Heavy Metal Pollution in Ganga basin: A Risk Factor for Amyotrophic Lateral sclerosis

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Abstract

The association between heavy metals and Amyotrophic Lateral Sclerosis (ALS) patients was explored with respect to the longest and pious Indian river Ganga. This study is designed to investigate the severity and heavy metal contamination in the blood of Gangetic (within 25 km from river Ganga) and non-Gangetic (> 25 km from river Ganga) ALS patients. Out of 65 recruited ALS patients, 36 from the Gangetic belt and 29 from the Non-Gangetic belt. Amyotrophic Lateral Sclerosis functional rating Scale (ALSFRS), Mini-Mental State Examination (MMSE) and Frontal Assessment Battery (FAB) were calculated to study disease progression. Lead (Pb), Manganese (Mn) and Cadmium (Cd) concentrations were estimated in the whole blood of 23 subjects from Gangetic belt, 19 subjects from Non Gangetic belt and 23 healthy controls via Inductively coupled plasma mass spectrometry (ICP-MS) technique. A significantly lower FAB score was obtained in Gangetic patients. Pb concentration was significantly higher in both the diseased group than control and Cd concentration was detected significantly higher in Gangetic ALS patients than non Gangetic patients and control group.

Introduction

ALS is a progressive neurodegenerative disease characterized by degeneration of neurons of corticospinal tract and in the brainstem and spinal cord. The worldwide incidence rate of this disease is approximately 1–3 per 100,000 persons, out of which 5–10% of ALS patients may be inherited from the family (familial ALS) and 90–95% are developed later in age (sporadic ALS) [1, 2]. The symptoms are widespread, ranges from muscle twitching, cramping, stiffness, or weakness to complexity in chewing or swallowing. On the basis of origin, ALS is categorised into Bulbar ALS which initiate with slurred speech and progress to weakness of muscle strength. Another is Limb onset (upper or lower limb onset), which usually begin with weakness in one or both of the hands, shoulders, arms or legs. As ALS progress, individuals face challenges in terms of cognitive impairments and overall quality of life. Approximately 20 percent of individuals with ALS also develop frontotemporal dementia (FTD) that is change in personality and behaviour worsen with time. ALS patients die from respiratory failure within 2 to 10 years after diagnosis. Although the accurate etiology of ALS is debated, reports have suggested that alteration in the gene environment interaction such as alterations in C9orf72, SOD1, TDP-43 and FUS genes are associated with toxins like Pb, Cd and pesticides, which may be the risk factors for sporadic ALS forms [3]. Manganese (Mn) have been potentially associated with ALS pathogenesis through alteration in levels of specific neurotransmitters in the brain, such as GABA, dopamine, and glutamate [4]. Although conclusive evidence is still lacking, But the observation of rising ALS rates over time provides evidence in favor of the theory that ALS is a condition brought on by the environment. Interestingly, the long journey of rivers are an asset for human exposure that acts as source as well as sink for metals, if not taken care of.

The longest and spiritually most pious Indian river Ganga, has experienced such kind of deterioration in its water quality over time, where discharged metals pass through the vertical stretch into the river, precipitated and accumulated onto river sediments and ultimately enters the food chain [5, 6]. People
living in its vicinity may experience the effect of these metal pollution over time. Our population based study with reference to river Ganga in north India, aimed at evaluating the role of Gangetic metal load as risk factors in ALS etiology as well as in the cognition of ALS patients.

This is the first hospital based and case control population study, where ALS patients were diagnosed, categorized on the basis of their residence from river Ganga as Gangetic (< 25 km from river Ganga) and non Gangetic (> 25 km from river Ganga), estimated Pb, Mn and Cd levels in their blood and compared their concentration levels in both the groups and healthy control, seeking whether Gangetic metal contamination is a possible risk factor for developing ALS in north India.

**Material and Methods**

The present study was conducted after obtaining ethical approval from the Institute ethical committee of IMS, BHU, and written consent was taken from each enrolled patient for this study.

