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Clinical and Electrophysiological Profile of Amyotrophic Lateral Sclerosis and study of plasma levels of neurotoxic metals as a significant risk factor in development of Amyotrophic Lateral Sclerosis.

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ABSTRACT:
Introduction: *Amyotrophic Lateral Sclerosis (ALS) is a rare and debilitating disease. The hypothesis tested is that neurotoxic metals contribute significantly to the risk of ALS. In this study we investigate the metallotoxic etiology of ALS in the region of north coastal AndhraPradesh.*
Method: *We studied the clinical and electrophysiological profile of the patients diagnosed with motor neuron disease (MND) in the tertiary care hospital of North Coastal AndhraPradesh. We also studied the association of blood level of heavy metals in patients, diagnosed with MND by comparing with controls. Diagnostic criteria used were Awaji criteria. We measured concentration of 9 toxic metals in the plasma from 109 patients with MND. Total of 56 patients (51.4%) had ALS, concentration of toxic metals in the plasma from 56 ALS patients and from 42 age matched controls was measured by inductively couples plasma mass spectrometry(ICP-MS). Independent sample T-test was applied to find statistical difference between meansof heavy metal levels in ALS patients and controls. P-value of <0.05 was considered statistically significant.*
Results: *We found that higher blood levels of Arsenic (As), Lead (Pb) and lower levels of Cesium(Cs)are associated with ALS. Spinal onset ALS had association with high blood levels of Cadmium(Cd) and Lead (Pb) as compare to bulbar onset ALS.*
Discussion: *Results suggest that metal exposure contributes to the pathogenesis of ALS.Further heavy metal analysis should be carried out in the large population based multicenter studies, along with assay in common exposure sources like drinking water, air, soil,occupational areas for an in-depth investigation on their possible involvement in the pathogenesis and in course of ALS that can open a door to new therapeutic targets.*
Key words*: Amyotrophic Lateral Sclerosis, blood levels, toxic metals, pathophysiology.*

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Abbreviations: ALS Amyotrophic Lateral Sclerosis, **APB** Abductor Pollicis Brevis, **ADM** Abductor Digiti Minimi, **BAD** Brachial Amyotrophic Diplegia, **BMMA** Brachial Mono Melic Amyotrophy, **CMAP** Compound Muscle Action Potential, **CMMA** Crural Mono Melic Amyotrophy, **FTD** Fronto-Temporal Dementia, **IBALS** Isolated Bulbar Amyotrophic Lateral Sclerosis, **LAD** Leg Amyotrophic Diplegia**, MMA** Mono Melic Amyotrophy**, MMNCB** Multifocal Motor Neuropathy with Conduction Block, **MMND** Madras Motor Neuron Disease, **MMNDV** Madras Motor Neuron Disease Variant, **MND** Motor Neuron Disease, **MNDs** Motor Neuron Diseases**, PLS** Primary Lateral Sclerosis, **PMA** Progressive Muscular Atrophy , **PPS** Post-Polio Syndrome, **ROC** Receiver Operating Characteristic, **SOD** Superoxide dismutase, Upper Motor Neuron **UMN,** Lower Motor Neuron **LMN,** Selenium **Se,**Lead **Pb,**Arsenic **As,** Cesium **Cs,** Cadmium **Cd**

I. Introduction

ALS is a relatively rare disease, but with a strong impact on the life of patients, their families, and the society as a whole. The term motor neuron disease (MND) coined by Russel Brain in 1969 refers to a specific disorder of both upper and lower motor neurons, otherwise known as amyotrophic lateral sclerosis(ALS).The term motor neuron diseases (MND) refers to a broader family of disorders that may affect the upper and/or lower motor neuron system.[1]

Classical ALS worldwide incidence rates varied between 0.42 and 5.3 per 100,000 person per year.[2] The average age of onset ranges from 60.7 to 64.3years and an average time from symptoms onset to diagnosis between 10.8 and 16.9 months.[3-7]ALS prevalence in United States and India is 5 and 4 cases per 100,000 persons respectively with mean age of onset of 46.2 years and survival of 114.8 months.[8, 9]

The cause of ALS is unknown. The current prevailing theories include genetic, viral, inflammatory, oxidative or toxic mechanisms. Some indications point toward metallotoxic etiologies. Clusters of ALS have been observed in regions where geological conditions cause elevated metal concentrations in water and soil. Several studies demonstrated that certain occupational and environmental exposures may contribute to the risk of developing ALS(REF?)

On the other hand, MNDs were least explored and thoroughly studied in the north coastal region of the Indian state Andhra Pradesh. We hypothesize that certain neurotoxic metals may contribute significantly to the risk of ALS. To further validateour hypothesis, we conducted a case-control study and measured the plasma concentration of 9 toxic metals in of 109 research participants with MND and matched controls. Out of 109 patients, plasma levels of toxic metals in 56 patients with ALS were compared with plasma levels of toxic metals from 42 age matched controls.

II. Aims And Objectives

1) To study clinical and electrophysiological profile of patients diagnosed with MND in the tertiary care hospital of North Coastal Andhra Pradesh.
2) To study the association of blood level of heavy metals in the patients diagnosed with MNDs by comparing with controls.

III. Method

We studied the clinical and electrophysiological profiles of the patients diagnosed with MND in the tertiary care hospital of North Coastal Andhra Pradesh from April 2016 to February 2018. In this cross sectional observational study we included 109 subjects, 56 patients with ALS, age >15years, fulfilling the diagnostic criteria of the Department of Neurology, King George Hospital, Viskapathnam.(Awaji Criteria). Age matched healthy controls were recruited for only heavy metal analysis. Any patient with MNDs with genetic predispositions as well as secondary causes or MND mimics were excluded from the study.

Informed written consent from patient/parent/guardian was taken after explaining in their understandable language. After recruiting subjects into the study, detailed history was taken regarding occupation and symptoms. A through clinical examination was done. For all patients, routine hematological investigations like hemogram, serum creatinine, random blood sugar, HIV screening, nerve conduction studies, needle EMG in bulbar, cervicothoracic, lumbosacral segments was done using Nicolet Viking machine. Other investigations like MRI brain and spinal cord, thyroid profile, serum parathyroid hormone, serum calcium, and serum phosphorus were done if indicated to rule out mimics of MNDs.

