



Tumor Necrosis Factor-Alpha and Interleukin-4 Levels in Patients With Idiopathic Sudden Sensorineural Hearing Loss

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특발성 돌발성 감각신경성 난청 환자에서 종양 괴사 인자-알파 및 인터루킨-4에 대한 연구

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Background and Objectives The pathogenesis of idiopathic sudden sensorineural hearing loss (ISSNHL), an otologic emergency disease, remains unclear. Several studies have attempted to illustrate the association between cytokines and ISSNHL. The purpose of this study was to evaluate the prognostic significance of various cytokines in patients with ISSNHL.

Subjects and Method In this case-control study, a total of 55 patients with ISSNHL underwent treatment with oral prednisolone for 2 weeks. Serum cytokine levels, including interleukins (ILs) (IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12), and tumor necrosis factors (TNF- α and TNF- β), were measured using human cytokine panels at first visit. Patient characteristics such as age, gender, the status of hypertension, diabetes mellitus, vertigo, time from onset to visit, and initial hearing levels were also evaluated.

Results Serum levels of cytokines were correlated with the prognosis of ISSNHL patients. IL-4 ≥ 0.225 (pg/mL) and TNF- $\alpha \geq 5.155$ (pg/mL) were significantly associated with poor therapeutic outcomes (OR=44.317, $p=0.015$ and OR=269.465, $p=0.006$, respectively). In addition, age and initial hearing levels were also significant prognostic factors.

Conclusion Patients' age, initial hearing levels, and serum levels of IL-4 and TNF- α prior to treatment are associated with hearing recovery and can be used as prognostic factors for patients with ISSNHL.

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Keywords Interleukins; Sensorineural hearing loss; Sudden deafness; Tumor necrosis factor-alpha.

Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is one of the most important emergency diseases in otolaryngology, defined as a sensorineural hearing loss which occurs within 3 days.¹⁻³⁾ The definite cause of ISSNHL is unknown

in most cases; it is usually idiopathic. Although many mechanisms, including infectious diseases, immune-mediated mechanisms, vascular occlusion, and injuries in the inner ear structures, are suggested as etiologies of the disease, their roles have not been clarified yet.^{2,4,5)} The reported incidence of ISSNHL varies between 3.9–160 per 100000 individuals every year;⁶⁻⁸⁾ the recovery rate ranges between 45%–65% in patients without treatment, and between 49%–79% in those with treatment.^{1,8,9)}

Tumor necrosis factor-alpha (TNF- α) and interleukins (ILs)

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are cytokines that play major role in immune reactions and inflammatory processes; several studies on TNF- α and ILs have reportedly illustrated the etiology of ISSNHL. Although their mechanisms are still unclear, TNF- α production and ILs are known to regulate apoptosis of the inner ear cells, which affect microcirculation of the inner ear and contribute to the inflammatory response of the cochlea.¹⁰⁻¹²⁾ Previous studies demonstrated that serum cytokine levels, such as IL-6, IL-8, and TNF- α , significantly increased in patients with ISSNHL compared to controls.^{13,14)} In addition, many studies are underway that have suggested the potential use of serum cytokine levels, such as of IL-2, IL-6, IL-8, IL-10, IL-12, and TNF- α , to determine the prognosis of ISSNHL patients.^{11,15)}

However, previous studies failed to demonstrate the prognostic significance of specific serum cytokine levels. Therefore, the present study aimed to analyze serum cytokine levels in patients with ISSNHL, and to determine their prognostic significance. Additionally, we attempted to present the significant cutoff values for cytokine levels in order to enable proper treatment in patients with poor prognosis.

Subjects and Methods

Patients

The present study included 55 patients (31 males, 24 females) who visited our institution between March 2014 and March 2017. All patients underwent routine physical examination including otoscopic examination, pure tone audiometry, impedance audiometry, temporal bone CT, and posterior fossa MRI with gadolinium. Patients with hearing loss of ≥ 30 dB across at least three contiguous frequencies occurring within ≤ 3 day were included. The exclusion criteria were 1) middle ear disease, 2) retrocochlear pathology, 3) poor general condition, not amenable to steroid treatment, 4) family history of deafness, 5) bilateral hearing loss, 6) treatment at another hospital, 7) history of previous ISSNHL, 8) conductive or mixed hearing loss, 9) initial hospital visit occurring 10 days after hearing loss, and 10) other otologic illness such as Meniere's disease, perilymphatic fistula, and autoimmune inner ear disease.

Audiometric evaluation

All patients underwent a pure tone examination at their first visit. Averages of air conduction thresholds at frequencies of 500, 1000, 2000, and 4000 Hz were calculated. We classified the patients into four classes using the initial average thresholds as suggested by Demirhan, et al.¹⁵⁾ mild (30–50 dB), mod-

erate (51–70 dB), severe (71–90 dB), and profound (≥ 90 dB).³⁾

Hearing improvement

All patients were admitted to our hospital and treated with oral prednisolone for 14 days (1 mg/kg/day for 5 days, 0.8 mg/kg/day for 3 days, 0.5 mg/kg/day for 3 days, 0.2 mg/kg/day for 3 days). Pure tone audiometry was performed 2 weeks after the end of the treatment, and the outcome of patients were divided into 4 groups as 'complete recovery,' 'partial recovery,' 'slight recovery,' and 'no improvement' according to Siegel's criteria of hearing improvement.¹⁶⁾