**Study Population:**

The present study was conducted in the department of Neurology, a wing of tertiary care center in SSL Hospital, affiliated with BHU, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Total 65 ALS patients (as per El Escorial criteria [7]) and 23 age match healthy controls were recruited from February 2018 to February 2020. The healthy controls were mostly persons residing approximately in the same area without any neurological disease with an average age ± 5 years of patients. Among ALS patients, 36 were from the Gangetic belt and the rests 29 were from the non-Gangetic region. A detailed medical examination including clinical history of each patient was studied by an expert clinical neurologist of the department. All the 65 subjects and 23 healthy controls were interviewed for their demographic details and place of residence i.e distance from river Ganga. Information regarding the medical history of ALS was obtained by a questionnaire administered to ALS patients. Hindi Mental Status Examination (HMSE) [8], Frontal Assessment Battery (FAB) [9], and ALSFRS [10] were performed on all the ALS subjects. However, 13 subjects from Gangetic belt and 10 subjects from non Gangetic group either denied to donate their blood, or could not be traced for follow ups or died or moved elsewhere before blood sample collection. Therefore, only 32 ALS patients from Gangetic and 19 from non-Gangetic belts were subjected to venous blood extraction to study the accumulation of Pb, Mn and Cd in their blood.
Table 1
Descriptive characteristics of ALS subjects in Gangetic and non Gangetic region and healthy control.

<table>
<thead>
<tr>
<th></th>
<th>Gangetic</th>
<th>Non Gangetic</th>
<th>Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>32</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>57.73 ± 8.40</td>
<td>54.73 ± 5.75</td>
<td>44.52 ± 6.40</td>
</tr>
<tr>
<td>Residence</td>
<td>Urban = 13</td>
<td>Urban = 11</td>
<td>Urban = 9</td>
</tr>
<tr>
<td></td>
<td>Rural = 19</td>
<td>Rural = 18</td>
<td>Rural = 14</td>
</tr>
<tr>
<td>Life style</td>
<td>Smoker = 6</td>
<td>Smoker = 5</td>
<td>Smoker = 3</td>
</tr>
<tr>
<td></td>
<td>Alcoholic = 2</td>
<td>Alcoholic = 3</td>
<td>Alcoholic = 0</td>
</tr>
<tr>
<td></td>
<td>Tobacco chewer = 9</td>
<td>Tobacco chewer = 8</td>
<td>Tobacco chewer = 4</td>
</tr>
<tr>
<td>Mean duration of the disease (years)</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
<td></td>
</tr>
<tr>
<td>Sex (no.)</td>
<td>Male</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Types of ALS on the basis of onset of disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limb onset</td>
<td>16</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Lower limb onset</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Bulbar onset</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>FTD-ALS</td>
<td>01</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The values are represented as Mean ± SD

Sampling:

2 milliliter of blood was drawn with a sterile syringe from the upper limb's superficial vein and stored at -20°C till analysis. Previous to conducting the lab investigation, the samples were kept for 10 minutes at 4°C and lastly bring to room temperature.

Sample Preparation:

Approximately 1 ml of blood was digested with 7 mL of HNO₃ (65% v/v) in acid-prewashed PTFE vessels. Digestion was carried out using a closed-vessel microwave digestion system at 80°C with a power of
1,000 W for 30 min. After cooling down to room temperature, the digested samples were quantitatively transferred into pre-cleaned 25 mL volumetric flasks, dilution was done using deionized water and stored at room temperature.

**Instrument:**

**Quality assurance and quality control (QA/QC):**

The quality assurance and quality control analytical estimation was performed using ICP multi element standard solution VIII with purity of 99.99%. The ICP MS Perkin Elmer Optima 7000 DV was calibrated with three point calibration in triplicates. The samples were analysed in triplicate with mandatory blank sample for all the estimations during this study.

<table>
<thead>
<tr>
<th>Element</th>
<th>Detection limit, mg/L*</th>
<th>Recovery%</th>
<th>RSD*%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>1.802</td>
<td>93.6-106.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Mn</td>
<td>0.176</td>
<td>103.8-106.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Cd</td>
<td>0.012</td>
<td>95.6-108.7</td>
<td>9.5</td>
</tr>
</tbody>
</table>

*Detection limit is calculated 3 times of standard deviation of the blank

*Relative Standard Deviation

**ALS FRS score:**

ALSFRS is a validated rating instrument for screening the progression of disability in patients with ALS. This is systematically planned to endow with a rapid and consistent estimation of patients and establish how the disease is affecting them at any specified time. It envelops four functional areas: speech, swallowing, upper and lower movement of the extremities. The ALSFRS for each function is scored from 4 (normal) to 0 (no ability), with a maximum total score of 48 and a minimum total score of 0.

