Journal of the Neurological Sciences, 118 (1993) 38–42

JNS 04059

Mercury and selenium contents in amyotrophic lateral sclerosis in Hokkaido, the northernmost island of Japan

F. Moriwaka [a], H. Satoh [b], A. Ejima [b], C. Watanabe [b], K. Tashiro [a], T. Hamada [c], A. Matsumoto [d], K. Shima [e], T. Yanagihara [c], T. Fukazawa [c], H. Okumura [f], Y. Maruo [g], K. Itoh [h] and K. Yoshida [i]

[a] *Department of Neurology, Hokkaido University School of Medicine, Sapporo, Japan,* [b] *Department of Environmental Health Sciences, Tohoku University School of Medicine, Sendai, Japan,* [c] *Hokuyukai Neurological Hospital, Sapporo, Japan,* [d] *Department of Neurology, Sapporo City Hospital, Sapporo, Japan,* [e] *Department of Neurology, National Sapporo Minami Hospital, Sapporo, Japan,* [f] *Department of Neurology, Kushiro Rousai Hospital, Kushiro, Japan,* [g] *Department of Neurology, Hakodate City Hospital, Hakodate, Japan,* [h] *Department of Neurology, Bibai Rousai Hospital, Bibai, Japan, and* [i] *Division of Neurology, Otaru Second City Hospital, Otaru, Japan*

(Received 8 October, 1992)
(Revised, received 2 February, 1993)
(Accepted 15 February, 1993)

Key words Amyotrophic lateral sclerosis, Mercury content, Selenium content, Plasma; Blood cell, Scalp hair, Japanese

Summary

We evaluated the pathogenicity of mercury (Hg) and selenium (Se) which are supposed to be one of the risk factors in the development of amyotrophic lateral sclerosis (ALS). Hg and Se contents were measured in plasma, blood cells, scalp hair samples of 21 sporadic ALS patients and 36 controls, who included 19 patients with other neurological diseases, in Hokkaido, the northernmost island of Japan. Hg and Se levels in plasma and blood cells of ALS patients were significantly lower in advanced staged ALS patients than controls Low Hg and Se contents in ALS, being correlated with their disabilities and nutritional conditions, would rather reflect the disease contracted states than the pathogenic roles in ALS.

Introduction

The cause of amyotrophic lateral sclerosis (ALS) remains unknown, and several hypotheses have been proposed for its pathogenesis. Among them, heavy metals and trace elements have been widely discussed, and mercury (Hg) and selenium (Se) are supposed to be one of the risk factors in the development of ALS (Brown 1954; Kantarjian 1961; Currier et al. 1968; Adams et al. 1983; Roelofs-Iverson et al. 1984; Mano et al. 1989, 1990, Mitchell et al. 1991)

To clarify the pathogenicity of Hg and Se in ALS, we measured their contents in plasma, blood cells and scalp hair samples of sporadic ALS patients in Hokkaido, the northernmost island of Japan

Geography and background

Hokkaido, the second largest island and northernmost part of Japan, is located at latitude from 42 and 46 degrees North, with an area of 83500 km^3 and a population of about 5.7 million. This island was reclaimed about 120 years ago by immigrants from the rest of Japan, and so many mines could constitute one of the characteristics of this district. One of the biggest Hg mines in Japan, the so-called "Itomuka" mine, which produced 200 tons of high grade cinnaber per year until 1970, was located on this island, while the "Yamato" mine is in the middle of Kii Peninsula, a well known endemic area of ALS in Japan (Mano et al 1989; Moriwaka et al 1991) Hg contamination in water and fish is recognized in these areas, but Hokkaido is a non-endemic area of ALS (Mano et al. 1989; Okumura et al.1992).

Patients and methods

We examined 21 patients with ALS under our care (12 men and 9 women, mean age with standard deviation 59.6 ± 12.2 years, range 31–76 years). The clinical diagnosis was based on neurological examination by Japanese board certified neurologists, EMG showing diffuse denervation, normal nerve conduction velocity, and muscle biopsy if available (Okumura et al. 1992, Moriwaka et al. 1993) We selected sporadic ALS, having been born and living their entire lives in

Correspondence to Fumio Moriwaka, Department of Neurology, Hokkaido University School of Medicine, North 14, West 5, Kita-ku, Sapporo 060, Japan Tel 81-11-716-1161, ext 6028, Fax 81-11-757-2671

Hokkaido, and excluded familial ALS, questionable diagnosis, and patients born outside Hokkaido for this study.