Cytokine measurement

Blood samples were obtained from all patients before the initiation of treatment. Serum samples were derived from the blood samples by centrifugation and were stored in a freezer at -80°C . Milliplex[®] MAP Human Cytokine Panel (HCYTO-MAG-60K; Millipore, Billerica, MA, USA), a stable and precise tool for detection of biomarkers, was used to measure serum levels (pg/mL) of 11 different cytokines, including IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, TNF- α , and TNF- β , according to the manufacturer's instructions.¹⁷⁾ In brief, 96-well plates were pre-wet with 200 μL of wash buffer for 10 min at room temperature (20°C – 25°C). Wash buffer was decanted from all wells by turning over the plates and tapping onto absorbent towels. Standards or serum samples (25 μL) were added to appropriate wells, followed by addition of assay beads. Plates were incubated with agitation on a plate shaker overnight at 4°C or for 2 h at room temperature. The contents of wells were then removed and detection antibodies were added to each well. Plates were incubated for 1 h at room temperature. The fluorescent conjugate (Streptavidin-Phycoerythrin) was added to each well and plates were incubated for 30 min at room temperature. Fluid was then removed by vacuum and wells were washed twice. Each sample analysis was performed in duplicate. Data were collected and analyzed using Luminex 200[™] (Luminex Corp., Austin, TX, USA), High Throughput Screening (HTS) System (Luminex Corp), FLEXMAP 3D[®] (Luminex Corp) and MAGPIX[®] with xPONENT[®] software (Luminex Corp).

Ethics

This study was approved by the Institutional Review Board (IRB) of our institution (Korea University Medical Center IRB: KUGH16327), and informed consent from all patients was obtained before investigation.

Statistical analysis

We used the t-test and linear-by-linear association test to analyze the difference between groups. We also used the Mann-Whitney U test to compare the cytokine levels between the recovered and unrecovered groups. In order to analyze the effect of cytokine levels on hearing recovery, we obtained the area under receiver operating characteristic (ROC) and used multivariate logistic regression. The best cut-off point was determined among the proposed points using Youden index. At the determined cut-off point, sensitivity and specificity were calculated and their range was estimated with 95% confidence interval. Statistical analysis was performed using the SPSS Software ver. 22 (IBM Corp., Armonk, NY, USA), and $p < 0.05$ was considered statistically significant.

Results

This study included 55 patients (31 males and 24 females). The mean age of all the patients was 51.0 ± 15.9 year and the

Table 1. Baseline characteristics of the study population (n=55)

Age (yr)	
Mean \pm SD	51.0 \pm 15.9
Range	17–81
Sex	
Male	31 (56.4)
Female	24 (43.6)
Hypertension	
Yes	15 (27.3)
No	40 (72.7)
Diabetes mellitus	
Yes	9 (16.4)
No	46 (83.6)
Vertigo	
Yes	15 (27.3)
No	40 (62.7)
Duration from onset (days)	3.6 \pm 2.8
Initial PTA	
Mild (30–50 dB)	20 (36.4)
Moderate (51–70 dB)	11 (20.0)
Severe (71–90 dB)	15 (27.3)
Profound (\geq 90 dB)	9 (16.4)
Hearing recovery	
Complete recovery	20 (36.4)
Partial recovery	9 (16.4)
Slight recovery	6 (10.9)
No improvement	20 (36.4)

Data are presented as the mean \pm SD or n (%). SD, standard deviation; PTA, pure tone audiometry

time from disease onset to the initial visit was 3.6 ± 2.8 day. Of all the patients, 15 patients (27.3%) experienced vertiginous dizziness, 15 patients (27.3%) had hypertension, and 9 patients (16.4%) had diabetes. When the patients were categorized according to initial hearing levels, 20 (36.4%) patients had mild hearing loss, 11 (20.0%) had moderate hearing loss, 15 (27.3%) had severe hearing loss, and 9 (16.4%) had profound hearing loss. When the patients were categorized according to the degree of hearing recovery based on Siegel’s criteria, 20 (36.4%) patients achieved complete recovery, 9 (16.4%) achieved partial recovery, 6 (10.9%) achieved slight recovery, and 20 (36.4%) showed no improvement (Table 1).

In Table 2, the ‘recovered group’ consists of patients who achieved ‘complete recovery,’ ‘partial recovery,’ and ‘slight recovery,’ while the ‘unrecovered group’ consists of patients who showed ‘no improvement.’ The characteristics of both groups were compared. The mean age of the patients in the unrecovered group was significantly higher than that of the recovered group patients (t-test, $p = 0.002$), and patients with underlying hypertension were more likely to be in the unrecovered group (t-test, $p = 0.026$). Also, there was a tendency for worse initial

Table 2. The characteristics of the recovered group and unrecovered group (n=55)

