Bull. Environ. Contam. Toxicol. (1994) 52:109–116

Environmental Contamination and Toxicology

Association of Metal Concentrations in Drinking Water with the Incidence of Motor Neuron Disease in a Focus on the Kii Peninsula of Japan

Okujou Iwami,[1] Chan-Seok Moon,[1] Takao Watanabe,[2] and Masayuki Ikeda[1]

[1]Department of Public Health, Kyoto University Faculty of Medicine, Kyoto 606-01, Japan and [2]Miyagi University of Education, Sendai 980, Japan

Three high incidence foci of motor neuron disease (MND) have been reported in the western Pacific area, i.e., the Kii Peninsula in Japan (Hohara and Kozagawa), Guam, and West New Guinea (Yase 1972, Reed & Brody 1975, Gajdusek & Salazar 1982). The etiology of MND in the foci is yet to be established, but it is suggested that environmental factors play an important role in its high incidence in the foci (Yase 1972, Reed & Brody 1975, Gajdusek & Salazar 1982).

The mineral deficiency hypothesis was first suggested by Kimura (1965). One theory postulates that low calcium, low magnesium, and high aluminum in the water and soil are MND-inducing factors in the foci (Yase 1980, Gajdusek & Salazar 1982). However, the studies conducted up to the present have often been deficient in suitable controls to show that the above metal concentrations are related to the incidence of MND in the three foci.

The present study was initiated to determine whether the concentrations of 8 metals in drinking water are related to the incidence of MND, by comparing the water in the Hohara area with the water in the neighboring areas. In the neighboring areas of Hohara in Nansei–cho, the incidence of MND is not remarkable (*Iwami et al., unpublished data*). By considering the neighboring areas as controls, the elucidation of how the regional environment affects the incidence of MND was expected. The results of this study suggest that the low concentration of magnesium in the drinking water is one of the risk factors associated with MND in this focus.

MATERIALS AND METHODS

Nansei–cho (109 km^2 in area) in the Mie prefecture is located on the Kii peninsula of Japan, facing the western Pacific Ocean (Fig.1), and is divided

---

Reprint requests to M. Ikeda at the above address.

into 19 regions (Table 1). Hohara is one of 5 administrative areas and consists of 5 regions. Since 1975, the Nansei Public Office has been supplying disinfected water to the inhabitants (supplied water). Even after the beginning of the supplied water, many private or community water sources (wells, streams, and springs) have been used for drinking and non-drinking purposes.

Water samples were obtained from water sources (which were used for drinking currently or previously) throughout Nansei-cho by visiting some 130 home and community sources accompanied by a public health officer in the spring of 1991. The water sources not in use were excluded. Thus, 117 samples were obtained (Table 1). Samples of 100 ml were collected in polypropylene bottles directly from the tap in homes and from pump outlets of community water sources after rinsing of bottles with sample water. The water samples were frozen at −20°C until analysis. Ten ml of sample were digested with 1.0 ml concentrated nitric acid (pollutant analysis grade; Wako Pure Chemicals, Osaka, Japan) in Teflon-PFA 50 ml tubes (Iuchi & Co., Osaka, Japan) according to EPA (1979). After digestion, 0.1 ml of concentrated nitric acid was added to each sample. Samples were then diluted with ion exchanged and Q-PAK (Japan Millipore Limited, Tokyo, Japan)-filtrated water to 10 ml, and subsequently stored in polypropylene tubes. Calcium and magnesium concentrations were determined with inductively coupled plasma-emission spectrometry (ICP) (Seiko SPS 1100). Iron, aluminum, manganese, lead, cadmium, and chromium concentrations were determined with flameless atomic absorption spectrophotometer (AAS) (Hitachi Z8100). The standard solutions of the metals were obtained from Wako Pure Chemicals, Osaka, Japan. Standard addition method was used for the determination of concentrations by AAS, and the measurement was repeated when the r-value of the regression was less than 0.99. The concentrations of metals were determined after the substraction of the concentration in reagent blank samples. All bottles and tubes were washed with metal free detergent (Wako Pure Chemicals, Osaka, Japan) and then presoaked in 1.5 *N* nitric acid solution for 24 hrs and finally rinsed repeatedly with ion exchanged water. The incidence of MND in the areas for 1961−1990 was surveyed by death certificates (*Iwami et al., unpublished data*). The crude annual death rates of MND were calculated with the population data from 1975, which were obtained from Nansei Public Office. The neighboring regions with populations of less than 200 were combined to form one region for the evaluation (Table 1).

