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한국인 허혈성 뇌중풍 환자에서 염증표지자의 발현 양상

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Inflammatory Marker Expression and Its Implication in Korean Ischemic Stroke Patients

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Background : Ischemic stroke is a complex condition influenced by many factors. Previous studies have demonstrated that inflammatory markers might play a role in such vascular diseases. Therefore the purpose of this study was to compare the expression of inflammatory markers in Korean ischemic stroke patients and to investigate their relationship to APOE polymorphism.

Methods : The patient group consisted of 275 patients with large artery atherosclerosis (LAA, n=169) and small artery occlusion (SAO, n=106). One hundred and nineteen age matched healthy subjects were recruited as the control group. Serum levels of three inflammatory markers (matrix metalloproteinase, MMP-9; tissue inhibitor of metalloproteinase-1, TIMP-1; and high-sensitivity C-reactive protein, hs-CRP) were measured in each patient by using commercially available kits. Comparison of clinical risk factors, inflammatory marker levels, and APOE genotypes between the stroke patient group and control group and between the two patient subgroups was assessed.

Results : Comparison of the stroke group to control group showed significantly elevated levels of circulating MMP-9 (P<0.01) and hs-CRP (P=0.01). Comparison between the individual subgroups revealed a significantly higher level of only TIMP-1 in the LAA subgroup compared to the SAO subgroup (P<0.01). There was no significant difference in inflammatory marker levels among each allele carrier.

Conclusions : The present study revealed the obvious tendency of increased circulating inflammatory markers in the patients with acute ischemic attack, especially MMP-9 and hs-CRP. Our observations suggest that measurement of serum MMP-9, TIMP-1, and hs-CRP levels may be useful in the diagnosis of ischemic stroke patients. ***(Korean J Lab Med 2007;27:197-204)***

Key Words : *Ischemic stroke, Inflammatory markers, ApoE polymorphism*

INTRODUCTION

Ischemic stroke is a complex condition influenced by inflammatory process, environmental risk factors, and genetic factors. Identification of these risk factors and their relationships are critical in the understanding of the pathogenesis and etiology, diagnosis, and treatment of this condition[1-5].

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One known genetic factor, the apolipoprotein E (apoE), has exhibited a central role in influencing the inflammatory response[6, 7]. It has also been postulated that certain apoE alleles are a risk factor for the initiation and/or development of atherosclerosis[8, 9]. However, in discrepancy to the previous reports, case-control studies in the Korean population showed controversial results. Some reports announced that apoE ε4 allele was not associated to ischemic stroke[10]. A recent study revealed a strong association of the apoE ε4 allele to ischemic stroke but did not show an association between specific apoE alleles and atherosclerosis in subtypes of ischemic stroke with different pathogenesis[11]. Therefore, the presence of other effects resulting in atherosclerosis is strongly suspected. Inflammatory markers, which have been reported to be associated to ischemic stroke and atherosclerosis are such possible candidates.

To date, many experiments on inflammatory cytokines involved have been attempted, yet still there is continuous controversy in their significance and actual role in this multifactorial disease. Furthermore, no systematic study on the Korean population has yet been attempted. Therefore, the purposes of this study were to investigate and compare the expression of inflammatory markers, matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and high-sensitive C-reactive protein (hs-CRP) in Korean ischemic stroke patients and to investigate the relationship between inflammatory marker expression and apoE polymorphism.

MATERIALS AND METHODS

Subjects were recruited from 550 ischemic stroke patients who visited the Department of Neurology at Kyung Hee Medical Center from January 2002 to April 2003. The patients were evaluated clinically for risk factors of vascular disease and physical examination was done. Using magnetic resonance imaging and angiography, patients classifiable based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria were selected[12]. A final 275 patients, classified as large artery atherosclerosis (LAA, n=169) subgroup and small artery occlusion (SAO, n=106) subgroup were enrolled as the ischemic patient group. The LAA subgroup included subjects who had atherothrombotic infarction with atherosclerotic vascular lesion in multiple or single vessel and the SAO subgroup included subjects who had small artery disease without atherosclerotic vascular lesion.

