ejpmr, 2015,2(4), 1159-1167

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ANALYSIS OF NEUROTOXIN METALS IN THE CSF SAMPLE OF MOTOR NEURON DISEASED PATIENTS.

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Article Received on 18/06/2015 **Article Revised on 09/07/2015** **Article Accepted on 30/07/2015**

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ABSTRACT

Motor neuron disease (MND) is a disorder of the nervous system characterized by atrophy of skeletal muscle and sclerosis of motor pathways in brain and spinal cord. Toxic metal have been implicated in the pathogenesis of MND however the exact mechanism is not known here we have analyze 3 neurotoxin metals in two type of MND cases total 35 patients of MND were taken out of which 20 were of ALS &15 were of JASSMA. Metal analyses were performed with high-resolution inductively coupled plasma mass spectrometry. Statistically significant difference in the level of heavy metal can be observed in both type of MND this result support the hypothesis that heavy metal may play an important role in the pathogenesis of MND.

KEYWORD: juvenile asymmetric segmental spinal muscular atrophy.ICP, QEMG,CSF

INTRODUCTION

MND was first described by the French neurologist Charcot in 1874 it is a group of incurable, adult onset, progressive, neurodegenerative condition which involves upper and lower motor neurons in different body region resulting in progressive weakness of limbs leading gradually to partial or complete paralysis, including loss of speech.[1] The majority of MND cases are classified as sporadic with no related family history of the disease, while gene mutations have been discovered in 5-10% of the affected population (familial type).[2] Here we have taken

two types of MND cases one is **Amyotrophic lateral sclerosis**, and other one is **juvenile asymmetric segmental spinal muscular atrophy.** The clinical features of amyotrophic lateral sclerosis indicate the loss of neurons at all levels of the motor system—from the cortex to the anterior horn of the spinal cord. Cerebral frontal lobes are involved in degeneration, axonal lose can be detected by quantitative electromyography (QEMG). On the other hand JASSMA is characterized by progressive muscular weakness and atrophy of distal upper limbs, followed by spontaneous arrest within several years. Although the cause of cervical myelopathy remains unclear, neuropathologic and neuroradiologic findings suggest a forward displacement of the posterior cervical Dural sac during neck flexion, causing compression of the cervical cord, and results in atrophic and ischemic changes in the anterior horn, The anterior horn lesions were characterized by central necrosis without cavity formation, decrease in the number of large and small nerve cells in the periphery with degenerative changes, and mild astrogliosis without macrophage infiltration.[3] the major difference in both type of MND is ALS is progressive while JASSMA stops progression after reaching hands the etiology of both disease is unknown one hypothesis which may be a cause of sporadic cases of MND is toxin which enters in to the neurons and get accumulated which hindered the signal to pass on from one neuron to other and also damage them. Evidences of these comes from animal experiments which shows metal toxin play an important role in degenerating the neurons.[4] accumulation of neurotoxin metal in nerve cell of spinal cord and /or in CSF could explain some of the features of MND. The aim is to analyze the metal concentration in CSF in patients diagnosed either ALS or JASSMA using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS)

MATERIAL AND METHODS

Study Population

The present study is conducted in Department of Neurology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Total 35 Patients of motor neuron disease were included in this study, out of which 20 were of ALS and 15 were of JASSMA from patients attending Neurology OPD and IPD. Patients were enrolled from oct.2012 to April 2014.

Inclusion Criteria

❖ All the cases of motor neuron disease.

Exclusion Criteria

❖ Patients other than motor neuron disease

Valid informed consent was taken from all patients. The entire patients were subjected to a detailed clinical history, physical and neurological, electrophysiological and neuroradiological examination, as per the standard protocol prepared by us. The past history of any illness, history of chronic illness, personal history, history of addiction, drugs/ toxin exposure, occupational, dietary habits and family history is to be taken in detail. The patients will undergo routine blood counts, blood sugar estimation, liver function test, renal function test, test for collagen vascular diseases, HIV ELISA, screening for malignancy, EMG, MRI scan of Brain and cervical spine were done in all patients.

Cerebrospinal fluid (CSF) examination done by lumber puncture with all aseptic precaution and sent to normal routine microscopy in central lab in IMS BHU and for detection of heavy metals (As, Hg, Pb) CSF was sent to department of chemical engineering. IIT BHU by ICP monitor machine.