Blood heavy metal analysis for Lead, Mercury, Arsenic, Cadmium, Barium, Caesium, Chromium, Selenium (Se), and Cobalt were done in the same laboratory for all patients who fulfilled inclusion criteria and age matched controls by inductively coupled plasma mass spectrometry(ICP-MS) method. Laboratory was blinded for patients and control information. An Awaji diagnostic criterion of various MNDs was used in this study.

Clinical&Electrodiagnosticcriteria,aftercarefulexclusionofothercausesforBrachialAmyotrophicDiplegia(BAD),wastedlegsyndromeorcruralMMA,PMA,Madras MND wereincluded.

InNCS,sensorystudiesofmedian,ulnar,suralandmotorstudiesofmedian,ulnar,tibial,peronealnervesweredoneinallpatients.CMAPsofAPB,ADMandratioofAPB/ADMwerecalculated.CMAPAPB/ADM<0.6(correlateofsplithand sign)&APB/ADM>1.4(correlateofreversesplithandsign)wereconsideredabnormal.

DatawasenteredintoMicrosoftExcelsheetandanalyzedusingIBMSPSSStatistics forWindows,Version22.0.Descriptivestatisticswereexpressedasmeansandpercentages.IndependentSampleT-testwasappliedtofindstatisticaldifferencebetweenmeansofheavymetallevelsinALSpatientsandcontrols.P-Valueof<0.05wasconsideredstatisticallysignificant.ROCcurvesshowingAUC(areaundercurve)wereplottedforallheavymetalsineachsubgroupofMNDstocomputesensitivityandspecificityoftheirblood levelsinMNDsbasedon thecoordinates.

IV. Results

Total numbers of 109 patients with different variants MNDs were included in this study. Out of 109 patients56 were diagnosed with ALS using Awaji criteria (Table 1).

Table 1:

Awaji Criteria
Clinically Definite
UMN + LMN signs in bulbar region + ≥ spinal regions; or
UMN + LMN signs in 3 spinal regions

Clinically Probable UMN + LMN signs in ≥ spinal regions and "with some UMN signs necessarily rostral to the LMN signs"
Clinically Possible UMN + LMN signs in 1 spinal region; or UMN signs in ≥ spinal regions; or LMN signs are found rostral to UMN signs, only after the appropriate neuroimaging and laboratory tests are performed to exclude other possible differential diagnosis that may mimic ALS

Geographic distribution:
87%oftotalcaseshailfromnorthcoastaldistrictsofAndhraPradesh,outofwhich32% (n=35) werefromVisakhapatnam district,28%(n= 31) fromVizianagaramdistrict,27%(n =29) fromSrikakulamdistrict.WestBengalandOrissa patients constitute 3 %(n=3).79%(n=86)oftotalcasesresideinruralareasandtheremaining21%(n=23) in urban areas (Figure 1).
Occupation:
Outoftotal109patients,22%(n =24) werefarmers,15%(n =16) werehomemakers, 13%(n =14) weremanuallaborerswhocarryheavyweightsonhead,13%(n =14) werestudents,4%(n=4)werewelders,2%eachwereprofessionaldriversandPharmaceuticalcompanyemployees,1%ea chwereNavyofficer, electrician,pipefitter andtheremaining26% didother jobs (Fig. 1).

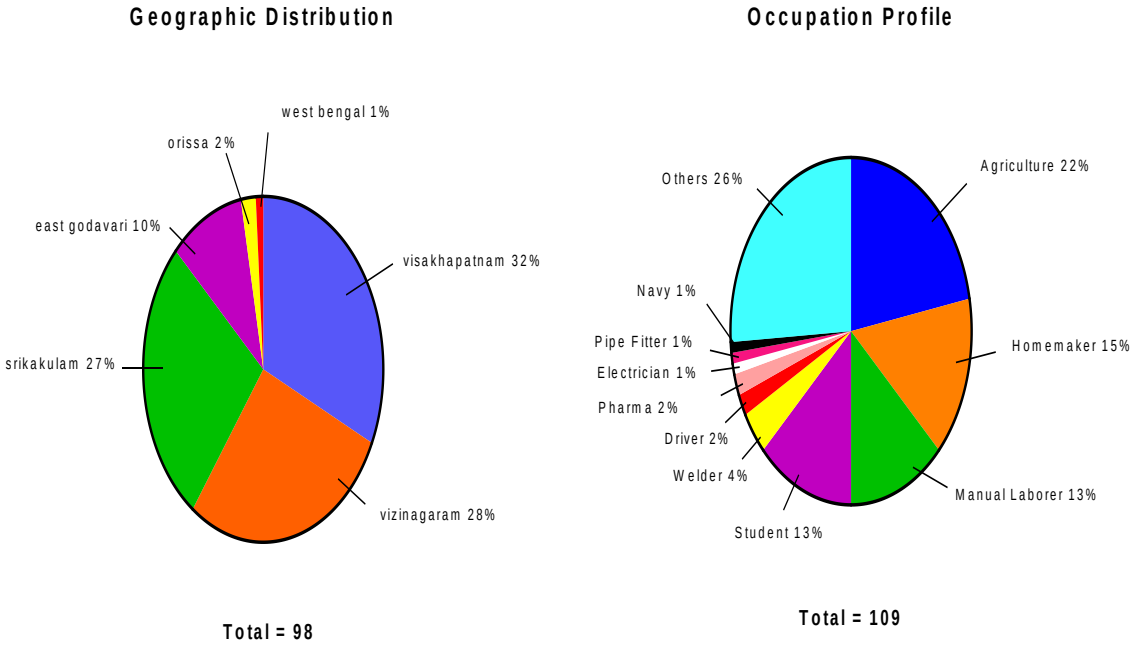

Fig. 1. Geographic distribution and occupation profile of patients with motor neuron disease.