For controls, 36 subjects (25 men and 11 women, mean age 61.1 ± 13.2 years, range 30–85 years) were examined. They consisted of 17 normal healthy subjects and 19 patients with other neurological diseases; 13 patients with Parkinson's disease (PD), 4 spinocerebellar degeneration (SCD), and 2 cerebrovascular disease (CVD). They were also born and living all their lives in this area.

Specimens of blood were obtained by venipuncture and centrifuged in a test tube, which had been washed in 10% nitric acid and distilled water, separating plasma and blood cells and stored at −80°C until analysis. Scalp-hairs were taken from frontal, temporal and occipital areas, cutting close to the skin at a length of 5–10 cm and stored at room temperature until measuring.

Total Hg contents were analyzed by heating decomposition gold amalgamation (MS-IS & D-2, Nippon Instruments, Tokyo) or semi-automated cold vapor atomic absorption after wet incineration (HG-4000, Pasco, Tokyo and D-2, Nippon Instruments, Tokyo) as well as standard reference materials such as NIES No. 5 (hair), IAEA MA-A 2 (fresh fish) and BCR No 185 (bovine liver). Se contents were measured by a fluorometric method (821-FP, Jasco, Tokyo) (Suzuki et al. 1980; Moriwaka et al. 1991; Satoh, 1992). The sensitivity in our assay was 0.1 ng Hg and 3.95 ng Se.

To evaluate the clinical profiles, the disability of daily activities was graded on an ordinal scale with four categories: stage 1 is normal daily activity without any assistance, stage 2 is slightly handicapped with occasional assistance, stage 3 is a moderately disabled state becoming wheel-chair bound, but having regular diets by themselves or by assistance, and stage 4 is bed-ridden with or without taking liquid diets by nasogastric tube feeding and ventilator support. The birth place, current residencies, dietary lives, occupational history, and exposure to chemicals and herbicides were also inquired. For statistical analysis, the Student *t*-test and Spearman's statistic were used

Results

(1) Clinical findings and risk factors

Eight out of 21 ALS patients and 26 of 36 controls were living in Sapporo, the capital city of Hokkaido, while the others were distributed in different parts of this island.

On interviews concerning Hg exposure, 6 out of 21 ALS (28 6%) and 5 of 36 controls (13.9%) were presumed to be exposed.

For dietary life, most of ALS and controls preferred to have sea food (boiled or baked fishes and also raw fish, so-called "sashimi" including tuna, squid and others) as Japanese traditional meals, and their dietary attitudes were similar except severely disabled ALS patients who were restricted to liquid diets.

Duration of illness in ALS patients ranged from 2 months to 9 years (mean 52.5 ± 48.8 months), which was not relevant to Hg or Se levels.

As for disability in ALS patients, 2 patients were in stage 1, 4 in stage 2, another 6 stage 3, and 9 patients stage 4. Seven of 9 patients, being stage 4 on a ventilator, had been taking liquid diets through nasogastric tubes and the mean duration of tube feeding was 46 0 ± 31.7 months (range; 2 months to 8 years)

In controls, most of PD were in stage 2 (consistent with Hoehn and Yahr's stage 2 and 3 except one patient with stage 4), 3 patients with SCD were wheelchair-bound and the others were ambulatory.