	Recovered group (n=35)	Unrecovered group (n=20)	p value
Age (yr)			
Mean \pm SD	45.9 \pm 13.8	60.1 \pm 15.7	0.002*
Range	17–73	17–81	
Sex			
Male	19 (54.3)	12 (60.0)	0.688
Female	16 (45.7)	8 (40.0)	
Hypertension			
Yes	6 (17.1)	9 (45.0)	0.026*
No	29 (82.9)	11 (55.0)	
Diabetes mellitus			
Yes	5 (14.3)	4 (20.0)	0.308
No	30 (85.7)	16 (80.0)	
Vertigo			
Yes	10 (28.6)	5 (25.0)	0.780
No	25 (71.4)	15 (75.0)	
Duration from onset (days)			
	3.3 \pm 2.9	4.3 \pm 2.5	0.186
Initial PTA (dB)			
30–50	17 (48.6)	3 (15.0)	0.005*
51–70	7 (20.0)	4 (20.0)	
71–90	8 (22.9)	7 (35.0)	
\geq 90	3 (8.6)	6 (30.0)	

Data are presented as the mean \pm SD or n (%). *statistical significance. SD, standard deviation; PTA, pure tone audiometry

hearing levels in the unrecovered group, as compared to the recovered group (linear-by-linear association, $p=0.005$). However, there were no statistical differences between the two groups according to sex, diabetes, dizziness, and the time from disease onset to the initial visit. Based on these results, age, hypertension, and initial hearing levels were associated with the degree of hearing recovery.

Table 3 shows the comparison data of serum cytokine levels between the two groups. Since the cytokine levels do not follow a normal distribution, data was presented with the median, first quartile, and third quartile. When the two groups

Table 3. Statistical comparisons of cytokine levels in recovered group and unrecovered group (n=55)

	Recovered group (n=35)	Unrecovered group (n=20)	p value
IL-1 α	0.11 (0.07, 0.16)	0.31 (0.12, 0.71)	0.217
IL-1 β	0.71 (0.54, 0.90)	0.51 (0.39, 0.77)	0.468
IL-2	0.53 (0.47, 0.68)	0.55 (0.43, 0.71)	0.599
IL-4	0.14 (0.04, 0.37)	0.60 (0.48, 1.30)	0.005*
IL-6	0.10 (0.06, 0.18)	0.56 (0.42, 0.95)	0.297
IL-7	0.99 (0.73, 1.87)	0.15 (0.07, 0.32)	0.252
IL-8	1.61 (0.47, 3.21)	1.53 (0.76, 2.78)	0.570
IL-10	0.05 (0.02, 0.15)	0.86 (0.24, 7.02)	0.330
IL-12	0.19 (0.14, 0.36)	0.10 (0.03, 0.43)	0.204
TNF- α	4.09 (2.57, 6.47)	44.31 (28.71, 82.08)	0.031*
TNF- β	0.02 (0.01, 0.04)	0.03 (0.01, 0.07)	0.721

pg/mL, median (25th percentile, 75th percentile), *statistical significance. IL, interleukin; TNF, tumor necrosis factor

were compared using the Mann-Whitney U test, the IL-4 and TNF- α levels of the unrecovered group were significantly higher than those of the recovered group ($p=0.005$ and $p=0.031$, respectively). Although there were no significant differences in the other cytokines, IL-1 α , IL-6, IL-10, and TNF- β were higher in the unrecovered group. Interestingly, there was no significant difference in IL-4 and TNF- α among the groups according to initial hearing level (one-way analysis of variance, $p=0.484$, $p=0.645$).

To identify the specific levels of IL-4 and TNF- α that could affect patient prognosis, the ROC curves based on IL-4 and TNF- α levels of all patients were calculated. By analyzing this, we tried to find the optimal cutoff value considering both sensitivity and specificity. The area under curve (AUC) of IL-4 was 0.727 (pg/mL) ($p=0.005$) (Fig. 1A) and the AUC of TNF- α was 0.676 (pg/mL) ($p=0.031$). When the cutoff value of IL-4 was set to 0.225 (pg/mL), the sum of sensitivity (88%) and specificity (66%) was the highest. Similarly, when the cutoff value TNF- α was set to 5.155 (pg/mL), the sum of sensitivity (70%) and specificity (71%) was the highest. The patients were categorized into two groups based on the cutoff values derived from the ROC curve analysis. Among the 28 patients with IL-4 ≥ 0.225 (pg/mL), 16 (57.1%) patients were in the unrecovered group, while among the 27 patients with IL-4 < 0.225 (pg/mL), 4 (14.8%) patients were in the unrecovered group. Therefore, the patients with IL-4 ≥ 0.225 (pg/mL) showed significantly more unrecovered cases ($p=0.002$) (Fig. 2A). Likewise, the