Age and gender: A total of 56 patients met the criteria for ALS diagnosis. Outof thoseALSpatients, 64.3%(36)wereabove50yearsof age (male to female ratio, M: F, 1.6:1),26.8%(15)were40-49years (M: F, 4:1)and the remaining 9% wereall males <40yearsofage (Figure 2).Table 2 classifies the ALS patients according to Awaji criteria.Out of total 56 ALS patients, 48% (27) had definite ALS (M: F, 2: 1), 36% (20) had probable ALS (M: F, 3: 1) and 16% (9) had possible ALS (M: F, 1: 2).

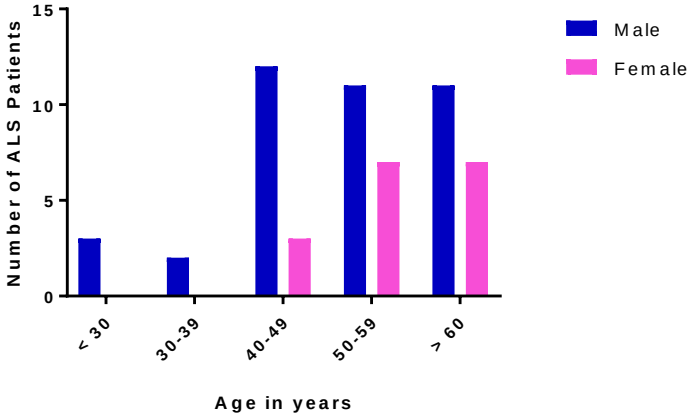

Fig. 2. Age and gender distribution

Table 2
Classification of ALS patients according to Awaji Criteria.

	Definite ALS	Probable ALS	Possible ALS
Male	18	15	3
Female	9	5	6
Total (N=56)	27 (48%)	20 (36%)	9 (16%)

Site of onset: Outoftotal56ALSpatients, ones with bulbaronsets ALS were 20(36%) (M: F,1.5: 1)andspinalonsetALSwere36(64%) (M: F, 3:1).Inpatientswithspinalonset ALS,symptomsstartedin upperlimbsin22(39%)(M: F,2.7:1),lowerlimb in 11(20%) (M: F, 4.5: 1),uncertainonsetin3(5%)(M: F, 2:1), (Table 3).

Age of onset: Meanageofpresentationin56ALSpatientswas51.7+/-15.5years.Mean age of presentation in bulbar onset ALS was 53.7years (males; 50.7years, females; 53.9years) and spinal onset ALS was 48.6 years (males; 47.4years, females, 48.7years) (Table 3).

Table 3: Descriptive characteristics and distribution of ALS subjects.

	Onset site	**Male**	**Female**	**Total**
	Bulbar	50.7 (15.7)	53.9 (15.7)	53.7 (15.7)
	Spinal	47.4 (15.7)	48.7 (15.7)	48.6 (15.7)
Mean age in years (SD)				
Mean symptom onset to diagnosis	Bulbar	9.2 (28.2)	10.2 (28.2)	8.7 (28.2)
	Spinal	18.7 (28.2)	22.9 (28.2)	19.6 (28.2)
Total N=56 (%)	Bulbar	12 (21.4)	8 (14.3)	20 (35.7
	Spinal-UL	16 (28.6)	6 (10.7)	22 (39.3)
	Spinal- LL	9 (16.1)	2 (4)	11(19.6)
	Uncertain	2 (4)	1 (2)	3(5.4)

Duration of symptom onset to diagnosis (in months): Mean duration of symptom onset to diagnosis (in months) in 56 ALS patients was 15.2+/- 28.5 out of which bulbar onset was 8.7 months. In bulbar onset ALS mean duration of symptom onset to diagnosis in males and females was 9.2 months and 10.2 respectively. In spinal onset ALS mean duration of symptom onset to diagnosis in males and females was 18.7 and 22.9 months respectively.

Symptoms: Out of total 56 patients with ALS,history of weakness in the limbs was reported distally (46%), proximally (9%), both proximally and distally (21%) and no weakness (25%)History of stiffness of limbs (64%), flabbiness (16 %),stiffness& flabbiness (14 %),No history of stiffness or flabbiness in limb (5 %).Patients reported following symptoms thinning of the limb being most common (66%), fasciculation (57%), pseudobulbar symptoms (29%), fatigue (18%), UMN bladder symptoms (11%), cramps (5%),

impaired cognition in the form of executive and behavioral disturbance (5%). TwentybulbaronsetALSpatients,presentedwithdysarthria (60%), dysphagia (30%)&both (10%)

Cranial nerve involvement: Fig. 3 describes the percentage of ALS patients with different cranial nerves being involved in their disease. The most commonly involved cranial nerve was CN 12 (68%), second being CN 9 and 10 (41%),CN 5 and 7 (11% each). CN 11 was least commonly involved (5%). However, there was no cranial nerve involvement in 23% of the patients. 1 patient presented with atypicalpresentationsofunexplained unilateral partial CN 3 palsyand 1 patient presented with verticalgazepalsy.

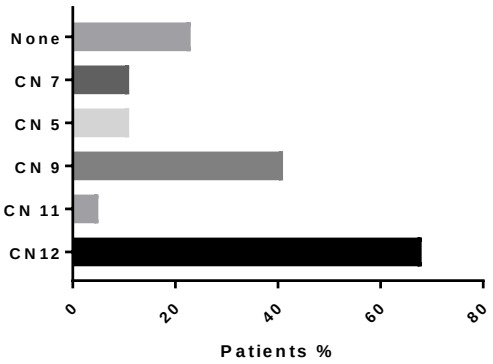

Fig. 3. Cranial nerve involvement on examination in ALS (N=56).

Clinical Signs: ThemostcommonclinicalsigninALSpatientswaspolyminimyoclonus(63%),splithandsign(57%) (Fig. 4),obliqueforearmwasting andexaggeratedjawjerk (52%),plantarresponsewasextensor(48%),flexorорequivocal(29%),andmute (23%).Reversesplithandwastheleastcommonclinical sign (7%).

Fig. 4. Split hand sign in one of the ALS patients. Disproportionate wasting of abductor pollicis brevis and first dorsal interosseous compared to abductor digiti minimi.

