대한침구학회지 제22권 제2호(2005년 4월)
The Journal of Korean Acupuncture & Moxibustion Society

원저

Tumor necrosis factor-α, interleukin-6 and interleukin-10 polymorphisms in the Korean stroke patients

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Abstract

Objective : With the onset of stroke, white blood cells release several proinflammatory cytokines, including interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α. It has been proven in previous studies that the release of these cytokines is related to the extent of damage to the brain and to overall prognosis. However, no studies have yet been performed to determine the connection with IL-6 and IL-10. Thus, this study is performed to see whether polymorphisms of IL-6, IL-10, and TNF-α genes that show increased serum concentration with the onset of stroke are related to stroke attack in Koreans.

Methods : Peripheral blood samples derived from patients with stroke (n=100) and healthy controls (n=100) were taken under informed consent. In subjects with stroke, blood samples were obtained within 24 hours of stroke onset. Genomic DNA was isolated using the Wizard DNA Purification Kit (Promega, Madison, WI).

Results :

1. Subjects with Heterozygote (GA) and Homozygote (AA) TNF-α gene types showed 2.433 and 20.457 times higher risks of being attacked by stroke, respectively, compared to subjects with wild type (GG) TNF-α gene type. The data was still statistically significant after adjusting for age, sex, history of smoking, and history of alcohol drinking.

2. Subjects with Homozygote (CC) IL-6 gene type showed 182.033 times higher risk of being attacked by stroke, compared to subjects with wild type (GG) IL-6 genes. This data was statistically insignificant (p=0.700). The data was still statistically insignificant after adjusting for age, sex, history of smoking, and history of alcohol drinking.

3. Subjects with Heterozygote (GA) and Homozygote (GG) IL-10 gene types showed 8.785 and 3.303 times higher risks of being attacked by stroke, respectively, compared to subjects with wild type (AA) IL-10 genes. The data was still statistically insignificant after adjusting for age, sex, history of smoking, and history of alcohol drinking.

* This work was supported by Korea Research Foundation Grant and Kyung Hee University (KRF-2003-005-E00001)

· **Acceptance** : 2005. 1. 7. · **Adjustment** : 2005. 3. 12. · **Adoption** : 2005. 3. 17.

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Conclusion : Our results suggest that the investigated TNF-α and IL-10 gene polymorphisms play an important role in stroke attack, but IL-6 gene polymorphism has not been found to be associated with stroke.

Key words : TNF-α, IL-6, IL-10, Polymorphism, Stroke

Ⅰ. Introduction

After cancer, stroke is the most frequent cause of death in Korea, more frequent than heart disease[1]. Stroke can have serious medical and social consequences including motor, speech, and mental impairments of the patient, disruption of the family and social and occupational activities. More than one half of them who survive from a stroke are disabled, and because of their functional or motor impairments would benefit from some form of rehabilitation. However, stroke rehabilitation is expensive and time consuming.

Stroke prevention, therefore, should take precedence over other medical issues and effective treatments should be administered early enough to take care of the onset, in order to minimize sequelae.

With the onset of stroke, white blood cells release several proinflammatory cytokines, including interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α. It has been proven in previous studies that the release of these cytokines is related to the extent of damage to the brain and to overall prognosis[2]. Also, in the Western countries, Acalovschi[3] and Rovilla[4] proved that, in stroke, changes in the serum concentrations of IL-6 are determined not only by the size of the infarct, but also by genetic control, and that this is related to the polymorphism of IL-6. Recent studies have shown that the polymorphisms of IL-1 and TNF-α act as the main risk factors related to the Korean stroke patients[5-7]. Other studies have also reported on the polymorphisms of the serotonin transporter gene[8], the angiotension converting enzyme(ACE) gene[9-10], the catalase gene[11], and ß-fibrinogen[12], analyzing recent genetic effects on the Korean stroke patients. However, no studies have yet been performed to determine the connection with IL-6 and IL-10.

Thus, this study reports on the results of the examination of stroke patients treated while hospitalized at the Hospital of Oriental medicine, Kyunghee University Medical Center, to see whether polymorphisms of IL-6, IL-10, and TNF-α genes that show increased serum concentration with the onset of stroke are related to stroke attack in Koreans.

