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**Transforming growth factor- $\beta$ 1 in the cerebrospinal fluid of patients with distinct neurodegenerative diseases**

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**Abstract**

A chronic inflammatory condition may underlie neurodegenerative disorders, including Parkinson's disease (PD) and Alzheimer's disease (AD). For example, both PD and AD patients show an increase in transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) levels in their cerebrospinal fluid (CSF). TGF- $\beta$ 1 is a cytokine that inhibits inflammation. In the present study, using an enzyme-linked immunosorbent assay, we tested the hypothesis that the level of TGF- $\beta$ 1 in the CSF of patients with amyotrophic lateral sclerosis (ALS), spinocerebellar degeneration (SCD), or multiple system atrophy-cerebellar subtype (MSA-C) would be elevated compared with that of normal controls. We found that TGF- $\beta$ 1 levels in the CSF were not significantly different between these patients and normal controls. Our data suggest that the level of TGF- $\beta$ 1 in the CSF is an unreliable biomarker of ALS, SCD, and MSA-C.

## 1. Introduction

Neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and spinocerebellar degeneration (SCD), are rare, adult-onset, fatal, progressive motor dysfunctions of mostly uncertain etiology. ALS is characterized by the selective and rapid degeneration of motor neurons [1]. SCD involves a variable degree of degeneration, mainly in the cerebellum, and is hereditary in some cases. Multiple system atrophy (MSA) presents clinically with progressive autonomic failure and parkinsonian, cerebellar, and pyramidal features in various combinations [2]. MSA is classified into a parkinsonian variant (MSA-P) associated with striatonigral degeneration and a cerebellar subtype (MSA-C) with olivopontocerebellar atrophy, if cerebellar features dominate. It is often difficult to make a differential diagnosis between SCD and MSA-C because both diseases exhibit predominant cerebellar symptoms [3]. Conquering these neurodegenerative diseases will require not only clarifying the pathophysiology of these diseases and determining therapeutic intervention but also identifying clinical biomarkers for diagnosis in the early stage of the diseases. Currently, however, there is no available and approved effective biomarker for the early-stage diagnosis of these diseases.

The extracellular space in the brain contains cerebrospinal fluid (CSF). Changes in the molecular constituents of the CSF are suggestive of neurological disease-related changes in the central nervous system [4]. Transforming growth factor- $\beta$  (TGF- $\beta$ ) proteins are secreted into the CSF. TGF- $\beta$ s are multifunctional cytokines that regulate the development and repair of many cell types, including the proliferation, cell death, and differentiation of neuronal cells [5]. In mammals, there are three different isoforms of TGF- $\beta$ s: TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. TGF- $\beta$ 1 is a 25-kD multifunctional polypeptide that mediates various cellular functions, such as the anti-inflammatory response, differentiation of glial cells and immune cells, and tissue repair [6–8].

Mounting evidence suggests that neurodegenerative diseases are a chronic inflammatory condition. For example, an elevated TGF- $\beta$ 1 level was found in the lumbar CSF of Alzheimer's disease patients [9]. Elevated TGF- $\beta$ 1 levels have also been reported in the ventricular CSF of patients with Parkinson's disease [10]. These findings suggest that elevated TGF- $\beta$ 1 levels in the CSF may be a biomarker for other neurodegenerative diseases, such as SCD and MSA-C. However, no study has compared the CSF-TGF- $\beta$ 1 levels between such patients and normal controls.

In the present study, we hypothesized that the TGF- $\beta$ 1 concentration in the CSF of patients with SCD or MSA-C was higher than that of normal controls. To prove this hypothesis, we performed a pilot study to measure the TGF- $\beta$ 1 levels in the CSF using an enzyme-linked immunosorbent assay.

## 2. Materials and Methods

### 2.1. Patient samples

After obtaining the local ethics committee's approval and informed consent, CSF samples were obtained from both male and female Japanese subjects (Table 1;  $n = 75$ ), which included normal individuals without a neurological disease as controls ( $n = 19$ , M/F; 8/11) and patients with ALS ( $n = 27$ , 17/10), SCD ( $n = 13$ , 9/4), and MSA-C ( $n = 16$ , 9/7). ALS and MSA-C was diagnosed according to the El Escorial Diagnostic Criteria [11] or Second consensus statement on the diagnosis of multiple system atrophy revised by Gilman et al. [3, 12], respectively. All SCD indicating the pure cerebellum symptom made gene analyses and were diagnosed as SCA6 ( $n = 4$ ), SCA31 ( $n = 3$ ), SCA3 ( $n = 1$ ), DRPLA ( $n = 1$ ) or Holms type cerebellar ataxia ( $n = 4$ ). The CSF samples were obtained using a lumbar puncture from patients after clinical suspicion of a neurological disease. Next, the CSF samples were centrifuged at 2,000 rpm for 20 min to sediment other insoluble material and cells. The supernatant was then frozen and stored at  $-80^{\circ}\text{C}$  until assayed.

