terminating migraine attacks, it may be supposed that along with the CGRP itself, the receptor site may be an important partner in pathophysiological mechanisms of migraine. Moreover, since AM is a multifunctional peptide, it may sustain its effect on migraine attacks through some other ways regardless of CGRP. Furthermore, this study is the first about the serum AM levels during the nature attack of migraine patients in literature.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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