Ⅱ. Material and Methods

1. Patients and specimens

Peripheral blood samples derived from patients with stroke (n=100) and healthy controls (n=100) were taken under informed consent. From December 2002 to June 2003, we recruited stroke patients, who were admitted to the Department of Acupuncture and Moxibustion, Hospital of Oriental medicine, Kyunghee University Medical Center, Seoul, Korea. Inclusion criteria for this study were: (i) diagnosis of cerebral infarct (CI) or intracerebral hemorrhage (ICH)/intraventricular

hemorrhage (IVH) upon admission. Neurological evaluation was performed by a stroke neurologist. We included patients with neurological symptoms lasting more than 24 h and accompanied by corresponding focal density changes detected by brain computed tomography (CT) or magnetic resonance imaging (MRI). Patients suffering from epidural (or subdural) hematoma, brain tumors, infectious or inflammatory disease and accidental or iatrogenic stroke were excluded. Final diagnoses of stroke subtypes were confirmed by serial CT or MRI findings.

Healthy controls without past history of stroke or present evidence of cervical pathology were those who underwent health examination at the Kyunghee University Medical Center. In the case of stroke patients, demographic and social information (age, sex, smoking status, and alcohol intake) were obtained by a well-trained interviewer immediately after onset. Case and control subjects were all born in Korea, which means that they shared a same geographic origin and culture. The age distribution was uniformly matched in the two groups.

Whole blood samples were collected into tubes containing EDTA. In subjects with stroke, blood samples were obtained within 24 h of stroke onset. Genomic DNA was isolated using the Wizard DNA Purification Kit (Promega, Madison, WI).

2. Determination of TNF-α (-308) genetic polymorphism (Table 1)

DNA fragments containing the 308 polymorphisms were amplified in reactions using methods adapted from papers by Grove et al. The following primers were used:

Forward Primer

5′-AGGCAATAGGTT TTGAGGGCCAT-3′

Reverse Primer

5′-CATC AAGGATACCCCTCACACTC-3′

Each reaction used a primer with a single base pair mismatch (underlined), introducing a restriction site into the wild type nucleotide sequences after amplification. Incubations were performed in 50 μL of KCl reaction buffer (Bioline UK, London, UK) containing 200 μmol L^{-1} dNTP, 25 μmol of each primer, 1 μg of DNA and 2 U of Taq polymerase (Bioline UK). The 308 amplification was achieved by 35 cycles of denaturation, annealing, and extension for 1 minute at 94 °C, 60 °C and 72 °C, respectively, to achieve a 134-base pair product. The presence of the G-to-A substitution was assessed by restriction digest of the PCR products. For the 308 substitution, NcoI, which recognizes CCATGG, was used, resulting in 20- and 114-base pair fragments. The cleaved wild type was then differentiated from the uncleaved mutant homozygote or partially cleaved heterozygote by gel electrophoresis

3. Determination of IL-6 (-174) genetic polymorphism (Table 1)

The following primer pair was used for PCR amplification of genomic DNA samples:

Forward Primer

5′-ATGACTT CAGCTTTACTCTT-3′

Reverse Primer

5′-ATAAATCTTTGTTGGAGGGT-3′

Amplification reactions were carried out with 500 ng genomic DNA in a total volume of 50 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 M of each primer, 200 M each dATP, dCTP, dGTP and dTTP, 1.5 mM MgCl2, and 2.5 U Taq DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). The reaction was followed by 40 cycles of 1 min at 94 °C, 1 min at 58 °C, and

Table 1. PCR Primer Sequences, Cycling Parameters, Restriction Enzymes, and Agarose Gel Concentrations

Primer	Sequence (5′-3′)	Cycling parameters	Restriction enzyme	Agarose gel, %
TNF-α (-308) Foward Reverse	AGGCAATAGGTTTTGAGGGCCAT CATCAAGGATACCCCTCACACTC	35 cycles of 94℃ for 60 seconds, 60℃ for 60 seconds, 72℃ for 60 seconds	Nco I	4
IL-6 (-174) Foward Reverse	ATGACTTCAGCTTTACTCTT ATAAATCTTTGTTGGAGGGT	40 cycles of 94℃ for 60 seconds, 58℃ for 60 seconds, 72℃ for 90 seconds	Hsp92 II	3
IL-10 (-1082) Foward Reverse	CTCGCTGCAACCCAACTGGC TCTTACCTATCCCTACTTCC	40 cycles of 94℃ for 60 seconds, 58℃ for 60 seconds, 72℃ for 60 seconds	Mnl I	4