For exclusion of confounding effect of age, individuals over 55 yr of age were screened from subjects who visited the Health Promotion Center at Kyung Hee Medical Center. One hundred and nineteen individuals without any history of Alzheimer's disease, previous stroke or ischemic heart disease (IHD), hypertension and diabetes mellitus (DM) were ultimately selected as the control group.

Informed consent was obtained from all study participants and the present study was approved by the institution's research ethics committee. Inflammatory markers, MMP-9 and TIMP-1 were measured using commercially available kits (R&D Systems, Minneapolis, USA) employing the quantitative sandwich enzyme-linked immunosorbent assay techniques, according to the manufacturer's instructions. Hs-CRP levels were measured with commercially available kits (Nittobo, Tokyo, Japan) using the latex nephelometry assay technique on the automated chemistry analyzer Hitachi 7600 (Hitachi, Tokyo, Japan). Genomic DNA was extracted and purified from peripheral blood leukocytes using the automated nucleic acid isolation and purification system (Magtration System 6GC: Precision System Science, Chiba, Japan) according to the manufacturer's instructions. A commercially available LightCycler ApoE Mutation Detection kit (Roche Diagnostics, Mannheim, Germany) was utilized for apoE genotyping using the LightCycler (Roche Diagnostics), which employs the fluorescence resonance energy transfer (FRET) principle of the real time PCR assay.

Comparison of clinical risk factors, inflammatory marker levels, and apoE genotypes between the stroke patient group and control group and between the two patient subgroups was assessed by chi-square test for nominal variables and student's t test for continuous variables. Reported P values were considered statistically significant at $P<0.05$. Statistical analysis was performed using the SPSS for Windows version 11.0 (SPSS Inc., Chicago, USA).

RESULTS

1. Patient characteristics

The patient group consisted of 169 LAA and 106 SAO

cases. The sex ratio was 181:94 and the median age was 62.5±8.9 yr. There was no significant difference in age between the ischemic stroke and control groups. There were no significant differences in the frequencies of risk factors such as hypertension, DM, previous stroke and IHD between the LAA and SAO subgroups (Table 1).

2. Expression of inflammatory markers

Comparison of the stroke group to control group showed significantly elevated levels of circulating MMP-9 (P=0.00) and hs-CRP (P=0.01). Although statistically insignificant (P=0.06), TIMP-1 levels were apparently higher

Table 1. Distribution of clinical characteristics and risk factors in ischemic stroke group and control group

Characteristics	Ischemic stroke group				Control group	$P^{\dagger}$
	LAA	SAO	P^{*}	Total		
Number	169	106		275	119	
Sex (M:F)	113:56	68:38	0.64	181:94	62:57	0.01
Age	63.3 (±8.9)	61.2 (±9.0)	0.12	62.5 (±8.9)	61.5 (±7.5)	0.08
Smoking history	23 (13.6%)	9 (8.5%)	0.21	32 (11.6%)	20 (16.8%)	0.13
Hypertension	109 (64.5%)	68 (64.2%)	0.96	177 (64.3%)		
DM	47 (27.8%)	24 (22.6%)	0.36	71 (25.8%)		
Previous stroke history	17 (10.0%)	19 (17.9%)	0.06	36 (13.0%)		
IHD history	7 (4.1%)	8 (7.5%)	0.22	15 (5.5%)		

*, between LAA and SAO; †, between ischemic stroke and control group.
P value, two sample t-test for continuous variables and chi-square test for nominal variables.
Abbreviations: LAA, large artery atherosclerosis; SAO, small artery occlusion; DM, diabetes mellitus; IHD, ischemic heart disease.