(2) Hg contents

Significant low Hg contents were seen in ALS patients, i.e 1.33 ± 0.85 ng/ml (mean ± SD) in plasma ($P = 0.0056$), 13.56 ± 11.08 ng/g in blood cells ($P = 0.0033$) compared to that of controls (2.11 ± 1.07 ng/ml and 24.69 ± 13.39 ng/g, respectively) (Table 1). Hg contents in scalp hair of ALS were lower than in controls, but were not statistically significant. Decreased Hg levels in ALS patients were related with their disabilities. Advanced ALS patients in stage 4 showed significantly reduced Hg concentrations in comparison with not only controls but also milder disabled ALS patients in blood cells, but not in plasma

TABLE 1

MERCURY CONTENTS

n = number of cases Values are presented as mean ± SD Other indicates other neurological diseases and *P* values are estimated in comparison to controls. * $P = 0\ 0056$, ** $P = 0\ 0033$, *** $P = 0\ 0023$, **** $P = 0\ 0004$

	n	Plasma Hg (ng/ml)	*n*	Blood cell Hg (ng/g)	*n*	Scalp hair Hg (ppm)
Control	36	2 11 ± 1 07	35	24.69 ± 13 39	20	2 50 ± 1.29
ALS	21	1 33 ± 0 85 *	21	13 56 ± 11 08 ***	11	2.38 ± 1 34
ALS (1–3)	13	1 52 ± 0.92	12	18 60 ± 11 27	7	2.90 ± 0 82
ALS (4)	9	0 95 ± 0.63 **	9	6 85 ± 6 61 ****	4	1 45 ± 1 66
Other	19	1 63 ± 0.78	18	18 90 ± 9 20	19	2 40 ± 1 25

TABLE 2

SELENIUM CONTENTS

* $P = 0.0001$, ** $P^* < 0.05$, *** $P = 0.0015$

	n	Plasma Se (ng/g)	n	Blood cell Se (ng/g)	n	Scalp hair Se (ppm)
Control	35	120 61 ± 20 55	35	191 55 ± 35 88		
ALS	21	81 20 ± 46 42 *	20	134 63 ± 73 36 ***	2	0 51 ± 0 11
ALS (1–3)	11	102 75 ± 36 00 **	11	176 03 ± 58 03	1	0 43
ALS (4)	9	50 50 ± 43 24 *	9	84 02 ± 57 73 *	1	0 59
Other	18	120 44 ± 26 55	18	187 05 ± 22 37	8	0 59 ± 0.10

(Table 1) Patients with other neurological diseases did not differ significantly from controls.

(3) Se contents

Se contents were also significantly decreased in ALS patients, i.e. 81 20 ± 46.42 ng/g in plasma ($P = 0.0001$), 134 63 ± 73.36 ng/g in blood cells ($P = 0.0015$) in comparison with 120 61 ± 20 55 ng/g and 191 55 ± 35 88 ng/g in controls, respectively (Table 2), while Se levels of hair samples could not be evaluated because of too few available samples

Decreased Se levels in ALS were also related with their disabilities. ALS patients in stage 4 revealed significantly reduced Se concentrations, being different from controls and mildly disabled ALS in both plasma and blood cells. Se contents in patients with other neurological diseases were similar to controls.

TABLE 3

RATIO OF MERCURY TO SELENIUM

* $P < 0.05$

	n	Plasma (ng/ml)	n	Blood cells	n	Scalp hair
Control	35	0.018 ± 0 001	35	0 133 ± 0 083		
ALS	21	0 043 ± 0 119	20	0 091 ± 0 066	3	4 338 ± 1 530
ALS (1–3)	12	0 018 ± 0 010	11	0 099 ± 0 055		
ALS (4)	9	0 080 ± 0 181 *	9	0.082 ± 0 079		
Other	18	0 015 ± 0 007	18	0 099 ± 0 040	7	3 221 ± 1 334

TABLE 4

REPORTS OF MERCURY AND SELENIUM CHANGES IN ALS

I significant increase, D significant decrease, NS not significant, (+) detectable NS * not significant, but increase in one case, NS ** not significant, but increase in 2 cases, I(M) increase in men, D(W) decrease in women D(?) tendency of decrease

Author (year)	Mercury					Selenium					
	Scalp hair	Plasma	Blood cells	Spinal cord	Urine	Scalp hair	Plasma	Blood cells	CSF	Spinal cord	Urine
Kantarjian (1961)	(+)										
Currier (1968)					I						
Shirakawa (1976)	NS										
Kilness (1977)											I
Norris (1978)											D
Mitchell (1984)									NS		
Takasu (1985)	I										
Nagata (1985)								I			
Mitchell (1986)										I	
Katsui (1987)								I			
Mano (1989)	I										
Mano (1990)	I					D					
Oishi (1990)	NS *					NS **					
Khare (1990)		I(M)	I	D(?)			D	D(W)		D(?)	
Mitchell (1991)										I	
Our study	D(?)	D	D				D	D			

(4) Ratio of Hg to Se

The ratio of Hg to Se of plasma was significantly higher in ALS stage 4 (Table 3).