Nerve Conduction Studies: Table 4 describes, in the 56 ALS patients, themeanCMAPofAPB(millivolts)was5.0+/-4.07,ADMwas7.2+/-4.18,and CMAPratioofAPBtoADMwas0.62+/-1.08.46%(26)oftotalALSpatientshadAPB/ADMCMAPratioof<0.6whichcorrelateswithclinicalsplithandsignandAPB/ADM>1.4in7%(4)correlatingwithreversesplithandsign.
ThemeanCMAPofAPBandADMinspinalonsetALSwaslesscomparedto bulbaronset(3.94,6.37Vs6.83,8.7).Electrophysiologicalcorrelateofsplit handsign(APB/ADM<0.6) wasseenin46%oftotalALS,55%(20)ofspinal- onsetALScomparedto 30%(6)of bulbar-onsetALS.

Table 4
Nerve conduction studies of CMAPs of APB, ADM, and APB/ADM ratio in ALS. (Mean ± SD)

CMAP (in mv)	**Bulbar-Onset ALS(N=20)**	**Spinal-Onset ALS (N=36)**	**Total ALS (N=56)**
Mean APB	6.83 ± 4.07	3.94 ± 4.07	5.0 ± 4.07
Mean ADM	8.7 ± 4.18	6.37 ± 4.18	7.2 ± 4.18

APB / ADM RATIO	0.76 ± 1.08	0.56 ± 1.08	0.62 ±1.08
APB / ADM < 0.6	6 (30%)	20 (55%)	26 (46%)
APB /ADM > 1.4	1 (5%)	3 (8%)	4 (7%)

EMG: Table 5 describes the EMG evidence of LMN involvement as defined byAwaji Criteria. There was an involvement of bulbar region in 72% (41) of ALS patients, 84% (47) in cervicothoracicregion and 75% (42) in lumbo-sacral region. Out of total 20 bulbar onset ALSpatients, EMG evidence of LMN involvement was seen in 95% (19) patients, 65% (13) in cervico-thoracic region and 45% (9) in lumbo-sacral region. Out of total 36 spinal onset ALS patients, EMG evidence of LMN involvementwas seen in 61% (22) patients, 94%(34) in cervico-thoracic region and 92% (33) in lumbo-sacral region.

Table 5
Evidence of LMN involvement region wise by EMG

Segment	**Bulbar-Onset ALS (N=20)**	**Spinal-Onset ALS (N=36)**	**Total ALS (N=56)**
Bulbar	19 (95%)	22 (61%)	41 (73%)
Cervico-Thoracic	13 (65%)	34 (94%)	47 (84%)
Lumbar-Sacral	9 (45%)	33 (92%)	42 (75%)

Heavy Metal Analysis: In our study we observed that mean blood values in microgram per liter of Arsenic and Leadin ALS cases (2.18, 64.5 µg/l) were significantly higher than age matched controls(1.26, 39.8µg/l) with the significant p value of 0.00and 0.03 respectively, whereas mean blood level of Caesium in ALS cases (1.24µg/l) was lower than controls with (1.93µg/l) with a significant p value of 0.00 (Table 6 &Fig.5). Chromium,BariumandSemeanbloodlevelsinALSpatientswerelowerthancontrolsbutwithaninsignificantpvalue.Cadmium,MercuryandCobaltmeanbloodlevelswerehigherinALScomparedtocontrolswithaninsignificantpvalue.Therefore,higherlevelsofArsenicandLeadandlowerlevelsofCaesiumcorrelatedwithrisk ofdevelopingALS.

Table 6
Metal level in blood-plasma of ALS subjects and age-matched controls.

	Cases			**Controls**		
	Mean (ug/l)	**SD**	**N**	**Mean (ug/l)**	**SD**	**N**
ALS						
Arsenic***	2.18	1.24	56	1.26	1.26	42
Cadmium	0.58	0.34	56	0.46	0.46	42
Mercury	0.87	0.66	56	0.85	0.85	42
Lead *	64.51	59.25	56	39.81	47.77	42
Chromium	1.59	1.24	56	1.97	1.97	42
Barium	7.89	9.97	56	9.34	11.72	42
Cobalt	0.59	0.38	56	0.46	0.46	42
Caesium **	1.24	0.53	56	1.93	1.93	42
Selenium	144.31	49.08	56	148.57	148.57	42
Bulbar-Onset ALS						
Arsenic	2.05	1.20	20	1.45	1.33	20
Cadmium	0.47	0.22	20	0.48	0.44	20
Mercury	0.85	0.75	20	0.98	0.90	20
Lead	46.19	29.15	20	41.49	65.08	20
Chromium	1.63	1.40	20	2.11	1.36	20
Barium	7.35	11.14	20	6.39	10.48	20
Cobalt	0.63	0.49	20	0.50	0.31	20
Caesium **	1.10	0.58	20	2.14	1.20	20
Selenium	140.21	49.69	20	131.99	47.11	20
Spinal-Onset ALS						
Arsenic **	2.25	1.27	36	1.22	1.04	36
Cadmium *	0.64	0.38	36	0.44	0.42	36

Mercury	0.88	0.62	36	0.79	0.73	36
Lead *	74.68	68.97	36	40.00	51.15	36
Chromium	1.57	1.17	36	1.94	1.40	36
Barium	8.19	9.41	36	7.10	8.90	36
Cobalt	0.57	0.30	36	0.44	0.25	36
Caesium *	1.32	0.50	36	1.86	1.00	36
Selenium	146.59	49.30	36	137.02	54.68	36

* Statistically different at the level of $p < 0.05$, ** at $p \leq 0.001$, *** at $p \leq 0.0001$