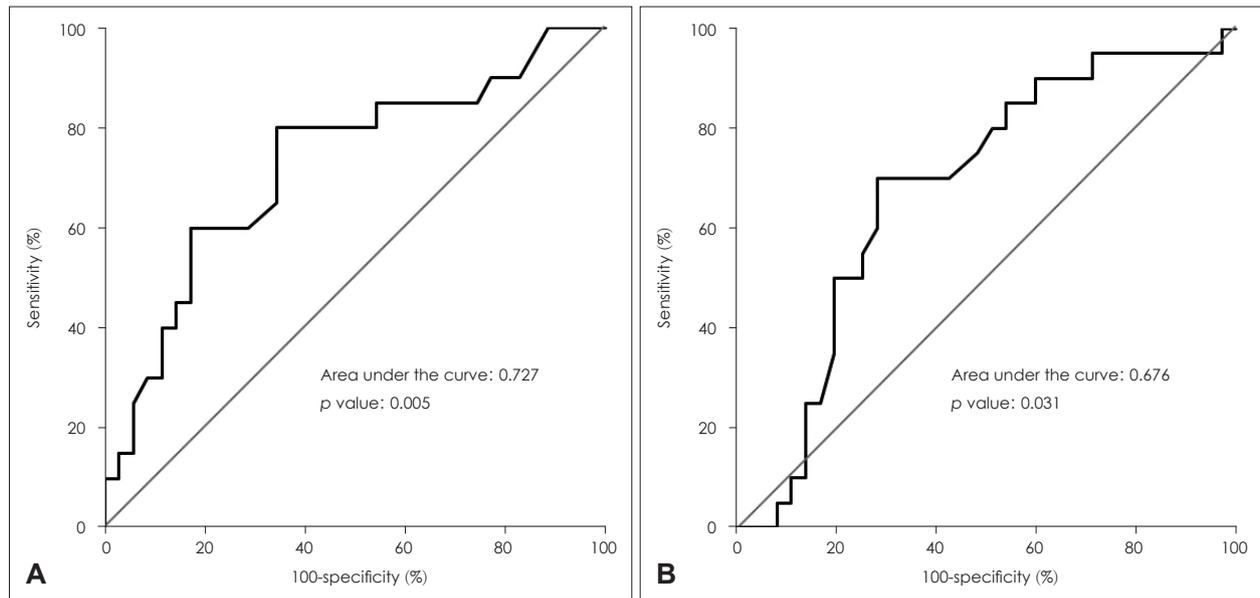


Fig. 1. ROC curves for sensitivity and specificity of IL-4 (A) and TNF- α (B). Area under ROC curve expresses the model explanation power, which was 0.727 ($p=0.005$) in IL-4 and 0.676 ($p=0.031$) in TNF- α , respectively. ROC, receiver operating characteristic; IL, interleukin; TNF, tumor necrosis factor.

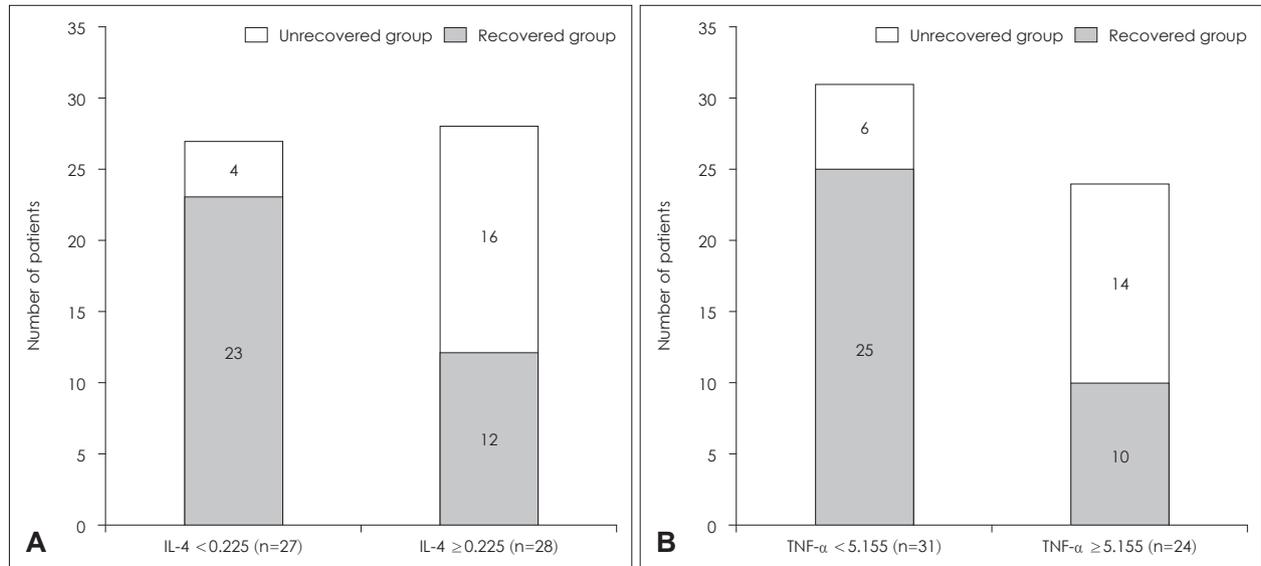


Fig. 2. Distribution of hearing recovery according to serum levels of IL-4 and TNF- α . A: When IL-4 ≥ 0.225 (pg/mL), the probability that the patient belongs to the 'unrecovered group' is significantly higher ($p=0.002$). B: When TNF- $\alpha \geq 5.155$ (pg/mL), the probability that the patient belongs to the 'unrecovered group' is also significantly higher ($p=0.004$). IL, interleukin; TNF, tumor necrosis factor.

patients with TNF- $\alpha \geq 5.155$ (pg/mL) showed significantly more unrecovered cases ($p=0.004$) (Fig. 2B).