Discussion

To clarify the pathogenic relation of Hg and Se to ALS, several tissue samples of ALS patients have been assayed, but the results are conflicting (Kantarjian 1961, Currier et al. 1968; Shirakawa et al. 1976; Kilness et al 1977; Norris et al 1978; Mitchell et al. 1984, 1986, 1991; Nagata et al. 1985; Mano et al 1989, 1990; Oishi et al. 1990; Khare et al. 1990) (Table 4).

Recently, Mano and his group reported high Hg and low Se contents in scalp hair of ALS in Kii Peninsula, a well-established endemic area of ALS in Japan (Mano et al. 1989, 1990). In Kii Peninsula, Hg contamination in water and fish was estimated to be as high as 0.008 mg/l which exceeds the Japanese environmental guidelines of 0.0005 mg/l (Mano et al. 1989).

We aimed to investigate the pathogenicity of Hg and Se in sporadic ALS in a non-endemic area, Hokkaido, where Hg contamination in water was estimated at 0.0015 mg/l.

Our results showed that Hg and Se contents in blood samples of ALS were significantly reduced. Decreased Hg and Se levels were more evident in severely disabled ALS patients. The differences between advanced ALS patients and those who were mildly or moderately disabled ALS and controls could be focused to their diets. The food of advanced ALS patients was restricted to liquid diets, consisting of powdered milk products, caseins and supplemental vitamins. In Japan, considering overall consumption of foods, the most contributing foods are supposed to be fish for the intake of Hg, and cereals, especially rice, for Se (Suzuki et al 1980) Hg and Se contents in liquid diets were below our detectable limits, although the average daily intake of Hg and Se in Japanese is estimated at 2–10 μg/day and 80–200 μg/day, respectively. We could assume that reduced Hg and Se levels in ALS might reflect their nutritional conditions taking mainly liquid diets rather than direct pathogenic roles of Hg and Se in ALS.

To support this assumption, our clinical survey of ex-mercury miners, who were poisoned by or exposed to Hg vapor about 18 years ago at the Itomuka mine, failed to disclose any pathogenicity of Hg in ALS in this cohort of 148 ex-miners (Moriwaka et al. 1991). Furthermore, the epidemiological survey of motor neuron disease in Hokkaido revealed a standard incidence rate of ALS in the neighborhood around Itomuka mine (Okumura et al. 1992)

In conclusion, we could not show any pathogenicity of Hg and Se in the development of ALS in Hokkaido, although our study does not fully rule out their pathogenic roles because the biologic onset of ALS may antedate the clinical onset by several years and the small number of early ALS patients in this study.

Acknowledgments We thank Drs S. Doi (Department of Neurology, National Sapporo Minami Hospital) and K Isonishi (Department of Neurology, Kashiwaba Neurosurgical Hospital) for their cooperation Part of this study was supported by a fund from the Research Committee of CNS degenerative diseases, the Ministry of Health and Welfare of Japan

References

Adams, C.R , D K. Ziegler and J T. Lin (1983) Mercury intoxication simulating amyotrophic lateral sclerosis JAMA, 250 642–643

Brown, I A (1954) Chronic mercurialism A cause of the clinical syndrome of amyotrophic lateral sclerosis Arch Neurol Psychiat., 72 674–681

Currier, R D and A F Haerer (1968) Amyotrophic lateral sclerosis and metallic toxins Arch Environ Health, 17 712–719

Kantarjian, A D (1961) A syndrome clinically resembling amyotrophic lateral sclerosis following chronic mercurialism Neurology, 11 639–644