### 2.2. Enzyme-linked immunosorbent assay (ELISA) analyses

To measure TGF- $\beta$ 1 concentrations in CSF samples, we first neutralized 20  $\mu\text{L}$  of each of the acidified CSF samples by adding 20  $\mu\text{L}$  of 1.2 N NaOH/0.5 M HEPES. After neutralization, the TGF- $\beta$ 1 concentrations were measured using a Quantikine ELISA Kit (SB100B; R&D Systems, Inc., MN, USA). The absorbance was measured at 450 nm (reference 570 nm) using a plate reader (Model 680; Bio-Rad Laboratories, Inc., CA, USA). We then compared the TGF- $\beta$ 1 levels in the CSF of normal subjects with those of ALS, SCD, and MSA-C patients.

### 2.3 Statistical analyses

Statistical analyses were performed using Tukey's method for multiple group comparisons using a statistical software program (JMP5.12-J, SAS Institute Inc., NC, USA). Differences with  $p < 0.05$  were considered statistically significant. This study was inspected and approved by the Clinical Studies Ethical Review Committee of the University of Tsukuba, and the approval number was H27-240.

## 3. Results

Using a different kit on an independent set of Japanese ALS patients and normal subjects, we initially attempted to replicate the results of Iłzecka et al. [13], who found no difference in the TGF- $\beta$ 1 concentration in the lumbar CSF between ALS patients and normal controls. In addition, we extended the analysis by testing the hypothesis that the CSF-TGF- $\beta$ 1 concentrations in SCD and MSA-C patients may be increased compared to those in control subjects.

Consistent with the previous report [13], the TGF- $\beta$ 1 levels in the CSF of ALS patients were not significantly higher than in the controls (Fig. 1, Table 2;  $p = 0.813$ ). Furthermore, the CSF-TGF- $\beta$ 1 concentration in the SCD and MSA-C patients did not differ markedly from control subjects (Fig. 1, Table 2;  $p = 1.000$  and  $p = 0.970$ , respectively).

#### **4. Discussion**

Consistent with the results of Iłzecka et al. [13], we did not find any marked differences in the CSF-TGF- $\beta$ 1 level between ALS patients and normal subjects. To our knowledge, our study is the first to measure the TGF- $\beta$ 1 level in the CSF of patients with SCD and MSA-C. SCD and MSA-C are neurodegenerative diseases that have been hypothesized to show an inflammatory condition. Thus, the TGF- $\beta$ 1 level in the CSF of SCD and/or MSA-C patients was expected to be higher than in normal subjects. However, we failed to detect any significant differences in the CSF-TGF- $\beta$ 1 level between these patients and normal subjects. Our results suggest that the TGF- $\beta$ 1 level in the CSF is an unreliable diagnostic marker for SCD and MSA-C.

The amount of TGF- $\beta$ 1 in the CSF is very small; therefore, careful performance of an ELISA assay is essential to obtain accurate data. As shown in Figure 1, the variation in the results for both the experimental and control groups was very small. Thus, our results are reliable.

TGF- $\beta$ 1 acts as both a growth-inhibiting and growth-promoting factor, although the former aspect is often more potent than the latter [10]. Previous studies have reported an increase in the TGF- $\beta$ 1 levels in ALS model mice [14]; however, we did not detect any marked elevation in the TGF- $\beta$ 1 levels in the CSF of ALS patients. Iłzecka et al. [13] observed a significant but modest increase in the TGF- $\beta$ 1 levels in the CSF of 1 of 4 groups of ALS patients. Taken together with those from our study, these findings suggests that the TGF- $\beta$ 1 level in some patients with ALS, SCD, or MSA-C may be elevated due to some yet unknown influence; however, the use of TGF- $\beta$ 1 as a clinical biomarker is impractical.

#### **5. Conclusion**

No significant differences in the TGF- $\beta$ 1 levels in the CSF were observed between the

control subjects and patients with ALS, SCD and MSA-C. These results indicate that measuring the CSF-TGF- $\beta$ 1 level is not practical as a biomarker of ALS, SCD, or MSA-C.

### **Conflicts of Interest/Disclosures**

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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## Figure legend

**Fig. 1.** An ELISA was used to measure the TGF- $\beta$ 1 concentration in the CSF for each diagnostic group. The mean values  $\pm$  SD are shown using horizontal magenta lines for each group.