Table 2. Expression of inflammatory marker levels and apoE polymorphism in ischemic stroke group and control group

Inflammatory marker	Ischemic stroke group				Control group	$P^{\dagger}$
	LAA	SAO	P^{*}	Total		
Number	169	106		275	119	
MMP-9 (ng/mL)	101.15 (±123.16)	114.64 (±106.15)	0.55	106.35 (±116.88)	68.64 (±68.33)	<0.01
TIMP-1 (ng/mL)	151.29 (±88.15)	90.89 (±45.45)	<0.01	128.01 (±80.14)	93.12 (±49.14)	0.06
hs-CRP (mg/dL)	0.40 (±0.85)	0.32 (±0.81)	0.66	0.37 (±0.83)	0.20 (±0.45)	0.01

*, between LAA and SAO; †, between ischemic stroke and control group.
P value, two sample t-test.
Abbreviations: LAA, large artery atherosclerosis; SAO, small artery occlusion; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitors of metalloproteinase-1; hs-CRP, high sensitivity c-reactive protein.

Table 3. Comparison of inflammatory markers between ε2/non-ε2 carriers and ε4/non-ε4 carriers in stroke cases and control group

	ε2 allele			ε4 allele		
	ε2 carrier	non-ε2 carrier	P^{*}	ε4 carrier	non-ε4 carrier	$P^{\dagger}$
MMP-9 (ng/mL)						
Stroke	181.8 (±215.8)	103.0 (±106.2)	<0.01	114.3 (±138.2)	113.0 (±125.2)	0.53
Control	77.3 (±78.1)	66.2 (±65.0)	0.51	58.1 (±49.0)	68.9 (±68.6)	0.52
$P^{\ddagger}$	0.06			0.05		
TIMP-1 (ng/mL)						
Stroke	149.0 (±106.0)	127.3 (±82.1)	0.08	131.5 (±82.4)	129.7 (±86.8)	0.95
Control	104.3 (±44.7)	93.9 (±49.8)	0.48	83.9 (±43.7)	96.7 (±49.8)	0.61
$P^{\ddagger}$	0.08			0.35		
Hs-CRP (mg/dL)						
Stroke	0.46 (±1.07)	0.34 (±0.75)	0.19	0.39 (±1.18)	0.35 (±0.64)	0.17
Control	0.07 (±0.56)	0.22 (±0.49)	0.10	0.09 (±0.13)	0.22 (±0.49)	0.22
$P^{\ddagger}$	0.04			0.15		

*, between ε2 and non-ε2 carriers; †, between ε4 and non-ε4 carriers; ‡, between stroke group and control group among ε2 or ε4 carriers.
P value, two sample t-test for continuous variables.

in the ischemic stroke patients than in the control subjects. Comparison between the individual subgroups revealed a significantly higher level of TIMP-1 in the LAA subgroup compared to the SAO subgroup ($P=0.00$). How-

Table 4. Comparison of inflammatory markers between ε4/non-ε4 carriers and ε2/non-ε2 carriers in large artery and small artery group

	ε2 allele			ε4 allele		
	ε2 carrier	non-ε2 carrier	P^*	ε4 carrier	non-ε4 carrier	$P^\dagger$
MMP-9 (ng/mL)						
LAA	208.8 (±272.7)	95.0 (±110.2)	<0.01	111.3 (±156.2)	105.3 (±133.4)	0.34
SAO	158.7 (±159.2)	115.4 (±99.3)	0.74	118.4 (±113.2)	124.2 (±112.2)	0.77
$P^\ddagger$	0.34			0.39		
TIMP-1 (ng/mL)						
LAA	198.3 (±125.8)	149.1 (±93.1)	0.11	156.9 (±92.1)	153.3 (±99.5)	0.80
SAO	106.8 (±63.8)	93.7 (±44.0)	0.06	97.2 (±51.5)	95.6 (±46.9)	0.91
$P^\ddagger$	0.07			0.15		
hs-CRP (mg/dL)						
LAA	0.83 (±1.52)	0.32 (±0.61)	<0.01	0.28 (±0.67)	0.41 (±0.78)	0.42
SAO	0.13 (±0.14)	0.37 (±0.94)	0.22	0.54 (±1.65)	0.26 (±0.33)	0.07
$P^\ddagger$	0.01			0.18		

*, between ε2 and non-ε2 carriers; †, between ε4 and non-ε4 carriers; ‡, between stroke group and control group among ε2 or ε4 carriers.
P value, two sample t-test for continuous variables.
Abbreviations: LAA, large artery atherosclerosis; SAO, small artery occlusion.

