ORIGINAL ARTICLES

Plasma and CSF Aluminium in Sporadic Motor Neuron Disease (MND)

MA HAYEE[a], M NUSRATULLAH [b]

Summary:

Study of aluminium in plasma and cerebrospinal fluid in motor neuron disease (MND) was carried out in the Neurology department of Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh. The duration of study was from January 2002 to June 2004. Seventy MND subjects between 16 to 65 years were selected. Among them 42 were male and 28 female. Same number of controls matched for age, sex, occupation and habitation (rural or urban) were also selected. Plasma and CSF were collected from the subjects and controls. Aluminium was estimated both from plasma and CSF. Cerebrospinal fluid aluminium level (18.09 ± 2.02 μg/dl) was significantly higher (p<0.001) in subjects as compared to the controls (12.22 ± 2.42 μg/dl). Plasma aluminium level of subjects and controls did not show any significant difference. Aluminium level in cerebrospinal fluid varied in different subtypes of MND and controls. The p-values were <0.01, <0.02 and <0.001 respectively in amyotrophic lateral sclerosis, progressive muscular atrophy and progressive bulbar palsy when compared with controls.

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Introduction:

Motor neuron disease is used to designate a progressive degenerative disorder of motor neurons in the spinal cord, brain stem and motor cortex, and manifest clinically by muscular weakness, atrophy and corticospinal tract signs in varying combinations[1].

The disease predominantly affects middle aged and elderly people and mean age of onset is 55 years, although middle-aged people are occasionally affected. There are three types of MND: (I) sporadic-which is the commonest form(90%), (II) familial, a small number of cases usually with an autosomal dominant mode of inheritance[2] and (III) Guamanian, a high number of cases of MND found in Guam and the Trusrt Territories of the Pacific.

The aetiology and pathogenesis of sporadic MND are not known. Numerous hypotheses about the pathogenesis/aetiology of MND have been proposed including environmental hypothesis, free radical hypothesis, immunological hypothesis, neurotropic factor deficiency hypothesis, altered neurofilament metabolism hypothesis, excitatory amino acid toxicity hypothesis, deficiency or toxicity of trace element hypothesis. Trace elements neurotoxicity has been implicated in the pathogenesis of MND. Several trace elements have been reported to be increased in the spinal cord of MND patients including lead[3], copper[4], iron[4], manganese[5] and selenium[5]. Epidemiological studies show that long term exposure to toxic trace elements are present in some MND patients[6-8]. Possible involvement of aluminium, calcium and manganese in ALS/Parkinsonism/dementia complex of Guam[9-11] and reports of MND-like syndromes in chronic intoxication with mercury[12] and lead also support the hypothesis[13].

Recently, many researchers found relationship of aluminium to sporadic MND. Aluminium is a widely dispersed metal being found in igneous rocks, shales, clays and moist soils. Aluminium is absorbed by many plants and occurs in plant products in diet. The daily ingestion of Al by humans is estimated to be 30 to 50 mg[14]. The general population is also exposed to Al from its widespread use in water treatment, as a food additive, from various Al-based pharmaceuticals, from occupational dusts, and from Al containers and cooking utensils[15]. The leaching of Al from the soils by acid rain increases free Al in the environment and in the surface waters. The increase exposure of the general population to Al has become

a. Dr. MA Hayee FCPS, MD, PhD, FRCP, Fellow (Interventional Neurology), Associate Professor and Head, Department of Neuromedicine, SSMC, Dhaka, Bangladesh.

b. Dr. M Nusratullah, Consultant, Department of Cardiology, University Hospital, Lausanne, Switzerland

Address of correspondence: MA Hayee FCPS, MD, PhD, FRCP, Fellow (Interventional Neurology), Associate Professor and Head, Department of Neuromedicine, SSMC, Dhaka, Bangladesh.

an increasing concern with publications that suggest possible association between exposure and neurological diseases[15-18].

Toxicity of Al in human is known to occur in at least two specific situations. Dementia in dialysis patient is related to Al exposure[16,19]. The Al intoxication in patients can be controlled by control of Al levels in dialysis fluids[20]. Chronic renal failure may lead to decreased Al excretion and enhances Al toxicity[21]. The pathogenesis of Al toxicity is complex and may be related to other factors such as impaired parathyroid function which affects Al absorption/or distribution[14]. A second prominent Al toxicity found in dialysis patients is osteomalacia or metabolic bone diseases[22,23].

Pre-term infants with parenteral administration of nutritional solutions are also at risk for Al-induced neurotoxicity[24,25]. Aluminium inhalation, especially in workers, may be associated with increased incidence of asthma[26,27]. Pre-term infants are also at risk for Al-induced metabolic bone diseases[28,29].