Sampling: Spinal tap was performed in the supine position from lumbar levels using a standard Spirocan 0.9×88-mm syringe with bevel according to Quincke (B. Braun Melsungen AG, Germany). Skin was disinfected using 4 % chlorhexidine gluconate in ethanol. No suction device or plastic tubing was used. CSF was collected dripping in free air in 1 ml aliquots into polypropylene CryoTubes with stopper and silicone gasket rinsed twice with ultrapure water using a mechanic whirlmixer. When performed in sequence with a routine spinal tap, the CSF samples for metal analysis were collected after the routine samples. When performed alone, the first 3 ml of CSF were allowed to drip and were discarded before the actual sampling for metal analysis Traumatic spinal punctures were excluded from the study.

Metal Analysis: CSF sample were Centrifuge at 3500rpm for 5min. and supernatant was taken 1000µl was approximately diluted with 0.5M HNO_3[5] then ICP test was performed. The spectrophotometer use charge couple Device technology which allows the instrument to measure the broad spectrum of elements. ICP Analysis can be performing on solid and liquid sample, but a solid sample must be converted to liquid before testing by dissolving the sample in a solvent to produce a solution.[6] The sample solution is introduced in to the ICP as a fine aerosol of droplets. The aerosol is produce by a nebulizer which aspirates the sample with high velocity to form a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed. Droplets are small enough to be vaporized in the plasma torch to

be passing in the torch body where the aerosol is mixed with more argon gas. A coupling coil is used to transmit radio frequency to the heated argon gas, producing argon plasma located in the torch. The hot plasma dries any remaining solvent and causes sample atomization. The ICP-ASE Spectrometer detects the atomic emission produces as light, with ICP Mass Spectrometry, the process uses ionization. The resulting mass of the ion indicates the elements present in the sample. Instrumental condition required to start the procedure is mentioned below in [table 1].

Table 1: instrumental condition for iCAP 6200*

Plasma gas	**Fixed 12l/ml**
Auxiliary gas	4 fixed flow 0,0.5 ,1.0 and 1.5 l/min
Nebulizer gas	Pressure control from 0-0.4 MPa
RF source Spectrometer	27.12MHz solid state 750-1500 watts out put simultaneous echelle type 52.9 groove /mm ruled 383 mm effective grafting focal length 9.5^0 uv fused silica cross dispersion prizm
Spectral band pass	7pm at 200 nm
Wave length range	193-769nm
Furnace temp	38^o c
Camera temp.	-46^o c
Optic temp.	38^o c
Gas Supplies	Ar plasma gas 5.5 bar (80psi)
Chiller temp.	5^oc below room temp. (and must be set between 15^o to 30^oc)
Torch is correctly fit in to the torch holder	
Ensure Adequate provision for waste sample is made	
Sample tubing particularly pump tubing must be good in condition	

*iCAP 6200 ICP Spectrometer Standard protocol

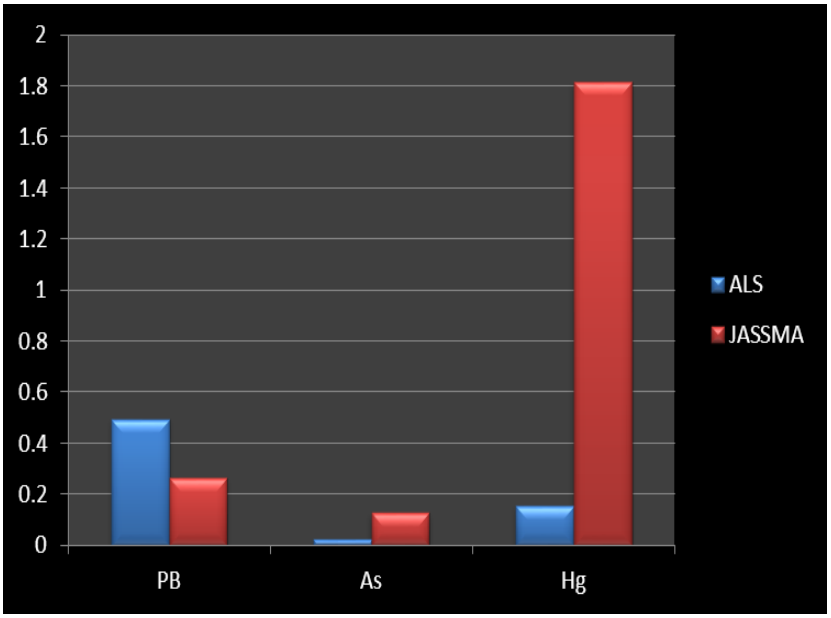
Statistical methods: Mean, median, standard deviation, minima, maxima of 3 metals for ALS, and JASSMA where determine. On the basis of the variability in data, it does not follow the assumption of the parametric test. So we apply the non parametric test name as Mann–Whitney test for intergroup comparison and to rank each metal according to its ability to separate the ALS patient group from the JASSMA patient's group confidence interval is of 95%. As a result of this test Z value & P value is mentioned in the table.[7,8]