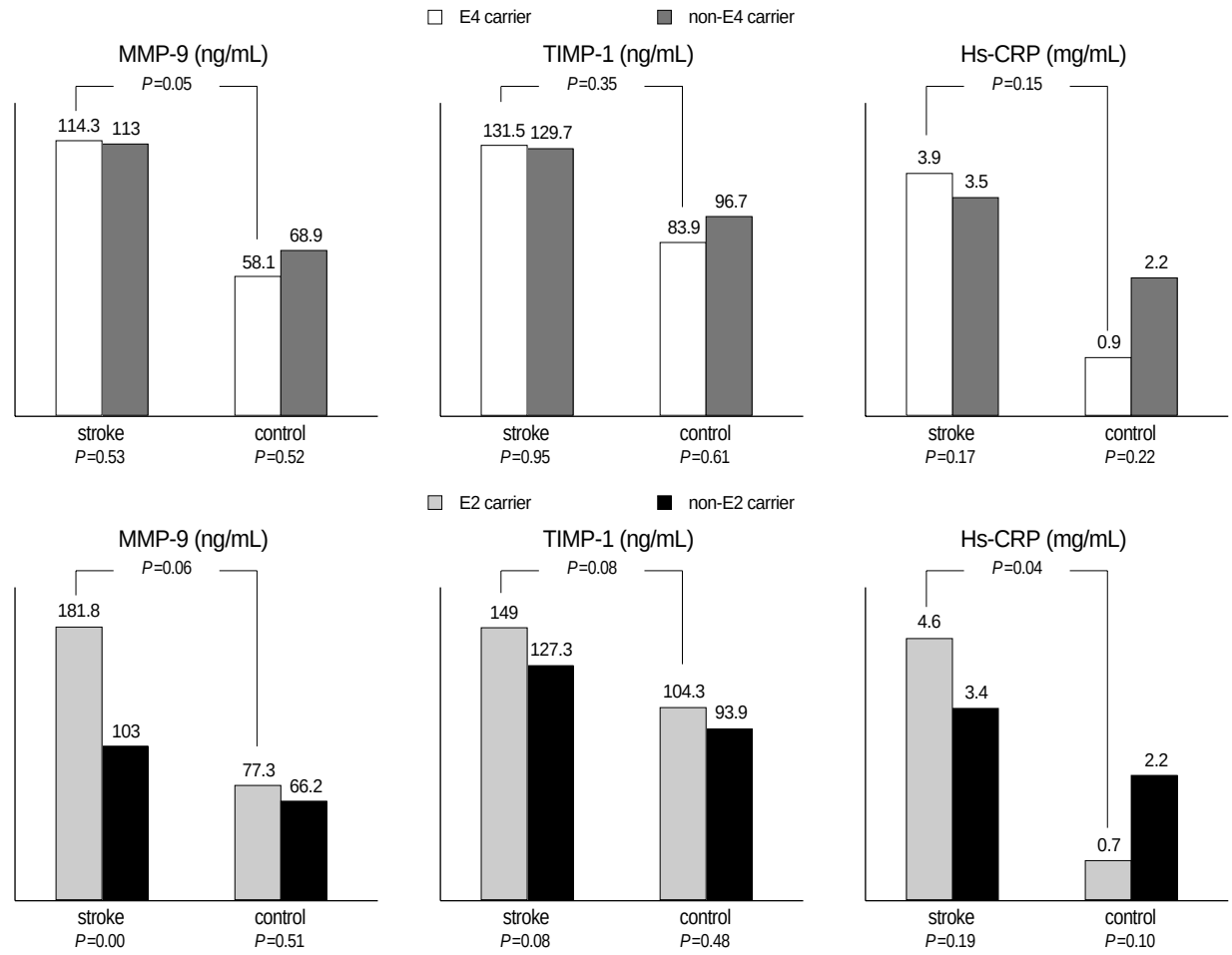

Fig. 1. Inflammatory marker levels between ε4/non-ε4 carriers and ε2/non-ε2 carriers in ischemic stroke group and control group.