Aluminium is clearly neurotoxic causing degeneration of astrocytes[19] and interferes with the metabolism of the neuronal cytoskeleton[30]. It causes encephalopathy in patients undergoing renal dialysis[16,31,32]. Cerebral dysfunction was reported in people exposed to drinking water that had been contaminated with Al-sulphate[17]. Aluminium has been implicated in a series of neurological diseases including amyotropic lateral sclerosis, dementia associated with Parkinson's disease, and suggested for Alzheimer's disease although this link is quite tenuous[30]. Animal studies indicate that oral exposure to Al leads to accumulation in the brain, bone, muscle, kidney and other organs.

No study on this issue has so far been done in Bangladesh and literature survey revealed very few works throughout the world. This study was done to find out whether deficiency or excess of alumimium is present in MND. The study may give a clue about the role of it in the causation of a disease and this may help planning further studies.

Materials and methods:

This was a prospective study. The study was carried out at the Neurology Department of Sir Salimullah Medical College and Mitford Hospital, Dhaka. The duration of the study was from January 2002 to June 2004. Seventy sporadic motor neuron disease subjects aged between 16 and 65 years were included in this study. Same number of controls matched for age, sex, occupation and habitation (rural or urban) were selected. The diagnosis of MND was done according to the E L Escorial World Federation of Neurology Criteria: (1) signs of lower motor neuron degeneration by clinical, electrophysiological or neuropathological examination, (2) signs of upper motor neuron degeneration by clinical examination and (3) progressive spread of signs within a region or to other regions. Together with the absence of: (1) electrophysiological evidence of other disease processes that might explain the signs of lower motor neuron and/or upper motor neuron degeneration and (2) neuroimaging evidence of other disease process that might explain the observed clinical and electrophysiological signs.

For confirmation of clinically detected motor neuron disease electrophysiological study was done and MND was confirmed by doing EMG and NCV. CT scan and MRI were also done to exclude other pathology. The controls were free from any major neurological diseases and were not suffering from functional disorders, tension headache, lumbar disc prolapse etc. None of the patients or controls had a history of exposure to heavy metals. Informed consent was taken before the individuals were included in the study. The patients of MND were grouped according to various clinical subtypes such as progressive bulbar palsy (PBP), progressive muscular atrophy (PMA), amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS) [34].

Blood (15 ml) was obtained through antecubital venepuncture by metal-free plastic syringes. It was collected in metal-free heparinised glass vials. Ten milliliters of the sample was centrifuged at 5000 rmp for 10 minutes and plasma was collected in a separate vial. Cerebrospinal fluid was collected by lumbar puncture done with steel needles which were previously washed with deionized water and sterilized. Samples were stored at –4° C for a variable period before estimation. To remove the organic

matrix, a measured amount of the sample was digested with 3 ml of digestion mixture containing metal free concentrated nitric acid and perchloric acid in a ratio of 6: 1. More digestion mixture was added if the sample did not digest completely with 3 ml of mixture. Samples were cooled and made up to 5 ml with deionized distilled water.

The estimations were done by direct current plasma emission spcectrophotometer (Beckman, USA) in which an electric arc was generated between two carbon anodes and one tungsten cathode. Pure argon gas was sustained as plasma between the electrodes which acted as a source of vapourisation, automization and excitation. The system first generated a straight line calibration curve by setting two standard solutions (a high and a low) of the element concerned which was used to convert subsequent signal measurements into concentration values. A blank sample of digestion mixture was run with each batch of samples. The method has been standardized in the laboratory; its accuracy being checked periodically by analyzing unknown samples. Aluminium was estimated in plasma and CSF. All the samples in the study group and controls were analyzed. Results were reported as mean ± standard error. Statistical significance was assessed by Student's *t*- test between patients and controls.

Results:

Seventy patients were evaluated in this study. After clinical assessment, all patients were investigated. Same number of healthy age and sex-matched controls were selected and their serum and CSF aluminium were estimated. In this study, the mean age of the patients was 41.60 (± 4.12) years. Age of onset of disease was 41.8 (± 3.7) years. There was slight difference of age of onset of disease between males (40.2 ± 3.38 years) and females (43.40 ± 4.02 years). Sex distribution showed male/female ratio 3: 2. None of the patients had a positive family history of MND and there was no evidence of geographic clustering of the disease. All of the patients were classed into clinical subtypes. Twenty seven (38.57%) patients presented as amyotrophic lateral sclerosis, 21(29.99%) as progressive bulbar palsy, 20 (28.57%) as progressive muscular atrophy and two (2.87%) as progressive lateral sclerosis. Cerebrospinal fluid aluminium level was estimated both in the patients and the controls. Aluminium concentration in patients was 18.09 (± 2.02) μg/dl and that in controls was 12.22 (± 2.42) μg/dl. Therefore, CSF aluminium concentration level of patients was significantly higher than that in controls i.e. $p<0.001$ (Table-I). CSF aluminium level among different subtypes was compared with their controls (Table – I). Statistical analysis revealed that CSF aluminium level was significantly higher in the patients than controls in each subtype. Plasma aluminium level of patients and controls were also estimated. Aluminium concentration in patients was 35.70 (± 3.71) μg/dl and that was 34.02 (± 4.21) μg/dl in controls. The difference was not statistically significant ($p>0.10$) (Table-II). Serum aluminium level in different subtypes was also compared with controls (Table – II). Statistical analysis revealed no difference between patients and controls.