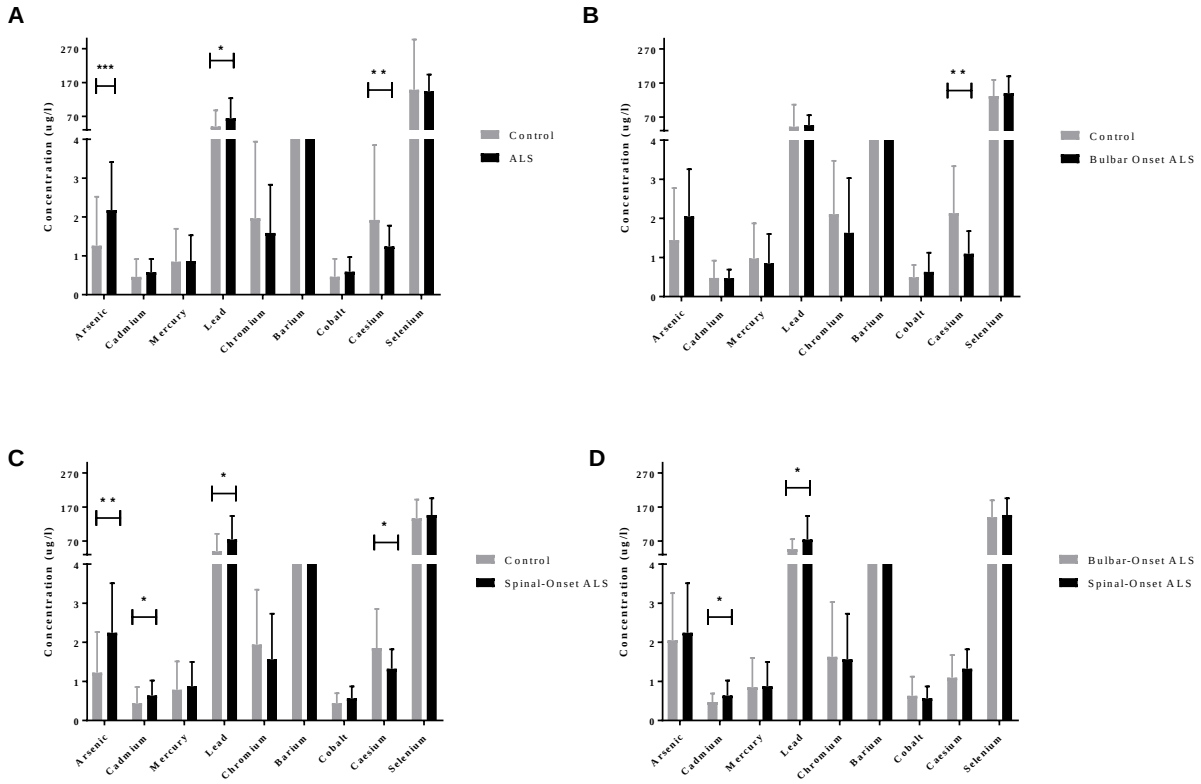

Fig. 5. A) Blood-plasma concentration of the heavy metals in ALS patients (N=56) versus age-matched controls (N=42). The data represents the mean blood concentration (µg/l) ± SD B) blood concentrations of the heavy metals in bulbar onset ALS patients (N=20) vs age-matched controls (N=20), C) heavy metals in spinal-onset ALS patients (N=36) vs age-matched controls (N=36), D) Inter-comparison between ALS patients with a bulbar-onset and those with a spinal-onset. * Statistically different at the level of $p < 0.05$, ** ≤ 0.001, *** ≤ 0.0001).

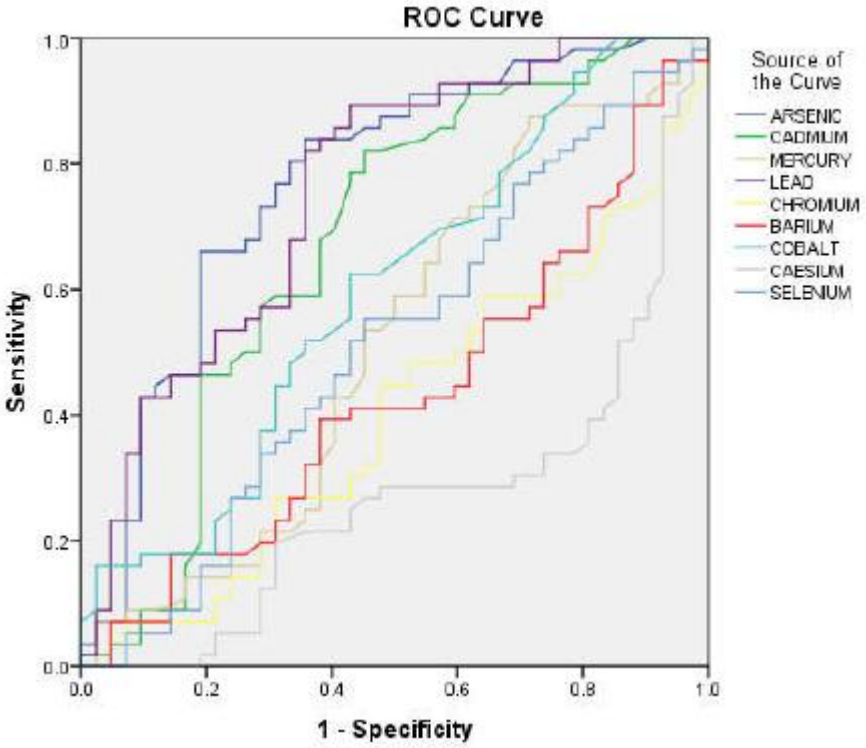

Fig.6. ROC curves for the measured heavy metals in ALS patients' blood-plasma.

As blood level>1.08µg/l had 84% sensitivity and 62% specificity in ALS. Lead blood level> 31.69µg/l had 82% sensitivity and 64% specificity. Cesium blood level> 1.8µg/l had 20% sensitivity and 69%specificity (Table 7).

Table 7
Cut off blood levels with corresponding sensitivity and specificity of arsenic, lead, cesium in ALS patients based on ROC curves.