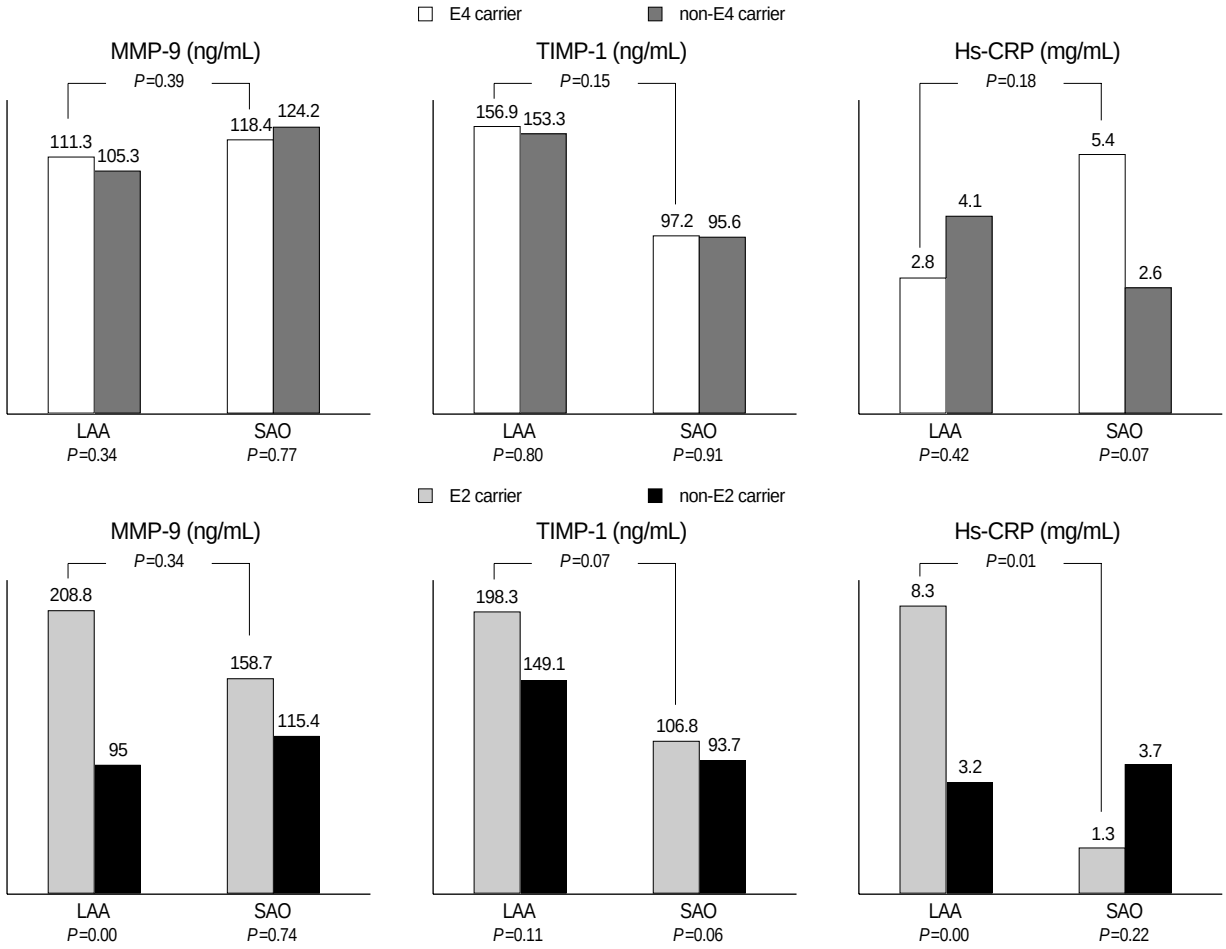

Fig. 2. Inflammatory marker levels between ε4/non-ε4 carriers and ε2/non-ε2 carriers in large artery group and small artery group.

ever no statistical differences in MMP-9 and hs-CRP levels were observed between the two subgroups (Table 2).