When univariate regression analysis was conducted on the unrecovered group with the previously mentioned variables, there were associations between age, hypertension, initial hearing, IL-4 ≥ 0.225 (pg/mL), TNF- $\alpha \geq 5.155$ (pg/mL), and poor hearing recovery (Table 4). However, other associations including diabetes, dizziness, and time from onset to the initial visit were not significant. Multivariate logistic regression was conducted with adjustment by the factors that showed association in the univariate regression analysis, age, hypertension, initial hearing, IL-4 ≥ 0.225 (pg/mL), TNF- $\alpha \geq 5.155$ (pg/mL), and poor hearing recovery. We also included duration from onset, since the p -value was marginally significant in univariate analysis. The odds ratio (OR) was 44.317 ($p=0.015$) for IL-4 ≥ 0.225 (pg/mL), and the OR was 269.465 ($p=0.006$) for TNF- $\alpha \geq 5.155$ (pg/mL). Furthermore, the OR with increasing age by 1 was 1.137 ($p=0.029$), the OR of profound initial hearing compared to mild initial hearing was 1431.676 ($p=0.027$), and the OR of duration by days from the onset of hearing loss was 2.228 ($p=0.020$) (Table 4). Therefore, we concluded that patients with older age, poor initial hearing, late onset of treatment, IL-4 ≥ 0.225 (pg/mL), and TNF- $\alpha \geq 5.155$ (pg/mL) are more likely to be classified into the 'unrecovered group' after treatment. Although only partially significant in our statistical analysis, hypertension and duration from onset are also considerable risk factors of poor recovery.

Discussion

The purpose of this study was to analyze the levels of various cytokines in patients with ISSNHL, and to identify cytokines that could predict hearing recovery in patients. Based on such study, patients who are expected to have poor prognosis could be screened before treatment, and be treated more aggressively. In this study, the levels of many cytokines were analyzed; the ORs of poor hearing recovery were found to increase with increased levels of IL-4 and TNF- α .

Although there have been many recent studies conducted to analyze the mechanism of ISSNHL, the role of cytokines in the onset of ISSNHL remains unclear. It is known that cytokines are produced by various types of cells, and they regulate the proliferation and differentiation of the target cell.^{3,18)} Based on previous studies, Ren, et al.¹⁴⁾ reported that SSNHL patients had higher serum TNF- α levels than controls. Demirhan, et al.¹⁵⁾ reported that patients who did not respond to the treatment had significantly higher serum TNF- α levels prior to treatment than patients who responded well to the treatment. Further on, Tsinaslanidou, et al.¹¹⁾ compared the expression of TNF- α between treatment days 1 and 8. The TNF- α levels of patients with good treatment results significantly decreased after treatment. This study had results similar to the previous study results, wherein the serum TNF- α levels were higher in patients with poor treatment results than in patients with good treatment results.

TNF- α is a strong pro-inflammatory cytokine, and is report-

Table 4. Univariate and multivariate logistic regression analysis of clinical variables and cytokine levels for unrecovered group in patients with idiopathic sudden sensorineural hearing loss

	β	SE	p value	OR	95% CI
Univariate analysis					
Age (years)	0.068	0.023	0.003*	1.071	1.023–1.121
HTN	1.375	0.635	0.030*	3.955	1.139–13.727
DM	0.405	0.739	0.583	1.500	0.353–6.382
Vertigo	-0.182	0.638	0.775	0.833	0.239–2.908
Duration from onset (days)	0.135	0.102	0.186	1.144	0.937–1.398
Initial PTA			0.065		
Mild (reference)				1.000	
Moderate	1.175	0.886	0.185	3.238	0.570–18.385
Severe	1.601	0.812	0.049*	4.958	1.009–24.370
Profound	2.428	0.945	0.010*	11.333	1.780–72.169
IL-4 ≥ 0.225	2.162	0.737	0.003*	7.667	1.678–36.798
TNF- $\alpha \geq 5.155$	1.899	0.705	0.007*	5.833	1.678–26.608
Multivariate analysis					
Age (yr)	0.128	0.059	0.029*	1.137	1.013–1.276
HTN	1.866	1.439	0.195	6.460	0.385–108.415
Initial PTA			0.148		
Mild (reference)				1.000	
Moderate	1.001	1.837	0.586	2.720	0.074–99.615
Severe	2.718	1.757	0.122	15.151	0.484–474.366
Profound	7.267	3.289	0.027*	1431.676	2.271–902696.106
Duration from onset (days)	0.801	0.344	0.020*	2.228	1.134–4.374
IL-4 ≥ 0.225	3.791	1.565	0.015*	44.317	2.061–952.807
TNF- $\alpha \geq 5.155$	5.596	2.040	0.006*	269.465	4.948–14673.902

*statistical significance. SE, standard error; OR, odds ratio; CI, confidence interval; HTN, hypertension; DM, diabetes mellitus; PTA, pure tone audiometry; IL, interleukin; TNF, tumor necrosis factor

ed to play an important role in the immune system, in the proliferation and differentiation of cells, and apoptosis.¹⁰⁾ Previous studies on TNF- α expression in ISSNHL patients suggest that ISSNHL is caused by either direct or indirect inflammatory damage, and that TNF- α has a crucial role in this process.^{11,14,15)} However, there are no studies that have been able to clearly identify the mechanism of TNF- α . Adams¹⁹⁾ found that TNF- α and NF-kB are abundant in the principal cell of the spiral ligament, and thus disruption of cytokine balance may result in hearing impairments. Henceforth, Keithley, et al.¹⁸⁾ conducted an animal study that demonstrated that TNF- α injected into the inner ear increased the number of inflammatory cells, thereby resulting in hair cell destruction. However, this study did not show a direct association to hearing impairment, and it did not provide a clear explanation of the mechanism. Further studies on the mechanism of TNF- α in the onset of SSSNHL are deemed necessary.