* PCR = polymerase chain reaction; TNF = tumor necrosis factor; IL-6 = interleukin-6, IL-10 = interleukin-10

90 seconds at 72 °C. Amplifications were carried out in a Perkin-Elmer GeneAmp 2400 thermal cycler. The products were digested with 1 U per 25 ㎕ reaction of Hsp92 II to detect allele G and allele C. Restriction products were visualized by electrophoresis on 3% agarose gels in 1 TBE (89 mM Tris-Borate, 89 mM boric acid, 2 mM EDTA).

4. Determination of IL-10 (-1082) genetic polymorphism (Table 1)

Three biallelic IL-10 promoter polymorphism were detected by polymerase chain reaction (PCR) using primers that amplified a short fragment of DNA containing the polymorphic sites. The primer sequences used in the present study were of the -1082 polymorphism. The following primers were used:

Forward Primer

5′-CTCGCTGCAACCCAACTGGC-3′

Reverse Primer

5′-TCTTACCTATCCCTACTTCC-3′

The product was obtained in 139 base pairs (bp). Amplifications were performed on a thermal cycler (Perkin-Elmer Inc., Wellesley, MA) in a 20 ㎕ reaction mixture containing 1 ㎍ template DNA, polymerase buffer (100 mM Tris-HCl (pH 9.0), 40 mM KCL, 15 mM MgCl2; Bioneer Inc., Korea), 2.5 mM each of dNTP, 5 pM of each primer, and 1 unit of Taq polymerase (Bioneer). The following three cycling conditions were used: firstly 94 °C for 7 min; secondly 30 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 60 s; and thirdly 72 °C for 10 min. A 5-㎕ aliquot of each PCR product was digested for 2 h at 37 °C with restriction enzyme Mnl I (New England Biolabs Inc., Beverly, MA) for the -1082 polymorphism. Two alleles, *A and *G, exist at the -1082 position. When the *A allele is present, no split occurs, but in the presence of the *G, splitting occurs, creating two fragments of 33 and 106 bp. Restriction products were visualized by electrophoresis on 4% agarose gels in 1 TBE (89 mM Tris-Borate, 89 mM boric acid, 2 mM EDTA).

5. Statistical analyses

Data was analyzed using SPSS version 11. Chi-square, odds ratios (OR) and 95% confidence interval (95% CI) by Mantel-Haenszel estimate, were used to test bivariate associations between cases and controls. The following variables were compared: age (<65 or ≥65 years), gender, educational level (<10 or ≥10 years of school), stroke type (CI or ICH), For multi variate analysis we used stepwise logistic regression, entering all the variables with a $p<0.15$ on the bivariate analysis. A p-value ≤0.05 was considered statistically significant.

III. Results

The objective of this study is to analyze the effects of genetic polymorphism and its individual sensibility in regards to stroke patients. One hundred stroke patients and one hundred healthy subjects were selected as subjects and were frequency-matched on age and sex. DNA separated from peripheral blood were examined of the gene type of major cytokines: TNF-α (-308), IL-6 (-174), and IL-10 (-1082) (Fig. 1).

Stroke patients were those which had been hospitalized in the Hospital of Oriental Medicine, Kyunghee University Medical Center, from December, 2002 to June, 2003 and diagnosed as stroke through CT or MRI. History of smoking or drinking was recorded upon the onset of stroke. The subjects of the control group were those who underwent health examination at the Health Examination Center, Kyunghee Medical Center, with no history of stroke.

As shown in Table 2, the distribution of sex and age of the experimental group and the control groups were 60% male with mean age