For statistical analysis, calcium and magnesium concentrations were assumed to be normally distributed. However, the concentrations in Kirihara were characterized by a log-normal distribution. For this reason, calcium and

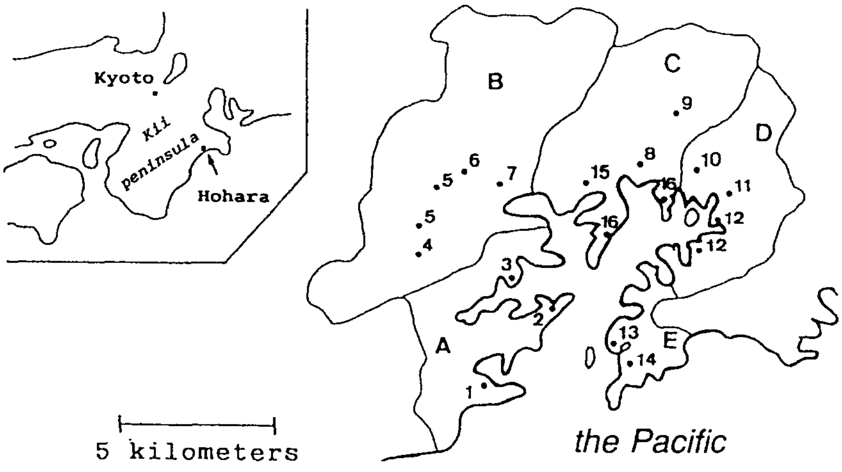

Figure 1. Location of Hohara in Japan, and the study areas in Nansei–cho. Nansei–cho is divided into five administrative areas: A) Nankai, B) Hohara, C) Gokasho, D) Kamihara, and E) Shukutaso. The numbers on the map are region code numbers provided in Table 1.

Table 1. The study areas, the crude annual death rates due to MND per 100,000 population for 1961–1990, and the numbers of the sampled water sources.

Region Code	Area	Region	Population in 1975	MND Deaths	Rate	Numbers of sources(c:p)*
1	Nankai	Ohkaura	1530	0	0.0	4(2:2)
2	*ibid.*	Sazaraura	1031	0	0.0	2(2:0)
3	*ibid.*	Hasamaura	1003	0	0.0	4(0:4)
4	Hohara	Oshibuchi	253	6	79.1	10(0:10)
5	*ibid.*	Saita & Hajikami	353	4	37.8	24(0:24)
6	*ibid.*	Iseji	685	14	68.1	35(0:35)
7	*ibid.*	Naize	526	3	19.0	8(0:8)
8	Gokasho	Gokashoura	2285	2	2.9	2(2:0)
9	*ibid.*	Kirihara	785	0	0.0	13(0:13)
10	Kamihara	Izumi	256	0	0.0	4(0:4)
11	*ibid.*	Konsa	357	0	0.0	3(0:3)
12	*ibid.*	Shimotsuura & Kitani	418	0	0.0	2(1:1)
13	Shukutaso	Shukuura	1794	0	0.0	2(2:0)
14	*ibid.*	Tasoura	2169	0	0.0	4(3:1)
15	Gokasho	Hunakoshi	780	0	0.0	No sample
16	*ibid.*	Nakatsuhama & Hanma	416	1	8.0	No sample

* c: Community water sources or private water sources which were used commonly by neighbors. p: Private water sources

magnesium concentrations were also treated with an assumption of log-normal distribution. Other metal concentrations were assumed to follow log-normal distribution. For analysis of the correlation between the incidence of MND and the metal concentrations, the arithmetic means (AM) and geometric means (GM) of metal concentrations were evaluated after logarithmic conversion. Multiple regression was applied by both step-up and step-down procedures with a p-value of 0.10.