ILs, including IL-2, IL-6, IL-8, IL-10, and IL-12, are known to be associated with the pathophysiology of ISSNHL.^{11,13-15)}

However, there have been no studies that specifically describe their mechanism. IL-6, one of the most studied ILs, tends to increase in most inflammatory states together with TNF- α . Ren, et al.¹⁴⁾ reported that the serum levels of TNF- α and IL-6 were higher in progressive ISSNHL patients than in controls, and suggested that this may have an important role in the pathogenesis of ISSNHL. Tsinaslanidou, et al.¹¹⁾ identified the expression of TNF- α , IL-2, IL-6, and IL-8 in ISSNHL patients using an enzyme-linked immunosorbent assay, and confirmed that TNF- α and IL-6 are associated with treatment prognosis. In particular, they reported that treatment results are better when the expression of TNF- α decreases while the expression of IL-6 increases. In addition, IL-6, as an inflammatory cytokine, promotes an inflammatory response via the IL-6 receptor (IL6R), which activates proinflammatory cascades, resulting in the hepatic production of C-reactive protein (CRP), fibrinogen, and other reactants.^{3,20,21)} It has been reported that IL6R is associated with various inflammatory conditions and vascular diseases; cardiovascular disease incidence also in-

creases when reactant levels increase (e.g., CRP, fibrinogen, and IL-6).^{22,23)}

Although no studies have reported association of serum IL-4 levels with the pathogenesis of ISSNHL, Nam, et al.²⁴⁾ reported that IL-4 receptor polymorphism is significantly related to increased risk of ISSNHL. IL-4, which is known for regulating cell proliferation and apoptosis in a variety of cells, plays a major role in immunoglobulin E secretion, resulting in the progression of various inflammatory conditions, such as systemic lupus erythematosus, pulmonary fibrosis, and allergic airway inflammation.^{25,26)} In addition, many studies have demonstrated that IL-4 interacts with TNF- α and IL-6 to amplify TNF- α levels, increase the expression of IL-6 mRNA, and enhance production of IL-6 proteins.²⁷⁻³⁰⁾ Although the exact role of IL-4 in the pathogenesis mechanism of ISSNHL is not clarified, it is considered that the interaction of IL-4, IL-6, and TNF- α contributes by inducing inflammatory responses in the inner ear cells or by affecting the blood supply to the inner ear. Therefore, further studies on the mechanism are deemed necessary.

Meanwhile, there are many studies on factors associated with treatment results of SSNHL patients, and recently studies suggesting models for predicting prognosis have been conducted. Based on these studies, age, tinnitus, dizziness, initial hearing levels, time from disease onset to the initial treatment, and comorbidity may be associated with prognosis of SSNHL.³¹⁻³³⁾ Although a few studies previously reported that age and recovery of ISSNHL are not associated,^{34,35)} recent studies repeatedly confirm that age is an important factor that can predict patient prognosis. Suzuki, et al.³¹⁾ studied the effect of patients' age on prognosis in 205 patients, and reported that the possibility of hearing recovery decreases by 0.07% as the age increases by 1 year. Gianoli and Li³⁶⁾ showed that patients aged below 60-year-old had a tendency of better treatment results, and Zadeh, et al.³⁷⁾ reported that patients aged below 40-year-old had higher hearing recovery rates. In this study, the mean age of the patients whose condition did not improve was significantly higher than of those whose condition improved. It was concluded that the possibility of no improvement was higher with increasing age. Furthermore, initial hearing was a very strong prognostic factor and many studies reported that as initial hearing worsens, prognosis also worsens.^{31-33,36,37)} This study also demonstrated that patients with profound hearing loss had a higher possibility of experiencing no improvement than patients with mild hearing loss. Based on these results, it is expected that patients with older age and increased

hearing loss show poor prognosis. In the current study, however, dizziness and time of treatment initiation did not affect treatment outcome, unlike in other studies. The limitations of this retrospective study are the small sample size and the possible inaccuracy of the medical records.

As with earlier studies, this study showed that inflammatory cytokines (e.g., TNF- α and IL-4) may have an important role in the pathogenesis of SSNHL, but was unable to clearly define the mechanism. Interestingly, initial hearing level and IL-4, TNF- α levels did not have significant association. Multivariate analysis reveals that elevation of IL-4 and TNF- α levels are independent risk factors for no recovery after treatment. In our study, we establish substantial evidence that specific cytokine levels hold significance in predicting post-treatment prognosis, even independent of initial hearing level. In other words, if the IL-4 and TNF- α levels prior to treatment are higher than the cutoff value, the patient is very likely to have no improvement in hearing after treatment. Therefore, through consideration of significant factors (e.g., age, initial hearing, and IL-4 and TNF- α levels), caregivers are likely to identify high risk patients, and inform patients the possibility of poor prognosis and the necessity for aggressive treatment.