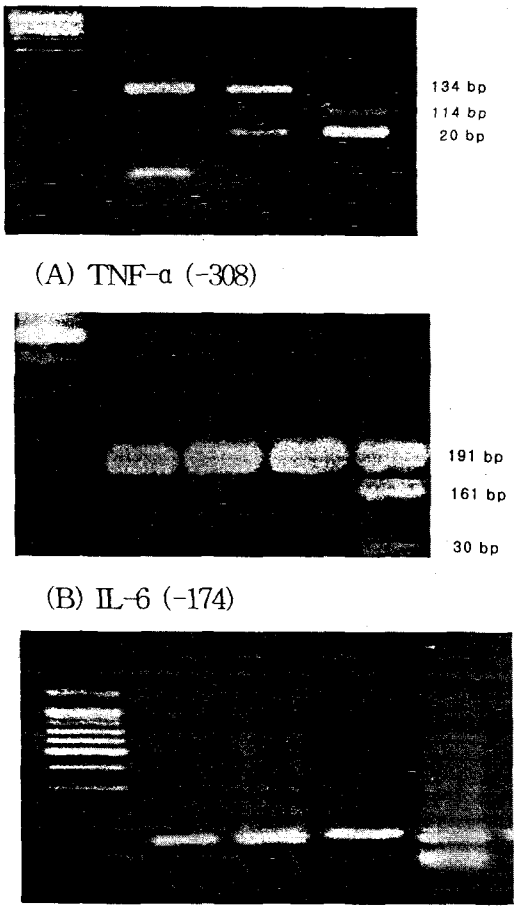

Fig. 1. Analyses of TNF-α (-308), IL-6 (-174), and IL-10 (-1082) polymorphism by PCR-RFLP. A 100bp ladder was used as a size marker (SM). (A) At position -308, a 134bp PCR fragment amplified and digested with Nco I. The -308 *A allele with an Nco I site demonstrated two fragments, 20bp and 114bp, whereas the -308 *G allele without an Nco I site produced no spitting. (B) At position -174, a 191bp PCR fragment amplified and digested with Hsp92II. The -174 *C allele with a Hsp92II site demonstrated two fragments 161bp and 30bp, whereas the -174 *G allele without an Hsp92II site produced no spitting. (C) At position -1082, a 139bp PCR fragment amplified and digested with MnlI. The -1082 *G allele with an Hsp92II site demonstrated two fragments of 106bp and 33bp, whereas the -1082 *A allele without an Hsp92II site produced no spitting.

Table 2. Demographic and Disease Characteristics of Stroke Patients and Controls

		No. of Subjects (%)		P value*
		Stroke patients (n=100)	Controls (n=100)	
Age [mean±S.D. (yr)]		60.44±12.54	60.21±13.05	0.899*
Sex				
male (%)		60	56	0.190**
female (%)		40	44	
Cerebral Infarction (%)		79	0	0.000*
Cerebral Hemorrhage (%)		21	0	0.000*
Weight (kg)		65.29±10.91	63.50±9.88	0.227*
Height (cm)		164.70±8.05	163.17±7.46	0.159*
Smoking status				
Never		55	54	0.957**
Current		41	41	
Ex-smoker		4	5	
Alcohol Intake				
No		55	57	0.776**
Yes		45	43	

* in comparison with controls using t-test; ** x2 test was used.

of 60.44 for the patient group, and 56% male with mean age of 60.21 for the control group. The difference in the distribution of sex and age between the two groups was not statistically significant (p>0.05).

In the patient group with 100 subjects, 41 (41.0%) patients had been smoking until hospitalization for stroke attack and 45 (45.0%) patients had history of drinking alcohol. Of the 100 subjects in the control group, 41 (41.0%) subjects were smokers and 43 (43.0%) subjects were alcohol drinkers. History of smoking or drinking alcohol were not statistically different between the two groups (p>0.05).

Below are the characteristics of the gene types of stroke patients and the control group, as shown in Table 3-5.

1. Analysis of TNF-α (-308) gene type

Among the experimental group, 39% of the patients had wild type (GG), 37% were Heterozygotic (GA), and 24% were Homozygotic (AA). In the control group, wild type (GG), Heterozygote (GA), and Homozygote (AA) were found in 70%, 28%, and 2% of the group, respectively. The difference in the gene type between the patient group and the control group was statistically significant (Table 3, x2=36.980, p<0.05).

Table 3. Characteristics of Study Population with Respect to TNF-α (-308) Genotype

	No. of subject (%)		
	Stroke patients (n=100)	Controls (n=100)	p value
TNF-α			0.000
GG	39 (39.0)	70 (70.0)	
GA	37 (37.0)	28 (28.0)	
AA	24 (24.0)	2 (2.0)	

x2 trend test was used.
n: The number of subjects studied

2. Analysis of IL-6 (-174) gene type

Of 100 stroke patients, 99 subjects had wild

type GG of IL-6 gene, no subject had the Heterozygote (GC) gene type, and one subject had the Homozygote gene type. All subjects in the control group had wild type GG gene type, and none showed gene types of Heterozygote (GC) or Homozygote (CC). The difference in the gene type between the patient group and the control group was not statistically significant (Table 4. x2=1.005, p>0.05).