**MMSE:**

MMSE is an examination of 30-point questionnaire to determine cognitive impairment (problem with thinking, communication, understanding and memory). It involves memorizing a few objects and then repeating the list back. Similarly, copying a drawing etc. A score of 25 or higher is classed as normal and below 24 is abnormal.

**FAB:**
FAB is cognitive test that integrate numerous clinical estimations to screen for frontotemporal dementia (FTD). Very low score of FAB denotes patients with FTD.

**Statistical analysis:**

For comparing the cognitive measurements, the unpaired t test was applied for both Gangetic and non Gangetic groups. One way ANOVA with non-parametric test and post hoc test was applied for calculating metal concentration in all the three groups for each metal. The p values at 0.05 were considered statistically significant. Pearson correlation was used to study age- metal and metal-metal correlation in both the groups. The Graph Pad Prism 5 was used as statistical plotting and calculation tool.

**Result and discussion**

Figure 1 shows the route of river Ganga in India, covering five states, Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, and West Bengal. Patients visiting to BHU SS Lal hospital, as in patients and out patient are mainly residence of North India, especially from the states of Uttar Pradesh, Jharkand and Bihar with a rare percentage from Madhya Pradesh as well. People living in the vicinity of river Ganga depend on the river directly or indirectly by either consuming river water as municipal water supply or by consuming contaminated edible fishes of the river. Untreated waste discharge is the major cause of heavy metal pollution in river Ganga. These heavy metals are bio-accumulated in the food chain, reaching edible fishes [11].

Table.1 gives the detailed description of ALS patients in both the groups, noticeably the mean age of patients in Gangetic belt is higher than non Gangetic belt, although early onset of ALS was also noticed in Gangetic belt, since standard deviation is high in this region. So, it cannot be verified that whether river Ganga is responsible for early onset of disease. However, in later part of the discussion, we correlated the age with metal concentration, which suggested a positive age correlation with Pb, Mn and Cd. Along with heavy metals correlation, this also supports the previous finding that age is positively correlated with ALS patients [12]. Female subjects are more in the non Gangetic compared to the other whereas only 2 females were observed in control. There is no particular cause following this anonymous. Three types of ALS on the basis of its onset is categorised with maximum number of patients with upper limb onset, followed by lower limb onset and bulbar ALS in both the groups. One patient with FTD-ALS was detected only in Gangetic group, supporting the involvement of Ganga basin in the severity of disease.
Table 3
The descriptive cognitive scores of ALS patients in Gangetic and non Gangetic groups. Unpaired t test applied with their level of significance measured at p < 0.05.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Gangetic</th>
<th>non Gangetic</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALSFRS</td>
<td>32.86 ± 2.94</td>
<td>33.03 ± 3.61</td>
<td>Non-significant</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.19 ± 1.48</td>
<td>28.58 ± 2.95</td>
<td>Non-significant</td>
</tr>
<tr>
<td>FAB</td>
<td>16.34 ± 2.46</td>
<td>17.55 ± 1.05</td>
<td>significant</td>
</tr>
<tr>
<td>Prevalence rate</td>
<td>0.3%</td>
<td>0.1%</td>
<td></td>
</tr>
</tbody>
</table>

The values are represented as Mean ± SD

Table 3 describes, ALSFRS score, MMSE score and FAB scores calculated in the patients of both belts with mean and standard deviation. Regarding ALSFRS and MMSE scores, when an unpaired t test is applied between both the groups, there was no significant difference obtained between them. However, FAB score was significantly higher (p < 0.05) in non Gangetic group than Gangetic category and because FAB score is deemed more pertinent in terms of authentication among other two [13], therefore Gangetic basin may have a significant influence on the progression of the disease. But limitation in data is insufficient to predict the severity and disease progression between both the diseased groups. Prevalence rate of ALS was observed more in Gangetic belt than non Gangetic belt, indicating higher flow of patients in the previous one. The reason for this could be attributed to either the Gangetic basin's influence or shorter distance between the patients' residences and the hospital.