RESULTS AND DISCUSSION

The incidence of MND focused in Hohara in Nansei-cho (Table 1).
The GMs of lead, cadmium, and chromium concentrations were lower than the lowest limit of determination (0.4 μg/l, 0.03 μg/l, and 0.2 μg/l, respectively). The other metal concentrations and the lowest limits of determination are shown in Table 2. When evaluation was made by study regions, the mean magnesium concentrations by regions in Hohara ranged from 0.93 mg/l to 1.67 mg/l in terms of AM, or from 0.90 mg/l to 1.62 mg/l in terms of GM. In other areas, only one region in Kamihara (No.11) had magnesium concentrations as low as in Hohara. When evaluated on a individual basis, 65 out of 77 samples (84%) had magnesium concentrations of less than 1.70 mg/l in Hohara, whereas only 3 (one each in Kirihara, Konsa, and Kitani) out of 40 samples (7.5%) in other areas (difference in distribution, $p < 10^{-10}$ by χ^2-test) were that low. The magnesium concentration was significantly ($p < 10^{-10}$) lower in Hohara than in the other areas (Table 3). Correlation between the crude annual death rate of MND and the magnesium concentration in the 14 regions is significant ($r = -0.82$, $p = 0.00035$ when evaluated by AM values, and $r = -0.78$, $p = 0.00096$ when evaluated by GM values) (Table 2), which is shown in Figure 2. Although the calcium concentration in Hohara was lower than in the other areas as a whole ($p = 0.00023$) (Table 3), the calcium concentrations do not significantly ($p > 0.05$) correlate with the rate of MND (Table 2). The concentrations of iron, aluminum, and manganese do not significantly ($p > 0.05$) correlate with the rate of MND (Table 2), and these 3 metals in Hohara do not differ from those in the other areas ($p > 0.05$) (Table 3).
Multiple regression analysis with the 5 metal (magnesium, calcium, iron, aluminum, and manganese) concentrations in the 14 regions by step-up and step-down procedures with p=0.10 resulted in the same equation as when simple regression analysis with magnesium was applied, as follows:
When AMs of magnesium and calcium, and GMs of iron, aluminum, and manganese are used;

$Y = 53.5 - 49.1\ X$ ($r = -0.82$, $p = 0.00035$)

where Y: Crude annual death rate of MND per 100,000 population, and X: Ln [Magnesium (mg/l)]

Table 2. Metal concentrations in drinking water and the correlations between the crude annual death rates of motor neuron disease per 100,000 population and the metal concentrations after logarithmic conversion.

Area	Region Code	Magnesium mg/l AM ±ASD[a]	Magnesium mg/l GM(GSD)[b]	Calcium mg/l AM ±ASD	Calcium mg/l GM(GSD)	Iron μg/l GM(GSD)	Aluminum μg/l GM(GSD)	Manganese μg/l GM(GSD)
Nankai	1	2.55 ±0.51	2.51(1.23)	3.75 ±0.46	3.72(1.13)	15(3.5)	29(2.2)	1.3(2.3)
ibid.	2	4.33 ±1.14	4.25(1.31)	3.90 ±0.45	3.89(1.12)	25(1.0)	31(3.9)	1.5(3.6)
ibid.	3	3.38 ±0.66	3.33(1.24)	5.07 ±2.46	4.60(1.69)	18(2.2)	87(2.6)	11.7(3.9)
Hohara	4	0.93 ±0.30	0.90(1.35)	3.90 ±1.43	3.66(1.47)	16(3.8)	41(3.1)	1.0(4.3)
ibid.	5	1.18 ±0.35	1.14(1.27)	4.27 ±1.35	3.99(1.52)	36(4.2)	31(4.8)	1.3(4.6)
ibid.	6	1.31 ±0.39	1.25(1.36)	4.52 ±1.99	3.91(1.86)	31(4.0)	18(6.4)	2.3(5.0)
ibid.	7	1.67 ±0.39	1.62(1.33)	7.68 ±1.49	7.55(1.22)	14(3.9)	19(3.9)	0.5(2.8)
Gokasho	8	2.77 ±1.33	2.60(1.65)	5.69 ±0.64	5.67(1.12)	15(1.9)	13(2.4)	0.8(4.7)
ibid.	9	2.75 ±1.27	2.53(1.51)	10.48 ±5.97	8.98(1.80)	21(3.4)	38(2.8)	1.4(3.2)
Kamihara	10	3.29 ±0.56	3.26(1.17)	8.64 ±1.97	8.48(1.24)	16(2.4)	26(2.5)	0.6(2.6)
ibid.	11	1.60 ±0.93	1.34(2.22)	3.54 ±2.66	2.69(2.69)	20(2.5)	66(1.5)	0.8(2.7)
ibid.	12	2.44 ±1.83	2.07(2.31)	4.57 ±2.70	4.15(1.88)	19(1.1)	89(1.2)	0.6(1.0)
Shukutaso	13	2.15 ±0.29	2.14(1.14)	10.06 ±1.12	10.03(1.12)	60(1.9)	74(1.8)	0.4(1.9)
ibid.	14	3.28 ±1.12	3.16(1.35)	11.44 ±7.23	10.03(1.77)	154(10)	22(5.1)	5.3(2.5)
Correlation	r	−0.82	−0.78	−0.35	−0.35	−0.08	−0.24	0.02
	p−value	0.00035	0.00096	0.22	0.22	0.78	0.41	0.95