	Arsenic (AUC-0.771)	**Lead (AUC-0.756)**	**Cesium (AUC-0.271)**
Blood Level (ug/l)	>1.08	>31.69	> 1.8
Sensitivity	84%	82%	20%
Specificity	62%	64%	69%

In spinal onset ALS, mean blood values in microgram per liter for Arsenic, Cadmium and Lead (2.25, 0.64, 74.68) were significantly higher than age matched controls (1.22, 0.44,40), with a significant p value of 0.00, 0.04 and 0.03 respectively (Fig. 5).Whereas mean blood levelof Cesium in spinal onset ALS patients (1.32) was lower than controls (1.86) with asignificantp value of 0.01. ChromiumbloodlevelsinspinalonsetALSpatientswaslowerthancontrolsbutwithaninsignificantpvalue.Mercury,BariumandSe mean blood levelswerehigher inALScomparedtocontrolswithaninsignificantpvalue.Therefore,higherlevelsofArsenic,Cadmium,Leadandlowerlevelsof Cesiumcorrelatedwithriskofdevelopingspinalonset ALS.
Table 8 reports thatbloodlevel>1.04µg/lhad83%sensitivityand61%specificityinspinalonsetALS,whereasCadmium>0.31ug/lhad81%sensitivityand62%specificity,Lead>31.69µg/lhad82%sensitivityand64%specificity.BloodCaesiumlevel> 1.8ug/lhad25%sensitivityand69% specificity.

Table 8
Cut off blood levels with corresponding sensitivity ad specificity of arsenic, cadmium, lead in ALS patients with spinal onset based on ROC curves.

	ARSENIC (AUC-0.79)	Cadmium (AUC-0.708)	LEAD (AUC-0.802)	CAESIUM (AUC-0.319)
Blood Level (ug/l)	>1.04	> 0.31	> 31.72	> 1.8
Sensitivity	83%	81%	89%	25%
Specificity	61%	62%	64%	69%

In bulbar onset ALS, mean blood values in micrograms per liter for Caesium (1.09) was lower than controls (2.13) with a significant p value of 0.001. Cadmium,Mercury,ChromiumandSemeanbloodlevelsinALSpatientswerelowerthancontrolsbutwithaninsignificant pvalue.Arsenic,Lead,Bariumandcobaltmeanbloodlevelswerehigherinbulbaronset ALScomparedtocontrolswithani nsignificantpvalue.Therefore,lowerlevelsofCaesiumcorrelatedwithriskofdevelopingbulbaronsetALS.
Based on coordinates of ROC curve Caesium blood level > 1.69µg/ml had 20% sensitivity
And 55% specificity in bulbar onset ALS(Figure 6).
Themeanbloodlevelsofarsenic,cadmium,Lead
andCaesiumwerehigherinspinalonsetcomparedtobulbaronsetALSwithasignificantpvalueof0.04forcadmiumandlea d.Therefore,highbloodlevelsoflead andcadmiumareassociatedwithspinal onset ALScomparedto bulbar onset.(Figure 3, E).

V. Discussion

This is the first study on ALS from North coastal Andhra Pradesh from a single center. The epidemiological, clinical and heavy metal blood levels in ALS patients in this study were compared with other studies from India, other developing countries and developed western ones.

In our study out of 56 patients with ALS, M: F ratio was 2.3:1. 64.3 % (36) were > 50 years of age (M: F, 1.6:1), 26.8 % (15) were between 40-49 year age (M: F, 4:1), 9% were all males < 40 years of age. According to a study by McGuire et al.[14] M: F in sporadic spinal ALS was 1.2-1.4: 1 with a slight female preponderance in the bulbar-onset variant with a peak incidence in 65-74-year age group. In the Saha et al.[60] study the M: F ratio was 4.3:1, with a maximum number of patients between 41-50 years of age. Our study is different from Saha et al, a majority of our patients were > 50years of age with less male preponderance. However, in Nalini et al.[9] study was done on 1153 ALS patients over 30 years, and M: F ratio was 3:1 with 50% between the age of 40-60year which is almost similar to our study.

In our study, the mean age of presentation of symptoms of ALS was 51.7+/- 15.5 years. Whereas in Nalini et al.[9] study it was 47.5+/- 13.9 years. This is almost a decade earlier as compared to other western studies (60.7-64.3 years).[3-7] .In our study, males with bulbar onset presented approximately 4 years earlier than females whereas in Nalini et al.[9] study females with bulbar onset presented 1 year earlier than males. In our study, the mean age of presentation of spinal onset ALS was approximately 5 years later than Nalini et al. study (48.6 vs 43.7). In our study, the mean age of presentation of spinal onset ALS was 47.4 and 48.7 year in males and females respectively, as compared to Nalini et al. study where it was 44 years for both males and females.

In our study, the mean duration of symptom onset to diagnosis in ALS patients was 15.2 months which was slightly lower (17.7 months) than Nalini et al.[9] but quite similar to many western population studies (10.8-16.9 months).[3-7]In our study, the mean duration of symptom onset to diagnosis in bulbar-onset ALS was 3.5 months earlier than Nalini et al. study[9] (8.7 vs 12.3 months), while in spinal on ALS it was almost similar in both studies (19.6 vs 19.7 months). In many studies from the USA, South East England, Italy and Ireland the average time from symptom onset to diagnosis was slightly earlier (10.8-16.9 months) when compared to our study.[3-7]

In our study out of 56 ALS patients, 36% had bulbar onset ALS which was higher as compared to Nalini et al.[9] study (27%) but similar to Saha et al.[60] (33.3%). Chopra et al.[61] study reported 29% bulbar onset ALS cases. In our study, M: F in bulbar-onset ALS was 1.5:1(similar to Nalini et al.[9], 1.6:1) and spinal onset ALS was 3:1 (similar to Nalini et al.9, 3.7:1). According to western literature[14]slight female preponderance was seen in bulbar-onset ALS which was not there in our study. Spinal onset ALS was seen in most of our cases (64%) and the majority had onset in upper limbs which were similar to Norris et al.[82] and Nalini et al.[9] studies.