Table 4. Characteristics of Study Population with Respect to IL-6 (-174) Genotype

	No. of subject (%)		
	Stroke patients (n=100)	Controls (n=100)	p value
IL-6			
GG	99 (99.0)	100 (100.0)	1.0
GC	0 (0.0)	0 (0.0)	
CC	1 (1.0)	0 (0.0)	

x2 trend test was used.
n: The number of subjects studied

3. Analysis of gene type IL-10 (-1082)

Of 100 stroke patients, 89, 8, and 3 patients had wild type AA, Heterozygote (GA), and Homozygote (GG) of IL-10, respectively. In the control group,wild type AA was found in 98 patients, and Heterozygote (GA) and Homozygote (GG) were each found in 1 patient. The difference in the gene type between the patient group and the control group was statistically significant (Table 5. x2 =6.878, p<0.05).

Table 5. Characteristics of Study Population with Respect to IL-10 (-1082) Genotype

	No. of subject (%)		
	Stroke patients (n=100)	Controls (n=100)	p value
IL-10			
AA	89 (89.0)	98 (98.0)	
GA	8 (8.0)	1 (1.0)	
GG	3 (3.0)	1 (1.0)	0.032

x2 trend test was used (x2 =6.878).
n: The number of subjects studied

The results of regression analysis on polymorphisms of each gene and stroke attack are shown in Table 6-8.

4. Correlation between polymorphism of TNF-α (-308) and stroke attack

Subjects with Heterozygote (GA) and Homozygote (AA) TNF-α gene types showed 2.433 and 20.457 times higher risk of being attacked by stroke, respectively, compared to subjects with wild type (GG) TNF-α gene type. The data was still statistically significant after adjusting for age, sex, history of smoking, and history of alcohol drinking (Table 6, p<0.05).

Table 6. OR and 95% Confidence Intervals for the Association between TNF-α (-308) Genotypes and Stroke

	No. of subject (%)		OR (95% CI)	Adjusted ORa (95% CI)
	Stroke patients (n=100)	Controls (n=100)		
TNF-α				
GG	39 (39.0)	70 (70.0)	1 (reference)	1 (reference)
GA	37 (37.0)	28 (28.0)	2.433b (1.359-4.356)	1.741 (1.021-4.213)
AA	24 (24.0)	2 (2.0)	20.457b (5.833-71.745)	18.320 (4.173-68.897)

Logistic regression analysis was used.
a; Adjusted for age, sex, smoking status and alcohol intake
b; Significantly higher in stroke patients than in controls
n: The number of subjects studied

5. Correlation between polymorphism of IL-6 (-174) and stroke attack

Subjects with Homozygote (CC) IL-6 gene

type showed 182.033 times higher risk of being attacked by stroke, compared to subjects with wild type (GG) IL-6 genes. This data was considered statistically insignificant (p=0.700). The data was still statistically insignificant after adjusting for age, sex, history of smoking, and history of alcohol drinking (Table 7, p<0.05).

Table 7. OR and 95% Confidence Intervals for the Association between IL-6 (-174) Genotypes and Stroke

	No. of subject (%)		OR (95% CI)	Adjusted ORa (95% CI)
	Stroke patients (n=100)	Controls (n=100)		
IL-6				
GG	99 (99.0)	100 (100.0)	1 (reference)	1 (reference)
GC	0 (0.0)	0 (0.0)	-	-
CC	1 (1.0)	0 (0.0)	182.033 (0.00-5.636E+13)	98.320 (0.00-4.387E+11)

Logistic regression analysis was used.
a; Adjusted for age, sex, smoking status and alcohol intake
n: The number of subjects studied

6. Correlation between polymorphism of IL-10 (-1082) and stroke attack

Subjects with Heterozygote (GA) and Homozygote (GG) IL-10 gene types showed 8.785 and 3.303 times higher risks of being attacked by stroke, respectively compared to subjects with wild type (AA) IL-10 genes. The data was still statistically insignificant after adjustment for age, sex, and history of smoking, and history of alcohol drinking (Table 8, p<0.05).