The lowest limit of determination was 0.01 mg/l for magnesium, 0.02 mg/l for calcium, 1 μg/l for iron, 1 μg/l for aluminum, 0.3 μg/l for manganese. Geometric means for lead, cadmium, and chromium were less than the lowest limit of determination (0.4 μg/l, 0.03 μg/l, and 0.2 μg/l, respectively). [a] Arithmetic mean ±arithmetic standard deviation. [b] Geometric mean (geometric standard deviation).

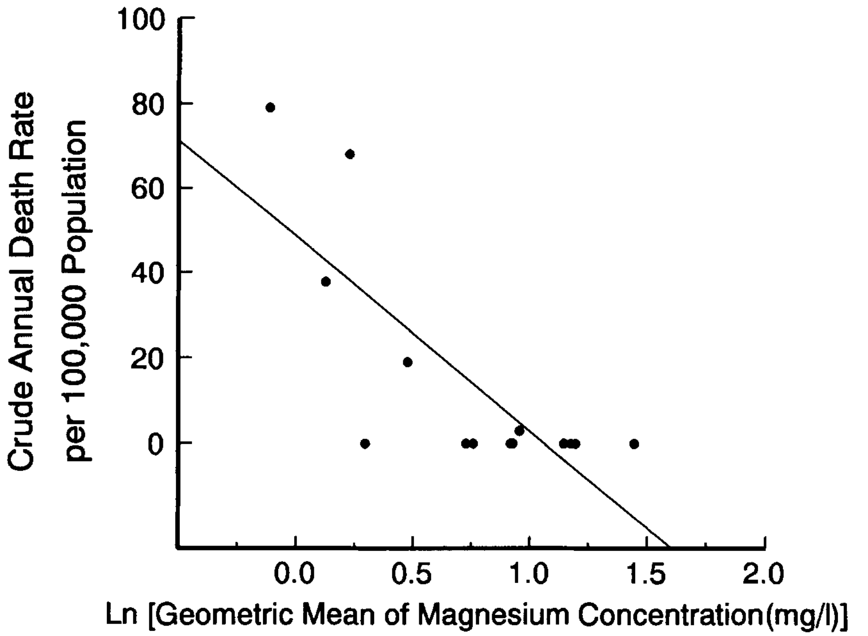

Figure 2. Correlation between the incidence of motor neuron disease and the magnesium concentration in drinking water with the data from 14 regions in Nansei-cho. The line in the figure is a calculated regression of :
$Y = 48.4 - 45.8 X$ ($n = 14$, $r = -0.78$, $p = 0.00096$), where X is ln [geometric mean of magnesium concentration (mg/l)], and Y is crude annual death rate of motor neuron disease per 100,000 population.

Table 3. The comparison of the metal concentrations in the drinking water between Hohara and the other areas on an individual basis.

	Magnesium mg/l	Calcium mg/l	Iron μg/l	Aluminum μg/l	Manganese μg/l
Hohara	1.20(1.37)	4.18(1.71)	27(4.1)	24(5.2)	1.5(4.8)
Other areas	2.62(1.55)	6.40(1.91)	25(3.6)	39(2.8)	1.5(3.8)
p–value	$<10^{-10}$	0.00023	NS*	NS	NS

Notes: The values in the table are geometric means (geometric standard deviations). Two–sided *t*–test employed when p–value of F–test is over 0.05, and Aspin–Welch's test when otherwise. Sample numbers were 77 in Hohara and 40 in the other areas. * $p > 0.05$

When GMs of the 5 metals are used;
$Y = 48.4 - 45.8\ X$ ($r = -0.78$, $p = 0.00096$)
The parameters of calcium, iron, aluminum, and manganese are not adopted into the multiple regression equation with $p = 0.10$.