In our study, the symptoms of thinning of limbs, twitching of muscles, limb stiffness and dysarthria were similar to Saha et al.[60], but dysphagia and nocturnal cramps were complained by fewer patients in our study. 18% of ALS patients in our study complained of fatigue and 11% complained of bladder symptoms, predominantly urinary urgency, hesitancy, and incontinence. These bladder symptoms might reflect the degeneration of Onuf's nucleus in the sacral spinal cord, with relative sparing of adjacent somatic motor neurons as it may occur in minority of patients in later stages of the disease. There was no full data from Nalini et al.9 There was no full data form Nalini et al.9 study regarding various symptoms of ALS as it was a retrospective study over 30 years. 5% of ALS patients in our study presented with executive and behavioral disturbances which were similar to cognitive disturbances in most series.50, 51

In our study, 2/3rd of the bulbar onset ALS patients presented with complains of dysarthria which was less than Nalini et al. [9]study. However, dysphagia at disease onset was higher in our study, on the other hand, the percentage of patients presenting with both dysarthria and dysphagia were similar in both studies. This difference might be due to less number of bulbar onset patients in our study (n=20) compared to Nalini et al.

Percentage of ALS patients with muscle weakness and atrophy in our study was similar to the above two studies. More than 2/3rd of ALS patients in our study had tongue fasciculation as compare to 1/5th in Nalini et al. and 50% in Saha et al. 63% of our patients had polyminimyoclonus as compared to nearly 40% in Nalini et al. Similar number of patients showed neck weakness in our study and Nalini et al study, whereas neck weakness was seen in 16% of patients in Saha et al. study. Facial and bulbar weakness was reported in similar numbers in all 3 studies. 25% of the patients in our study showed no evidence of cranial nerve involvement clinically. In > 50 % of ALS patients in our study, there was brisk jaw jerk which was higher than reported in the above two studies. The planter was extensor in about 50% of the ALS patients in all 3 studies including Norris et al.[82] study. In about 25% of ALS patients, the planter was mute in both our study and Saha et al. study.

In our study, more than 50% of ALS patients had oblique forearm wasting sparing brachioradialis and split hand sign. However, less than 1/10th had the reverse split hand sign clinically. These clinical features were not reported from other Indian studies.

Atypical features like deafness were reported in 2% of ALS patients by Nalini et al.9 However, no patient reported deafness in our study and Saha et al.[60] study. Sensory abnormalities in the form of paresthesias in feet were present in 1.8% of patients in our study, that is similar to Nalini et al.[9] and Bradly et al.[49] studies. One patient with paresthesia in our study had co-existing diabetes. Unexplained unilateral 3rd nerve and vertical gaze palsy were seen in one patient each in our study which was rarely described in the literature.[47, 48]

In our study, the mean CMAP of APB and ADM was higher than Singh RJ et al. study. The mean APB/ADM ratio of ALS patients in our study was almost equal to the landmark study done by Kuwabara et al study in Japan. APB/ADM <0.6 was seen in the similar percentage of patients in our study and in Kuwabara et al study, whereas the APB/ADM ratio was higher in Singh RJ et al. study. Interestingly, in our study, APB/ADM >1.4 was found in 7% of ALS patients. Our study did not compare the above variables of NCS with normal and other disease controls because the association of ALS with electrophysiological correlation of split hand sign (APB/ADM<0.6) was proven beyond doubt by Kuwabara et al. There was evidence of sensory neuropathy in 1.8% of ALS patients in our study, however, one of them had diabetes.

Certain muscles and muscle groups seem to be vulnerable to LMN loss early in the course of ALS and PMA and can be considered to be "index" muscles during the diagnostic process. Intrinsic hand muscles have received the most attention, in particular, those in the lateral hand (first dorsal interosseous and thenar group muscles). Both lower motor neuron and cortical dysfunction hypotheses have been postulated for split hand sign. Humans use more of the pincer grasp (involving the APB and FDI muscle) which places more oxidative stress/metabolic demand on the spinal motor neurons innervating these two muscles. Also, the corticospinal connections to APB and FDI far outnumber those of the ADM. This may result in more glutamate excitotoxicity to the APB and FDI spinal neurons.[54-56]Although the pattern of a dissociated or split hand is also seen in other disorders (normal aging, spinal muscular atrophy, spinocerebellar ataxia type 3,56 in the appropriate clinical context, atrophy, and weakness of these muscles are strongly supportive of ALS and PMA.[60,61]

In our study, Awaji criteria were used for diagnosis of ALS. A high percentage of our patients had definite ALS in our study similar to above-mentioned studies from India and Italy. Most of these patients were sent on a referral basis at a late stage of disease course and diagnosis of definite ALS was made in many patients before they visit our hospital.

The baseline characteristics of cases and controls in the above-mentioned studies were totally different from our study because of different geographical location. VALE study was conducted among US veterans and De Benedetti et al study among defined geographical area in Italy where acid mine drainage was reported.

The mean blood lead level of ALS cases was higher compared to controls in all the above-mentioned studies with a statistical significance between the means in the only VALE and our study. High level of lead was reported in De Benedetti study probably due to acid mine drainage exposure in that area. Wang et al 19 meta-analysis concluded that exposure to chronic occupational lead exposure carries a high risk of developing ALS with an odds ratio of 1.81 and level B strength of evidence.

An association between lead exposure and ALS is a long-standing hypothesis. Most previous studies have supported this relation but in general, have relied on indirect measures of lead exposure. Blood lead levels may reflect both current environmental lead exposure and mobilization of lead from bone. The distribution of lead between blood and bone may change during ALS progression as a patient's level of physical activity declines. VALE study had also taken bone turnover into account by using plasma markers of bone formation (CTX, ALA dehydrogenase etc.).

There was no occupational exposure to lead in any of the 56 ALS patients in our study. Further analysis of lead levels in CSF in ALS cases, water, air, soil samples from common point sources may give some clue regarding the exposure route.

On comparing our study with De Benedetti et al. the mean blood levels of heavy metals in cases and controls were low except that of Se.

Our study had higher levels of arsenic in cases compared to controls with a significant p-value. This implies that high arsenic blood level had an association with ALS. Though mercury and cobalt had higher levels in cases, they didn't have statistical significance. Chromium and Se blood levels were high in controls in both studies without statistical significance.