Table 8. OR and 95% Confidence Intervals for the Association between IL-10 (-174) Genotypes and Stroke

	No. of subject (%)		OR (95% CI)	Adjusted ORa (95% CI)
	Stroke patients (n=100)	Controls (n=100)		
IL-10				
AA	89 89.0)	98 (98.0)	1 (reference)	1 (reference)
GA	8 (8.0)	1 (1.0)	8.786b (1.080-71.484)	6.213 (1.785-69.335)
GG	3 (3.0)	1 (1.0)	3.303b (0.337-32.338)	2.859 (1.017-25.358)

Logistic regression analysis was used.
a; Adjusted for age, sex, smoking status and alcohol intake
b; Significantly higher in stroke patients than in controls
n: The number of subjects studied

IV. Discussion

Stroke is defined as the sudden occlusion or rupture of cerebral arteries or veins resulting in focal cerebral damage and neurological deficits. Strokes can either result from vascular occlusion or vascular rupture. Stroke is a multifactorial disease caused by the interactions of several genetic and environmental factors, as well as ischemic heart disease. Active studies and research on stroke are underway in the West, as well. Particularly concentrated studies are being performed regarding correlations with the polymorphisms of specific genes. Recently, it has become more evident that the inflammatory response plays an important role in the pathogenesis of cerebral lesions following stroke[5,7]. Activation of the inflammatory response plays a pivotal role in acute stroke[3].

Patients with acute stroke show increased production of several pro-inflammatory cytokines, including IL-1, IL-6 and TNF-α, in the cerebrospinal fluids (CSF) and the plasma[2]. Therefore, it is important to understand the regulation of the acute-phase response in stroke.

TNF-α, a potent immuno-modulator and proinflammatory cytokine, has been involved in many pathological processes. In addition to

autoimmune and infectious diseases, it also plays a significant role in obesity, insulin resistance, endothelial dysfunction, oxidative stress, and atherosclerosis. TNF-α, a marker of monocyte/macrophage activation, is elevated when the vascular wall is injured[13]. The TNF-α gene is located on the short arm of chromosome 6 between the class I and class II regions of the HLA complex. A number of polymorphisms have also been described for the TNF-α locus[14].

IL-6 has long been regarded as a pro-inflammatory cytokine which is induced by lipopolysaccharide, along with TNF-α and IL-1. It is often used as a marker for systemic activation of pro-inflammatory cytokines[15]. IL-6 is a pleiotropic cytokine that plays a pathogenic role in certain acute and chronic cerebral disorders. In stroke, increased plasma and cerebrospinal levels of IL-6 correlate with the size of the infarction and the functional prognosis of the patients[16-18]. A novel genetic polymorphism has recently been identified at the promoter region of the IL-6 gene and regulates the IL-6 gene expression[19-20].

IL-10 is the most important anti-inflammatory cytokine found within the human immune response. It is a potent inhibitor of Th1 cytokines, including both IL-2 and TNF-α. This activity accounts for its initial designation as a cytokine synthesis inhibition factor[21]. IL-10 attenuates the expression of TNF-α receptors and stimulates its release from the extracellular domains into the systemic circulation[22]. IL-10 is an anti-inflammatory molecule that exerts beneficial effects through interference with pro-inflammatory molecules (i.e. TNF and IL-1). External administration of IL-10 may inhibit the production of TNF and other proinflammatory cytokines by blocking gene transcription or through inhibition of nuclear stimulation on the transcription factor[23].

Recent studies on stroke investigated various polymorphisms and proved that genes, such as TNF-α gene[6-7], ß-fibrinogen[12], ACE gene[24], IL-1Ra gene[7], IL-1 alpha-889 gene[5,25], IL-6 gene[3], and IL-8 gene[2] were associated. It is also found that promoters of lipopolysaccharide receptor CD14[26], catalase gene[11], and serotonin transporter gene[8] are not related.

Previous studies on genetic polymorphisms with Korean stroke patients as subjects were focused only on TNF-α. No studies have yet been performed on IL-6 and IL-10.