An earlier study presented that a combination of hyperbaric oxygen therapy and oral steroid treatment can aid recovery in patients with profound initial hearing loss.³⁸⁾ There are other studies confirming the additional benefit of hyperbaric oxygen therapy.³⁹⁾ Furthermore, there are studies reporting the benefits of early intratympanic steroid injection in ISSNHL patients.⁴⁰⁾ Although hyperbaric oxygen therapy or intratympanic injections are still controversial with regard to treatment effects, the early addition of hyperbaric oxygen therapy and intratympanic steroid injection to high-risk groups is considered potentially beneficial. For this reason, further studies are required to further explore the benefits of the aforementioned treatment on high-risk patients with increased IL-4 and TNF- α .

The major limitation of this study is the small number of patients. Therefore, hearing improvement was only categorized into two groups (recovered and unrecovered). It is also possible that cytokines such as IL-1 α , IL-6, IL-10, and TNF- β might have shown insignificant results due to the small sample size. Also, due to the small number of patients, we could not consider subgroup analysis of some important factors. Comorbidities such as hypertension, diabetes mellitus, and obesity may alter cytokine levels, thus further studies including such considerations will clarify our findings. However, our results were generally consistent with previous studies suggesting that cer-

tain cytokines may play a major role in ISSNHL pathogenesis. The current study is exploratory, and is the first to suggest direct association between IL-4 and ISSNHL prognosis. Our findings may help to build insight about cytokine testing in sudden hearing impairment patients. Further studies are yet needed to better understand the mechanisms behind this relationship. Also, we attempted to set up initial cut-off values for IL-4 and TNF- α for the first time in order to give practical aid to caregivers to make decisions for aggressive treatment in the clinical setting.

In conclusion, the patient's age, initial hearing levels, and IL-4 and TNF- α plasma levels prior to treatment are associated with hearing recovery. Based on these, it is possible to predict patients who might have poor prognosis, warn the patients with possible poor prognosis, and provide more aggressive treatments, such as hyperbaric oxygen therapy and intratympanic steroid injection.

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REFERENCES

- Conlin AE, Parnes LS. Treatment of sudden sensorineural hearing loss: I. A systematic review. *Arch Otolaryngol Head Neck Surg* 2007;133(6):573-81.
- Merchant SN, Adams JC, Nadol JB Jr. Pathology and pathophysiology of idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2005;26(2):151-60.
- Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 2011;1813(5):878-88.
- Kassner SS, Schöttler S, Bonaterra GA, Stern-Sträter J, Sommer U, Hormann K, et al. Proinflammatory and proadhesive activation of lymphocytes and macrophages in sudden sensorineural hearing loss. *Audiol Neurootol* 2011;16(4):254-62.
- Merchant SN, Durand ML, Adams JC. Sudden deafness: Is it viral? *ORL J Otorhinolaryngol Relat Spec* 2008;70(1):52-60.
- Klemm E, Deutscher A, Mösges R. A present investigation of the epidemiology in idiopathic sudden sensorineural hearing loss. *Laryngorhinootologie* 2009;88(8):524-7.
- Teranishi M, Katayama N, Uchida Y, Tominaga M, Nakashima T. Thirty-year trends in sudden deafness from four nationwide epidemiological surveys in Japan. *Acta Otolaryngol* 2007;127(12):1259-65.
- Byl FM. Seventy-six cases of presumed sudden hearing loss occurring in 1973: Prognosis and incidence. *Laryngoscope* 1977;87(5 Pt 1):817-25.
- Seggas I, Koltsidopoulos P, Bibas A, Tzonou A, Sismanis A. Intratympanic steroid therapy for sudden hearing loss: A review of the literature. *Otol Neurotol* 2011;32(1):29-35.
- Scherer EQ, Yang J, Canis M, Reimann K, Ivanov K, Diehl CD, et al. Tumor necrosis factor- α enhances microvascular tone and reduces blood flow in the cochlea via enhanced sphingosine-1-phosphate signaling. *Stroke* 2010;41(11):2618-24.
- Tsinaslanidou Z, Tsaligopoulos M, Angouridakis N, Vital V, Kekes G, Constantinidis J. The expression of TNF α , IL-6, IL-2 and IL-8 in the serum of patients with idiopathic sudden sensorineural hearing loss: Possible prognostic factors of response to corticosteroid treatment. *Audiol Neurotol Extra* 2016;6(1):9-19.
- Fujioka M, Kanzaki S, Okano HJ, Masuda M, Ogawa K, Okano H. Proinflammatory cytokines expression in noise-induced damaged cochlea. *J Neurosci Res* 2006;83(4):575-83.
- Iguchi H, Anniko M. Interleukin 8 can affect inner ear function. *ORL J Otorhinolaryngol Relat Spec* 1998;60(4):181-9.
- Ren J, Li H, Lu Y. The determinations of tumor necrosis factor and interleukin 6 in serum of patients with sudden sensorineural hearing loss. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* 1998;12(7):311-3.
- Demirhan E, Eskut NP, Zorlu Y, Cukurova I, Tuna G, Kirkali FG. Blood levels of TNF- α , IL-10, and IL-12 in idiopathic sudden sensorineural hearing loss. *Laryngoscope* 2013;123(7):1778-81.
- Siegel LG. The treatment of idiopathic sudden sensorineural hearing loss. *Otolaryngol Clin North Am* 1975;8(2):467-73.
- Hermann N, Dreßen K, Schildberg FA, Jakobs C, Holdenrieder S. Methodical and pre-analytical characteristics of a multiplex cancer biomarker immunoassay. *World J Methodol* 2014;4(4):219-31.
- Keithley EM, Wang X, Barkdull GC. Tumor necrosis factor alpha can induce recruitment of inflammatory cells to the cochlea. *Otol Neurotol* 2008;29(6):854-9.
- Adams JC. Clinical implications of inflammatory cytokines in the cochlea: A technical note. *Otol Neurotol* 2002;23(3):316-22.
- Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis* 2000;148(2):209-14.
- Schütt H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. *Thromb Haemost* 2009;102(2):215-22.
- Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, et al. Interleukin-6 receptor pathways in coronary heart disease: A collaborative meta-analysis of 82 studies. *Lancet* 2012;379(9822):1205-13.
- Swerdlow DI, Holmes MV, Kuchenbaecker KB, Engmann JE, Shah T, Sofat R, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: A mendelian randomisation analysis. *Lancet* 2012;379(9822):1214-24.
- Nam SI, Ha E, Jung KH, Baik HH, Yoon SH, Park HJ, et al. IL4 receptor polymorphism is associated with increased risk of sudden deafness in Korean population. *Life Sci* 2006;78(6):664-7.
- Kelly-Welch AE, Melo ME, Smith E, Ford AQ, Haudenschild C, Noben-Trauth N, et al. Complex role of the IL-4 receptor alpha in a murine model of airway inflammation: Expression of the IL-4 receptor alpha on nonlymphoid cells of bone marrow origin contributes to severity of inflammation. *J Immunol* 2004;172(7):4545-55.