Table-I

CSF aluminium level in patients with different types of MND and in controls

MND types	Patient Mean with SE(μg/dl)	Control Mean with SE(μg/dl)	t-value	p-value
All MND (n=70)	18.09 ± 2.02	12.22 ± 2.42	3.41	<0.001
ALS (n=27)	17.01 ± 2.98	11.98 ± 3.01	2.82	<0.01.
PBP (n=21)	20.12 ± 1.78	12.01 ± 2.12	5.12	<0.001
PMA (n=20)	17.15 ± 1.30	12.67 ± 2.13	2.48	<0.02

Table-II

Plasma aluminium level in patients of different types of MND and controls

MND types	Patient Mean with SE(μg/dl)	Control Mean with SE(μg/dl)	t-value	p-value
All MND (n=70)	35.70 ± 3.71	34.02 ± 4.21	1.51	>0.10 (Not sig.)
ALS (n=27)	36.01 ± 2.98	34.99 ± 3.99	1.71	>0.05 (Not sig.)
PBP (n=21)	34.96 ± 4.01	34.05 ± 4.29	1.71	>0.05 (Not sig.)
PMA (n=20)	36.13 ± 4.14	33.02 ± 4.35	1.96	>0.05 (Not sig.)

Discussion:

Majority of the subjects in this study were of age between 26 and 55 years. This findings correlate with the findings of a study in Bangladesh[34] but does not corroborates with the other Europian studies. This finding may be due to regional variation of MND.

The mean age of the patients was 41.6 (± 4.12) years which was similar to the findings of an Indian study[35] and also to that of a Bangladeshi study[34].

In this study the age of onset of disease was 41.8 (± 3.7) years which correlates with a Mexican study[36]. The distribution by gender (60% males and 40% females) concurred with the world pattern[37-39].

The distribution of the different types of MND in this study was similar to the Indian studies[35,40] but differed from other studies[38,41]. This dissimilarity is probably due to geographical variation of the disease. This can also be supported by similarity with the Indian study i.e. people of Bangladesh and India are in the same geographical area and expose to same risk factors which is different from that of European population.

The finding of higher CSF aluminium than controls correlates with the study of Sood[35]. Cerebrospinal fluid aluminium estimation in different subtypes of MND was also higher than controls. Each subtype showed significant difference and this finding is similar to that of other studies[35,36].

No study on this issue has so far been done in Bangladesh. In this field very few works had been done in the world. First study was carried out in India[35]. The other studies of muscle metals in MND did not show any difference in aluminium levels from a control popultion. The finding of high CSF aluminium in MND is important as aluminium is a potential neurotoxin. It has been proved that aluminium has been associated with some other degenerative neurological disorders like Alzheimer's disease[42] and dialysis dementia[43].

The neurotoxicity of Aluminium has already been established. Studies from Japan and Guam have reported a high content of aluminium in the spinal cord[44] and hippocampal cortex[48] of MND patients. Aluminium has also been seen to accumulate in significant amounts within the nucleoli of lumbar anterior horn cells of MND patients[44] and it has a positive correlation with the number of neurons showing neurofibrillary tangles and granulovacular degeneration[44].

Aluminium has been linked with the formation of neurofibrillary tangles in the cortical neurons in patients of Alzheimer's disease[42] and spinal cord of patients dying of Guamanian MND[33]. Intraventricular[45], subcutaneous[46] and intracellular[47] injection of aluminium in rabbits, cats and ferrets causes neurofibrillary degeneration which resembles neurofibrillary tangles seen in the disease states except for some morphological differences[48]. The studies on Guamanian MND patients have shown intraneuronal deposition of aluminium with calcium as a hydroxyapatite[11], which interferes with slow axonal transport by altering normal neurofilament production leading to excessive neurofilament accumulation and formation of neurofibrillary tangles[49].

High CSF aluminium in MND may be of relevance keeping in view the dynamics of this element in the body[50]. Aluminium is known to accumulate very slowly in cell nuclei and chromatin and large long-

lived cells e.g. neurons are most liable to this accumulation. This uptake is very slow (1mg in 36 years) and the amount once taken up by the brain, cannot be eliminated and therefore gets accumulated. The normal and lethally toxic brain levels of aluminium are narrow. When the aluminium concentration of brain neurons become three to 10 times the normal then it becomes toxic.

The exact role of high CSF aluminium in MND seen in this study is not clear. It is not certain if it indicates neurofibrillary pathology in patients and their resemblance with MND seen in the Western Pacific as no data are available on neuropathological changes in MND in Bangladesh or any reports of trace metal eliminations in brain tissue in MND.

Therefore, results of the present study provide some evidence of a causal relationship between aluminium and MND particularly to progressive bulbar palsy.

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