3. Relationship of inflammatory markers and apoE polymorphism

Data analysis revealed no significant differences in inflammatory marker levels among each allele carrier, although all markers showed a tendency of elevated levels in the ε2 and ε4 carrier compared to the ε3 carrier group (data not shown).

A comparative study of marker levels according to the presence or absence of specific alleles was done and all inflammatory markers did not show any difference between allele carriers and non-carriers in the control group, as expected. However, in the stroke patient group, MMP-9 showed significantly increased levels in ε2 carriers compared to the non ε2-carriers (Table 3). Subgroup study showed ε2 carriers to have higher levels of MMP-9 and hs-CRP in the LAA subgroup compared to the non ε2-carriers in this group. Such relationship was not noted in the SAO subgroup (Table 4)

Comparison between patient group and control group among ε2 carriers showed significantly higher levels of hs-CRP in the patient group ($P=0.04$)(Table 3, Fig. 1). Subgroup comparison also revealed a higher level of hs-CRP in the LAA subgroup compared to the SAO subgroup (Table 4, Fig. 2).

DISCUSSION

Stroke is a major cause of hospitalization, disability, and death in our society[13]. The cellular pathologic mechanisms involved in ischemic brain damage are still incompletely understood[14, 15]. Although a considerable amo-

unt of research between vascular diseases and indices such as inflammatory process, environmental and genetic factors have been attempted, its relationship is far from clearly defined. At present, the absence of a widely available and sensitive diagnostic test for acute cerebral ischemia remains a significant limitation in the diagnosis and management of stroke[13]. Previous studies have demonstrated evidence for a role of inflammatory markers in vascular disease and possibly in the pathogenesis of atherosclerosis. In this study, approach of the diagnosis and differentiation of ischemic subgroups with underlying atherosclerotic lesion by rapid blood-borne markers have been attempted.

There was no significant difference in age between the ischemic stroke and control groups. Comparison between the LAA and SAO subgroups, showed no difference in both age and sex. Although sex-matched group comparison is usually not attempted in ischemic stroke studies, such lack of sex- matched grouping between the patient and control group may be a limitation in the objective comparison between theses two groups. There were no significant differences in the frequencies of risk factors such as hypertension, DM, previous stroke and IHD between the LAA and SAO subgroups.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidase that degrades many molecules of the extracellular matrix. MMP-2 and MMP-9, in particular, specifically attack type IV collagen, laminin and fibronectin, which are the major components of the basal lamina around cerebral blood vessels[16]. Endogenous counter-regulators, TIMP-1, inhibit active MMPs by forming noncovalent stoichiometric complexes within the catalytic site. Several recent reports suggest a role of TIMPs in brain and peripheral nerve injury and repair[17]. Both MMPs and TIMPs are considered to be involved in the pathophysiology of acute stroke and excessive or inappropriate expression of MMP/TIMP-1 is known to contribute to the pathogenesis of cardiovascular diseases such as atherosclerotic plaque rupture and aneurysm formation[18, 19]. Another well-known marker of inflammation, hs-CRP, plays a critical role in all stages of atherosclerosis and subsequent cardiovascular events. Several large scale studies demonstrated hs-CRP as a strong independent predictor of future vascular events among healthy individuals and as a useful index for subsequent vascular events in patients with underlying cardiovascular disease[20-24].