RESULTS

Table 2: Value of Pb, As and Hg in both ALS and JASSMA Patients

Variables	Group1 (ALS) Mean ± SD	Group 2(JASSMA) Mean ±SD	P value*	Z value *
Pb	.4953± .2746	.2631 ± .2551	.036	-2.100
As	.0241 ± .0248	.1281 ± .1804	.002	-3.076
Hg	.1554 ± .1183	1.8180 ± 2.2569	.000	-4.051

***this p value and z value is of Mann-Whitney Test**



Statistically Significantly higher concentration of metal Hg and Pb was found in CSF of patients having JASSMA and ALS. Group 1 was of ALS patients with significantly higher Pb (p= 0.036) concentration in compare with JASSMA. And the group 2 was of JASSMA patients with significantly higher Hg (p=0.000) and As (p=0.002) concentration in compare with ALS. Metals having neurotoxin property are significantly higher in both the type of MND cut off limit of neurotoxic metal is compared, when the concentration at or above the limit was found that metal was indicated as neurotoxin.[9] Metals like Pb,As,Hg, Al, Mn are established neurotoxins.[10-17]

DISCUSSION

In this case study level of Pb was significantly higher in case of ALS and As, and Hg level was higher in case JASSMA, Caparison was done between these two diseases. These metals are well well-characterized as neurotoxins. Metal intoxications involving several metal combinations resulting in different clinical profiles is one possible explanation to these overlap situations.[18] The presence of metals in CSF is however not tantamount to axonal damage caused by these very metals and further analyses of the mechanisms of metal

neurotoxicity are warranted. The interpretation that observed elevated metal concentrations are a consequence of some other disease process rather than the cause of disease is also valid. In contrast to the increased metal levels in CSF of the MND patients, a corresponding elevation of Hg Pb, and AS in blood plasma was not noted. Barrier systems [19] protect the spinal cord and the CSF compartment from chemical injury, To what extent transporters for the other metals, significantly increased in ALS CSF (Pb, Hg and As), are active in MND remains to be elucidated. Respiratory exposure to mercury vapor in primate or rodent causes accumulation of the metal in anterior horn cells of the spinal cord implicating impaired barrier protection.[20] Lead is known to accumulate in the choroid plexus. The possibility in ALS of injuries to the barrier systems themselves from toxic metal molecules is another possibility to consider. Exposure to lead also causes a motor neuropathy characterized by weakness and atrophy of skeletal muscles without sensory involvement.[21,22] The spinal origin of the neuropathy following chronic low-dose lead intoxications in humans has been debated for more than a century, and the historical observations[23] from a series of 1,213 lead-intoxicated patients stating the anterior horn of the spinal cord as the site of injury are still valid. A recent large NIEHS study[22] It is necessary to consider the possible role of toxic synergisms and interactions between several metals in the molecular mechanisms that lead to MND. Metal synergisms may account for the clinical variability seen in the MND distribution of weakness and time span of progression. Each metal has a unique profile regarding environmental occurrence, exposure, uptake, metabolism and kinetics, i.e. tissue distribution and toxicity to the nervous system. In the risk assessment, all these factors need to be considered.[18,24,25] Increased concentration of Pd found to be in ALS and increased concentration of Hg and As is found to in JASSMA

CONCLUSION

In summery statistically significant higher amount of neurotoxin metals Pd, As, Hg were reported in the CSF Sample of MND patients. Synergistic effects of the neurotoxic metals are expected. We propose that accumulation of these metals may play an important role in the pathogenesis of the disease which is yet to be identified.

ACKNOWLEDGMENT

Ranveer yadav initiated the study and performed clinical and electromaygraphical examination and prepare the basic structure of the manuscript, we are pleased to have Garima Gupta who collected the sample and also performed metal analysis and prepared the first

draft of the manuscript. We are very thankful to prof. A.K. Sinha Department of chemical engineering for providing ICP Analyzer of metal analysis. Prof. T.b. singh was acknowledged for his statistical support.

CONFLICTS OF INTEREST

No conflicts of interest have been reported by the authors.

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