The present study was initiated to compare metal concentrations in drinking water between a focus of MND and neighboring areas. The GM and AM of magnesium concentrations in the drinking water were below 1.70 mg/l in Hohara, and the magnesium concentration in Hohara was significantly lower than that in the other areas as a whole ($p < 10^{-10}$). The statistical analysis clearly demonstrated significant correlation ($p < 0.001$) between the incidence of MND and the magnesium concentration in the drinking water. The results suggest that the low magnesium concentration in the drinking water should be considered as one of the important risk factors of MND in this focus, or more conservatively, the low magnesium concentration in the drinking water can be related to the risk factors of MND. Although the calcium concentration in Hohara was lower than that in the other areas ($p = 0.00023$), the correlation with the rate of MND was not significant ($p > 0.05$). Thereby, the role of the low calcium concentration is less conclusive at the moment and may need further investigation.

In other foci of western Pacific areas, magnesium concentrations in drinking water were reported as : 9.20 ±5.76 mg/l [as AM ±ASD] and 6.99 mg/l (2.53) [as GM(GSD)] in Guam; 0.68 ±0.47 mg/l [as AM ±ASD] and 0.52 mg/l (2.24) [as GM(GSD)] in West New Guinea by the ICP method [calculated from Garruto et al. (1984), after Piga spring data were excluded according to Heitz et al. (1988)]; and 0.34 ±0.13 mg/l [as AM ±ASD] and 0.32 mg/l (1.52) [as GM(GSD)] in Kozagawa by titration method [calculated from Yase (1972)]. The data in West New Guinea and Kozagawa support the conclusion of the present study that the low magnesium concentration in the drinking water can be epidemiologically considered as one of the important risk factors of MND in the western Pacific area. However, the absolute values of magnesium in Guam do not support this conclusion. The etiology of MND in Guam might be different from another foci. Or otherwise, the ratio of magnesium concentration in the drinking water to the burden of metals such as aluminum might be related to the incidence of MND, because the elutable aluminum in soil is 42-fold higher in Guam than in control areas (Crapper MacLachlan et al. 1989). A study of dietary intake in Hohara is now in progress in our study group.

Acknowledgments. We are grateful to Mr. Toshiki Hamaguchi.

REFERENCES

Crapper McLachlan DR, McLachlan CD, Krishnan B, Krishnan SS, Dalton AJ, Steele JC (1989) Aluminium and calcium in soil and food from Guam, Palau and Jamaica: implications for amyotrophic lateral sclerosis and parkinsonism-dementia syndromes of Guam. Environ Geochem Health 11:45-53

EPA (1979) Methods of chemical analysis of water and waste. EPA 600-4-79-020 Environmental monitoring and support laboratory, USEPA, Cincinnati

Gajdusek DC, Salazar AM (1982) Amyotrophic lateral sclerosis and parkinsonian syndromes in high incidence among the Auyu and Jakai people of West New Guinea. Neurology 32:107-126

Garruto RM, Yanagihara R, Gajdusek DC, Arion DM (1984) Concentration of heavy metals and essential minerals in garden soil and drinking water in western Pacific. In:Chen KM, Yase Y(eds) Amyotrophic lateral sclerosis in Asia and Oceania. National Taiwan University, Taiwan, p 265-329

Heitz L, Zolan W, Ellis-Neill L, Wood R, Kilmer F, Randel R, Workman A, Steele JC (1988) Guam's Piga spring: A source of confusion. ALS/parkinsonism-dementia complex. Neurology 38(suppl 1):310

Kimura K (1965) Studies of amyotrophic lateral sclerosis in the Kozagawa district of the Kii peninsula, Japan. Wakayama Medical Rep 9:177-192

Reed DM, Brody JA (1975) Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam,1945-1972. I. Descriptive epidemiology. Am J Epidemiol 101:287-301

Yase Y (1972) The pathogenesis of amyotrophic lateral sclerosis. Lancet II 292-296

Yase Y (1980) The role of aluminum in CNS degeneration with interaction of calcium. Neurotoxicology 1:101-109

Received March 1, 1993; accepted June 19, 1993.