Our study revealed increase in MMP-9 and hs-CRP levels in the ischemic stroke group. TIMP-1, although not statistically significant, displayed an increasing tendency in level expression in the patient group. An interesting finding was the increase of TIMP-1 in LAA subgroup, in comparison with the SAO subgroup. Along with the reports that show increased levels in arterial calcification, a marker correlating to plaque burden, these results support the careful speculation that TIMP-1 may play a role in the pathogenesis of atherosclerosis[19]. However, atherosclerosis itself is a complex condition involving multiple processes; therefore combinative interpretation of the other dynamic factors that regulate the endothelial dysfunction, inflammation, vascular proliferation and ECM degradation should also be considered.

Although no significant differences in inflammatory marker levels among each apoE allele carrier was observed, study involving the specific apoE allele showed higher levels of MMP-9 in $\varepsilon 2$ carriers compared to the non-$\varepsilon 2$ carriers of the patient group and LAA subgroup. Perhaps patients with the underlying $\varepsilon 2$ genetic background are more vulnerable to an inflammatory process involving the MMP-9, therefore more prone to the ischemic stroke condition. Further studies are necessary to validate such speculations.

The present study revealed the obvious tendency of increased circulating inflammatory markers in patients with acute ischemic attack, especially MMP-9 and hs-CRP. A significant increase of hs-CRP levels was also confirmed in patient groups compared to the control group among the $\varepsilon 2$ carriers. Also interestingly observed was the difference in TIMP-1 expression in the two subgroups with different underlying pathogenesis. Lack of difference in MMP-9 and hs-CRP between the two subgroups with different pathogenesis suggests an existence of common inflammatory pathway in both groups and also underscores the importance of illustrating the associated dynamic regulators of the pro-atherogenic or anti-atherogenic condition. Our observations suggest that measurement of serum MMP-9, TIMP-1, and hs-CRP levels may be useful in the diagnosis and management of ischemic stroke patients providing a more feasible blood-borne index. Considering the previous studies that state the limitation of sensitivity and specificity of an individual marker, a panel of markers rather than a single marker would be useful for clinical settings[13, 25]. Further studies employing other inflammatory markers as a potential index for the diagno-

sis and management of ischemic stroke patients are essential and our results should be of value as baseline data in such studies. Such further study should also be helpful in understanding the mechanism of atherosclerotic pathogenesis.

요 약

배경 : 허혈성 뇌중풍은 염증반응, 환경적 및 유전적 요인 등에 의해 영향을 받는 복합적 상태이다. 이러한 관련 위험요인들을 밝혀내는 것이 뇌중풍의 원인 및 기전, 진단 그리고 치료를 이해하는데 중요하다. 아직까지 이들 표지자의 정확한 작용이나 의의에 대해서는 논란이 있으며 더욱이 한국인을 대상으로 이러한 표지자들의 통합적인 연구는 아직 이루어진 적이 없다. 이에 저자들은 한국인 허혈성 뇌중풍 환자들을 대상으로 염증 표지자들의 발현 양상을 보고자 하였고 나아가 잘 알려진 유전적 요인인 아포 E 단백의 다형성과의 관계를 알아보고자 하였다.

방법 : 총 275명의 허혈성 뇌중풍 환자들과 119명의 건강한 대조군을 대상으로 하였다. 환자군은 169명의 LAA (large artery atherosclerosis)군과 106명의 SAO (small artery occlusion)군으로 분류하여 비교하였다. 염증 표지자 matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1)와 high-sensitivity C-reactive protein (hsCRP)의 혈장 농도를 측정하였다.

결과 : 모든 염증 표지자, 특히 MMP-9와 hs-CRP가 대조군에 비해 환자군에서 높게 측정되었다. 또한 환자군 내의 서로 다른 기전을 지닌 두 군을 비교한 결과 TIMP-1만 LAA군에서 유의하게 높게 측정되었다. 각 특정 아포 E 단백 이형접합체간에 염증 표지자의 수치에는 유의한 차이가 관찰되지 않았다.

결론 : 본 연구 결과 염증성 표지자가 허혈성 뇌중풍 환자군에서 유의하게 높게 관찰되었으며 이들 표지자의 측정은 손쉽고 빠른 지표를 제공함으로써 허혈성 뇌중풍 환자의 진단 및 치료에 유용할 것으로 생각된다.

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