Katsui, Y , H Nagata, S Miyata, S Nakamura and M Kameyama (1987) Heavy metal concentrations in blood cells of patients with amyotrophic lateral sclerosis – Study of five cases in Mie Clin Neurol (Tokyo), 27. 19–22

Khare, S S , W D. Ehmann, E J Kasarskis, and W.R Markesbery (1990) Trace element imbalances in amyotrophic lateral sclerosis. NeuroToxicology, 11 521–532

Kilness, A W and F H Hochberg (1977) Amyotrophic lateral sclerosis in a high selenium environment JAMA, 237 2843–2844

Mano, Y , T. Takayanagi, A Ishitani and T Hirota (1989) Mercury in hair of patients with ALS Clin Neurol. (Tokyo), 29 844–848

Mano, Y., T Takayanagi, T Abe, and Y Takizawa (1990) Amyotrophic lateral sclerosis and mercury – A preliminary report. Clin Neurol (Tokyo), 30 1275–1277

Mitchell, J.D , I A Harris, B W East and B Pentland (1984) Trace elements in cerebrospinal fluid in motor neurone disease Br. Med. J , 288 1791–1792

Mitchell, J D , B W East, I.A Harris, R J Prescott, and B Pentland (1986) Trace elements in the spinal cord and other tissues in motor neuron disease J Neurol Neurosurg Psychiat , 49 211–215

Mitchell, J D , B W East, I A Harris and B. Pentland (1991) Manganese, selenium and other trace elements in spinal cord, liver and bone in motor neurone disease Eur Neurol , 31 7–11.

Moriwaka, F , K Tashiro, R Doi, H Satoh and Y Fukuchi (1991) A clinical evaluation of the inorganic mercurialism – Its pathogenic relation to amyotrophic lateral sclerosis. Clin Neurol (Tokyo), 31 885–887

Moriwaka, F , H Okumura, K Tashiro, T Hamada, A Matsumoto, H Matsumoto, N Itoh, R. Shindo, N Takahata and ALS study group (1993) Motor neuron disease and poliomyelitis – Geographic study in Hokkaido, the northern-most island of Japan J Neurol , 240. 13–16

Nagata, H , S Miyata, S Nakamura N Kameyama and Y Katsui (1985) Heavy metal concentrations in blood cells in patients with amyotrophic lateral sclerosis J Neurol Sci , 67 173–178

Norris, F H and K S U (1978) Amyotrophic lateral sclerosis and low urinary selenium levels JAMA, 239· 404.

Oishi, M , T Takasu and M Tateno (1990) Hair trace elements in amyotrophic lateral sclerosis Trace Elements Med , 7 182–185

Okumura, H., F Moriwaka, K Tashiro, T Hamada, A Matsumoto, H. Matsumoto, N Itoh, R Shindo, N Takahata and ALS study group (1992) Epidemiological study of motor neuron disease in Hokkaido Island Its incidence, prevalence and regional distributions Brain Nerve (Tokyo), 44 727–732

Roelofs-Iverson, R A., D W Mulder, L R Elveback, L T Kurland and C.A Molgaard (1984) ALS and heavy metals a pilot case-control study Neurology, 34 393–395

Satoh, H (1992) From the experience of mercury analyses of biological materials. Biomed Res Trace Elements 3 61–62

Shirakawa, K, T Yuasa, K Hirota, T Tsubaki and M Hoshi (1976) A case of methylmercury poisoning with onset of a clinical syndrome resembling amyotrophic lateral sclerosis Neurol Med (Tokyo), 4 58–62

Suzuki, T, H Satoh, R Yamamoto and H. Kashiwazaki (1980) Selenium and mercury in foodstuff from a locality with elevated intake of methylmercury Bull Environm Contamin Toxicol, 24 805–811.

Takasu, T, M Oishi and M Tsuchiya (1986) Measurement of hair trace metals in amyotrophic lateral sclerosis and the other degenerative diseases In Nakanishi, T (Chairman), Annual report of the Research Committee of CNS Degenerative Diseases, the Ministry of Health and Welfare of Japan, pp 41–46