**Table 1** Diagnosis by gender and age.

Diagnosis	Gender	Age $\pm$ SD
Normal controls	Male (n = 8)	61.3 $\pm$ 12.1
	Female (n = 11)	
ALS	Male (n = 17)	65.7 $\pm$ 11.3
	Female (n = 10)	
SCD	Male (n = 9)	62.7 $\pm$ 10.3
	Female (n = 4)	
MSA-C	Male (n = 9)	62.9 $\pm$ 5.1
	Female (n = 7)	

SD; standard division

**Table 2** Mean TGF- $\beta$ 1 levels in the CSF of ALS, SCD and MSA-C patients and normal controls.

Diagnoses	TGF- $\beta$ 1 (pg/ml)		
	Mean $\pm$ SD	Range	<i>P</i> value*
Normal controls (n = 27)	96.53 $\pm$ 24.12	51.51 - 156.65	
ALS (n = 27)	103.75 $\pm$ 33.62	54.64 - 240.75	0.813
SCD (n = 17)	95.31 $\pm$ 22.82	61.74 - 139.52	1.000
MSA-C (n = 16)	92.39 $\pm$ 21.07	61.74 - 132.55	0.970

SD; standard division, \*Tukey's test

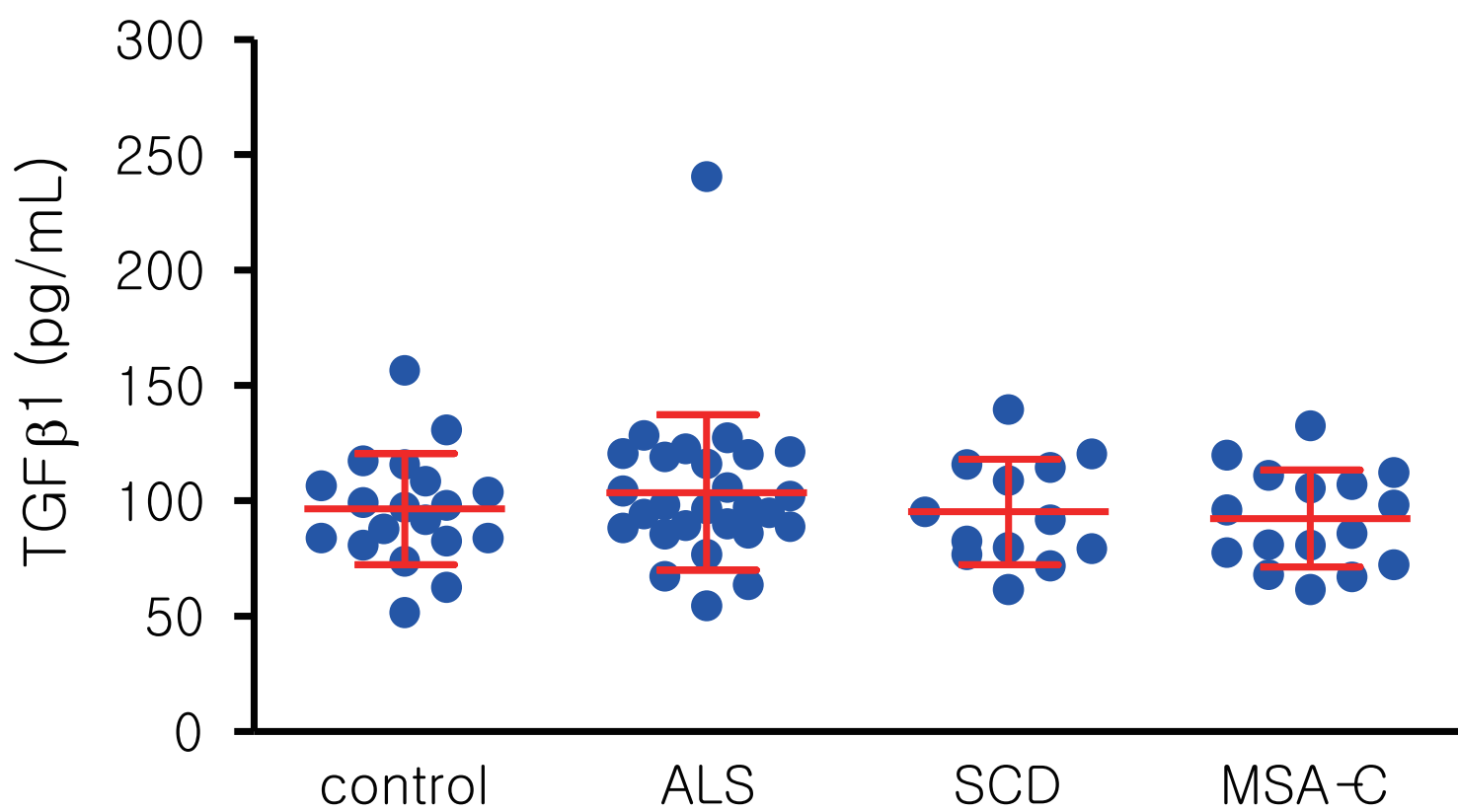


Fig. 1 Masuda et al.