Thus, this study aims to analyze the effects of genetic polymorphism and its individual sensibility in regards to stroke patients. One hundred stroke patients and one hundred healthy subjects, frequency-matched on age and sex, were selected as subjects. DNA separated from peripheral blood were examined for the gene types of major cytokines: TNF-α (-308), IL-6 (-174), and IL-10 (-1082) (Figure 1).

Analysis of TNF-α (-308) gene type among the experimental group determined that 39% of the patients had GG, 37% had GA, and 24% had AA. In the control group, GG, GA, and AA were found in 70%, 28%, and 2% of the group, respectively. The difference in the gene type between the patient group and the control group was statistically significant ($p<0.05$). Compared to the results obtained from the study performed by Um et al.[6] in 2004 on 366 subjects with cerebral infarction and 610 normal subjects as control group, this result shows similarity in the control group in terms of the frequency of gene type (GG: 83.4%, GA: 14.1%, AA: 2.5%). The control group also shows similarities with the results obtained by Chung et al. in 2001 on 109 normal subjects

(GG: 83.5%, GA: 16.5%, AA: 0%)[27] and Lee et al. in 2004 on 165 normal healthy controls (GG: 83.6%, GA: 15.2%, AA: 1.2%)[7]. However, the frequency of gene type in the experimental group with cerebral infarction showed difference with GG: 88.8%, GA: 10.9%, and AA: 0.3%. This is the similar results by Lee et al. in 2004 on 152 ischemic stroke patients (GG: 91.4%, GA: 8.6%, AA: 02%)[7].

Upon analysis of the IL-6 (-174)gene type among stroke patients, 99%, 0%, and 1% of the subjects had GG, GC, and CC type, respectively. All the subjects in the control group had GG gene type, and none showed GC or CC gene types. The difference in gene type between the patient group and the control group was not statistically significant ($p>0.05$). The relationship between stroke patient and gene polymorphisms of IL-6 has never been reported on. The control group did not agree with the result obtained by Lee et al. in 2001 in the study with 238 normal subjects (GG: 76.5%, GC: 13.9%, CC: 9.6%)[28].

Analysis of gene type IL-10 (-1082) among stroke patients, 89%, 8%, and 3% of the subjects showed the gene types AA, GA, and GG, respectively. In the control group, AA was found in 98%, and GA and GG types were each found in 1% of the group. The difference in the gene type between the patient group and the control group was statistically significant ($p<0.05$). There was no report on the relationship between the stroke patient and the gene polymorphisms of IL-10. The control group showed near accordance with the results obtained by Roh et al. in 2002 in a study performed on 179 normal subjects (AA: 100%, GA: 0%, GG: 0%)[29].

This study was undertaken to prove whether the polymorphisms of IL-6, IL-10, and TNF-α contribute to the initial stroke stage in Koreans. Our results suggest that the investigated TNF-α and IL-10 gene polymorphisms play an important role in stroke attack, but IL-6 gene polymorphism has not been found to be associated with stroke. Further studies performed on larger groups of patients are necessary to confirm the results of this study.

V. Conclusion

In order to determine whether or not gene polymorphisms of TNF-α, IL-6, and IL-10 influence stroke attack in Koreans, genetic characteristics of 100 stroke patients hospitalized in the Hospital of Oriental medicine, Kyunghee University Medical Center, and 100 healthy subjects with no history of stroke who visited the Health Examination Center, Kyunghee University Medical Center were investigated. The analytic result of the gene type is as follows below.

1. Subjects with Heterozygote (GA) and Homozygote (AA) TNF-α gene types showed 2.433 and 20.457 times higher risks of being attacked by stroke, respectively, compared to subjects with wild type (GG) TNF-α gene type. The data was still statistically significant after adjusting for age, sex, history of smoking, and history of alcohol drinking.
2. Subjects with Homozygote (CC) IL-6 gene type showed 182.033 times higher risk of being attacked by stroke, compared to subjects with wild type (GG) IL-6 genes. This data was statistically insignificant ($p=0.700$). The data was still statistically insignificant after adjusting for age, sex, history of smoking, and history of alcohol drinking.

3. Subjects with Heterozygote (GA) and Homozygote (GG) IL-10 gene types showed 8.785 and 3.303 times higher risks of being attacked by stroke, respectively, compared to subjects with wild type (AA) IL-10 genes. The data was still statistically insignificant after adjusting for age, sex, history of smoking, and history of alcohol drinking.

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