The one-way ANOVA with Bonferroni’s Multiple Comparison post hoc test shows no significant difference in the concentration of Pb level in ALS patients from Gangetic and non Gangetic groups, however, Pb concentration was significantly higher (p < 0.05) in ALS patients of Gangetic group as well as non Gangetic group, when compared with control group. Lead concentration [Fig. 2 (a)] in ALS patients in Gangetic belt ranges from 1.019 to 3.621 µg/l whereas it was 0.625 to 3.34 to µg/l in non Gangetic belt and 0.74 to 2.54 µg/l in control group. This suggest that Pb is an important element and may play role in the risk of ALS etiology. Higher Pb level in the CSF of ALS patients suggest that it can cross the tight junction of blood-brain barrier [4]. It gets accumulated in the hippocampal region of the brain, via bound red blood cells (RBCs). This is supported by a gestational study in rats where Pb exposed rats resulted in higher lipid oxidation products and higher catalase and superoxide dismutase activities in the brain cortex, hippocampus, and cerebellum compared to their corresponding controls [14]. Though the difference between the two ALS groups studied is non-significant, we got higher Pb concentration in Gangetic group of patients as compared to non Gangetic group. This could be a triggering factor for more number of patients and more severity in Gangetic belt. Although, whether increase in blood Pb level is the cause or consequence of ALS is debated, however this is more towards consequence because Pb get mobilized from the bones upon bone weakness. Because bone is the endogenous source of Pb accumulation and blood measurement only reflects a medium term exposure [15]. A few researchers have
published contradictory outcomes in the course of environmental pollution in disease etiology. In Reggio Emilia Province of the Italian population, in spite of severe long-term lead pollution in the environment, there doesn't occur any increased incidence of ALS [16].

Mn concentration was determined in the whole blood of ALS patients which is summarised in Fig. 2(b). We observed higher mean Mn concentration in control group than ALS patients of both the groups, although significant difference (p < 0.05) was observed only between non Gangetic and control group. Its concentration in ALS patients in Gangetic belt ranges from 0.765 to 4.96 µg/l whereas it was 0.764 to 3.629 µg/l in non Gangetic belt and 0.603 to 5.529 µg/l in control group. In present study, both the case groups (Gangetic and non Gangetic) witnessed lower Mn concentration than the control group, indicating the inadequacy of Mn as candidate element in cause or progression of ALS disease. However, in other way, whether deficiency of Mn is the probable cause for ALS is a question of concern. Also, it can be interpreted that deregulated metal concentrations may not be the cause rather the consequence of degenerative process. As Mn is an indispensable trace element and performs a significant function as a cofactor in enzymatic activities required by all living organisms [17]. Moreover, there was no significant difference in Mn concentration in ALS patients between Gangetic and non Gangetic category, infers that despite of high Mn contamination in river Ganga [18, 19], it cannot be blamed for disease etiology. Although, genetic susceptibility to environmental exposure may trigger the pathway for its onset which is an area of future study. Some previous findings have also revealed lower Mn concentration in motor neuron disease (MND) than in normal subjects and in another study, unchanged levels of Mn were found in the CSF and serum of ALS patients [20, 21]. However, a study in Japan supported role of Mn for the prevalence of ALS in two foci in western pacific Kii peninsula [22]. Contradictory result in chronic exposure to Mn decreases striatal dopamine turnover in human α-Syn transgenic mice and induces microglia activation and dystrophy in the SN in non-human primates was observed. [23, 24].