- 26) Yamamoto S, Kobayashi I, Tsuji K, Nishi N, Muro E, Miyazaki M, et al. Upregulation of interleukin-4 receptor by interferon-gamma: Enhanced interleukin-4-induced eotaxin-3 production in airway epithelium. *Am J Respir Cell Mol Biol* 2004;31(4):456-62.
- 27) Lee YW, Hirani AA. Role of interleukin-4 in atherosclerosis. *Arch Pharm Res* 2006;29(1):1-15.
- 28) Lee YW, Eum SY, Chen KC, Hennig B, Toborek M. Gene expression profile in interleukin-4-stimulated human vascular endothelial cells. *Mol Med* 2004;10(1-6):19-27.
- 29) Chen CC, Manning AM. TGF-beta 1, IL-10 and IL-4 differentially modulate the cytokine-induced expression of IL-6 and IL-8 in human endothelial cells. *Cytokine* 1996;8(1):58-65.
- 30) Lee YW, Kim PH, Lee WH, Hirani AA. Interleukin-4, oxidative stress, vascular inflammation and atherosclerosis. *Biomol Ther (Seoul)* 2010;18(2):135-44.
- 31) Suzuki H, Tabata T, Koizumi H, Hohchi N, Takeuchi S, Kitamura T, et al. Prediction of hearing outcomes by multiple regression analysis in patients with idiopathic sudden sensorineural hearing loss. *Ann Otol Rhinol Laryngol* 2014;123(12):821-5.
- 32) Suzuki H, Mori T, Hashida K, Shibata M, Nguyen KH, Wakasugi T, et al. Prediction model for hearing outcome in patients with idiopathic sudden sensorineural hearing loss. *Eur Arch Otorhinolaryngol* 2011;268(4):497-500.
- 33) Cvorović L, Deric D, Probst R, Hegemann S. Prognostic model for predicting hearing recovery in idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2008;29(4):464-9.
- 34) Moskowitz D, Lee KJ, Smith HW. Steroid use in idiopathic sudden sensorineural hearing loss. *Laryngoscope* 1984;94(5 Pt 1):664-6.
- 35) Mattox DE, Simmons FB. Natural history of sudden sensorineural hearing loss. *Ann Otol Rhinol Laryngol* 1977;86(4 Pt 1):463-80.
- 36) Gianoli GJ, Li JC. Transtympanic steroids for treatment of sudden hearing loss. *Otolaryngol Head Neck Surg* 2001;125(3):142-6.
- 37) Zadeh MH, Storper IS, Spitzer JB. Diagnosis and treatment of sudden-onset sensorineural hearing loss: A study of 51 patients. *Otolaryngol Head Neck Surg* 2003;128(1):92-8.
- 38) Liu SC, Kang BH, Lee JC, Lin YS, Huang KL, Liu DW, et al. Comparison of therapeutic results in sudden sensorineural hearing loss with/without additional hyperbaric oxygen therapy: A retrospective review of 465 audiotically controlled cases. *Clin Otolaryngol* 2011;36(2):121-8.
- 39) Alimoglu Y, Inci E, Edizer DT, Ozdilek A, Aslan M. Efficacy comparison of oral steroid, intratympanic steroid, hyperbaric oxygen and oral steroid + hyperbaric oxygen treatments in idiopathic sudden sensorineural hearing loss cases. *Eur Arch Otorhinolaryngol* 2011;268(12):1735-41.
- 40) Kim YH, Park KT, Choi BY, Park MH, Lee JH, Oh SH, et al. Early combination treatment with intratympanic steroid injection in severe to profound sudden sensorineural hearing loss improves speech discrimination performance. *Eur Arch Otorhinolaryngol* 2012;269(10):2173-8.