Cadmium showed interesting results The concentration of Cd in ALS patients of Gangetic belt was significantly higher (p < 0.005) than control group. ALS patients in Gangetic group were also observed with significantly higher (p < 0.05) Cd concentration than non Gangetic group. Although no significant difference was observed between non Gangetic and control group. This suggest that concentration in river Ganga may play role in developing Cd toxicity in ALS patients and Cd may be responsible element in disease progression in Gangetic category. Also, significantly higher Cd concentration in Gangetic group than non Gangetic group reveals that Cd contamination of river Ganga may be an important factor for disease etiology in Gangetic belt. Although the less number of subject in non Gangetic category is the limitation of our study to clearly draw any conclusion regarding the same. Similar area based study from Australia found higher cadmium levels in plasma of patients with sporadic ALS and a study carried out in province of Reggio Emilia, Italy investigated a tendency towards higher risk of ALS on increasing whole blood Cd concentration [25]. Animal studies have also detected neurotoxin role of Cd, but epidemiological studies yielded inconsistent results [26].

Figure 3 denotes Pearson correlation between age and metal concentration in both the groups. In Gangetic category, age of ALS patients showed positive correlation with all the three metals, highest with
Pb followed by Cd and Mn. Also, all the three metal concentration were positively correlated with each other. In case of non Gangetic category also, age was positively correlated with all the three metals, highest with Pb followed by Mn and Cd. However, Pb and Cd shows negative correlation and Mn and Cd also showed negative correlation. Highest and positive correlation of Pb with age of ALS patients would be because Pb is stored in bones and in later age, with weakness of bones, they get demineralized and reach to blood. Our finding is similar to an age based correlation of previous study that determines higher Pb concentration in blood can be associated with ALS development [12]. Here, very interestingly, we can also interpret that because all the metals are positively correlated with each other in Gangetic belt, so river Ganga may play important role for metal accumulation in ALS patients.

**Conclusion**

This robust study gives an idea of role of Pb, Mn and Cd in cause and progression of ALS. Pb could be a probable contestant for progression of ALS in elderly patients. Neither the role of Mn in ALS progression or river Ganga in Mn toxicity is accounted in this study. Interestingly, Cd could be a potential risk factor for ALS etiology in Gangetic belt as it is one of the toxic metal and is the element of concern for ecological threat. However, human is a complex organism and encounters with numerous factors in the environment, so metal exposure alone cannot be a conclusive remark for ALS disease. More likely, peculiar genetic profiling in Gangetic ALS patients could produce a more clear picture associated for disease etiology.

**Declarations**

**Author’s contribution**

V.N Mishra is the person behind the idea of this study. Experimental work and manuscript preparation were done by Bhargawi Mishra. Sooraj Patil and Priyanka Gautam collected data and samples from ALS patients. Geeta Gautam helped in designing of experimental protocol and scientific discussion of experimental data. Abhishek Pathak helped in valuable scientific discussions, Varun Singh, Anand Kumar, Deepika Joshi, and R.N Chaurasia helped in scientific discussions and detection and identification of ALS patients, and Vineeta Singh and Niraj Kumar Srivastava helped in data alignment and manuscript preparation.

**Ethical statement** The above clinical study was assessed and approved by the BHU ethical committee (ECR/Bhu/Inst/UP/2014/Re-registration-2017 dt.31.01.2017)

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Acknowledgment**
We are grateful to the interdisciplinary School of Life Sciences-BHU (ISLS) for providing the instrumental facility. We thank to all the patients and even healthy people who donated their blood samples for this study.

References


Figures
Figure 1

Population distribution of ALS patients in Gangetic (yellow) and non Gangetic (green) region along river Ganga.
Figure 2

Concentration range of (a) Pb, (b) Mn and (c) Cd in ALS patients from Gangetic and non-Gangetic belt and control group with level of significance. ns indicates non significance, * shows significance at p<0.05 and ** indicates significance at p<0.005.

<table>
<thead>
<tr>
<th>Gangetic ALS</th>
<th>Age</th>
<th>Pb</th>
<th>Mn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.191947</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.006347</td>
<td>0.245162</td>
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<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.091327</td>
<td>0.019678</td>
<td>0.060507</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non Gangetic ALS</th>
<th>Age</th>
<th>Pb</th>
<th>Mn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
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<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.313188</td>
<td>0.169565</td>
<td>1</td>
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</tr>
<tr>
<td>Cd</td>
<td>0.218722</td>
<td>-0.03191</td>
<td>-0.22495</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 3

showing Pearson correlation between age of ALS patients and their blood concentration levels of Pb, Mn and Cd in both Gangetic and non Gangetic groups.