There was no evidence of skin changes or neuropathy attributable to arsenic exposure in our study. Arsenic-induced neurotoxicity causes changes in cytoskeleton composition and hyperphosphorylation, leading to dysregulation of the cytoskeletal framework causing neurodegeneration. Chronic Arsenic toxicity is a global environmental health problem affecting millions of people worldwide. Outbreaks of chronic arsenic poisoning occurred in states of West Bengal, Bihar, and Uttar Pradesh through consumption of drinking water. Arsenic is also released into the environment by melting of various metals, combustion of fossils or fuels, as herbicide or fungicide in agricultural products. Long-term effects of arsenic in ALS was least studied in humans. According to our study result, there is an imminent need to study arsenic levels in drinking water in the affected patient area.

In our study, the mean blood level of Cesium in ALS patients was less compared to controls with the statistical significance indicating that low blood level of cesium was associated with ALS. This finding was contrary to Khare SS et al.[85]study in which they have shown a high level of cesium in erythrocytes of ALS patients. Some unpublished studies from the Massachusetts Institute of Technology (MIT, USA) revealed that cesium levels from postmortem samples of ALS patient's spinal cord and cerebellum were two times the amount found in controls. Atomic bomb exposure, spray paints, fuel combustion and water are some of the potential sources of cesium toxicity described in the literature.

In our study, the mean blood levels of Se were less compared to controls without any statistical significance. This was similar to Moriwaka et al.40study where an inverse relationship between disease progression and both serum and erythrocyte levels of Se was found. Excessive exposure to Se, a trace element with both toxicological and nutritional properties, has been implicated in the etiology of ALS. Two remarkable epidemiological investigations, which documented an increased risk of ALS in populations residing in areas with increased Se exposure, have allowed for supporting a probable causal link between Se exposure and ALS risk. The former epidemiological study was carried out by Kilness and Hochberg[38], who reported 4 cases of ALS in a scarcely populated county of the west-central South Dakota, characterized by a high incident of selenosis in farm animals. Therefore, the association between the high Se environmental levels and the numerous ALS cases detected in this area was supportive of the causal relationship between SE exposure and ALS. The latter epidemiological analysis was performed by Vincet et al.[39]in the Northern Italy municipality of Reggio Emilia, where high levels of Se were detected in the waters of two wells, which were the source of municipal tap water from 1972 to 1988. A follow-up study, carried out for 11 years in a cohort of 5182 inhabitants who drank this high Se tap water at least for 5 years revealed an increased ALS risk in this municipality (i.e. 4 newly diagnosed ALS cases, compared to 0.64 expected ones).

Our study is the first to compute a cut off blood levels of heavy metals associated with ALS. Arsenic blood level > 1.08µg/l and Lead>31.69 had > 80% sensitivity and 60% specificity to assess ALS association. Cesium > 1.8 µg/l had 20% sensitivity and around 70% specificity. More population-based studies in large numbers are required to calculate the odds ratio and to prove the role of heavy metals in the causation of ALS.

For the first time in our study blood levels of heavy metals in patients with bulbar-onset ALS and spinal onset ALS were compared along with controls. In addition to the association that was found in total ALS group with Arsenic, lead and Caesium, interestingly there was a positive association with high blood levels of Cd with a statistical significance in spinal onset ALS patients when compared to controls.

Vinceti et al.[41] finding lends limited support to a possible involvement of Cd, but not lead or Selenium in the etiology of sporadic ALS. Several compounds including heavy metals such as cadmium or lead have been identified in tobacco and cigarette smoke as well as residues of pesticides used in tobacco cultivation. The toxicity of Cd as food and cigarette smoking contaminant or industrial pollutant has been well established.[42] With regard to Cd involvement into neurodegenerative mechanism; Huang et al.[43]have demonstrated that Cu/Zn SOD1 activity may be strongly inhibited by Cd. In support of this, it has also been demonstrated that cadmium can replace Zn to reduce the SOD activity. Although heavy occupational exposure to Cd has been associated with the development of sporadic ALS44, some epidemiological evidence found limited support to a possible involvement of Cd or other trace heavy metals in the etiology of sporadic ALS35, [41]. So, there is a need for research in a large number of smokers and tobacco farmers to prove any causation of Cd in ALS.

In our study, on comparing the blood levels of heavy metals in the patients of bulbar onset ALS to Spinal onset ALS, low level of Cs was found to be associated with bulbar onset ALS with statistical significance. In addition, we found that those patients with spinal onset ALS had higher Cd and Pb levels with statistical significance. Hence, High blood levels of As, Cd, Pb and low levels of Cs were associated with ALS. Higher blood levels of Cd and Pb were associated with spinal onset ALS compared to bulbar onset ALS. Even though there have been many studies, proposed theories and case reports investigating the role of heavy metals in the blood, CSF, hair, nails and postmortem tissues of ALS patients but unfortunately, many of them had contradictory or indefinite findings.

VI. Conclusion And Summary

- ALS is the most common MNDs variant followed by MMA.
- Mean age of onset of ALS was a decade early when compared to the western population with high male preponderance.
- Atypical clinical features like bladder involvement, gaze palsy,3rd CN palsy, and sensory neuropathy are associated occasionally with ALS.
- In the appropriate clinical setting, electrophysiological evidence of split hand sign (CMAP ratio of APB / ADM < 0.6) is strongly supportive of ALS.
- The incidence of BMMA was high compared to other Indian studies with high male preponderance.
- The electrophysiological correlate of reverse split hand sign aids in the diagnosis of BMMA.
- There is an imminent need to identify and study the group of BMMA with electrophysiological correlate of split hand sign as a separate entity, as they may progress to ALS in near future.
- Electrophysiologic correlation with clinical signs aids in the diagnosis of MND & its variants and gives less margin of error in ruling out their mimics which are potentially treatable.
- Higher blood levels of As, lead and lower levels of Cs are associated with ALS in our study.
- Spinal onset ALS had an association with high blood levels of Cadmium and Lead compared to bulbar onset.
- Blood Arsenic > 1.08 ug/l and Lead > 31.69 ug/l had sensitivity of > 80%, with specificity of > 60% in ALS patients.
- High blood levels of Arsenic, Cadmium, Lead and low levels of Chromium, Barium, Caesium might be probably associated with MNDs regional variants like BMMA, CMMA and BAD.
- A high blood level of Caesium in patients with < 2 years duration between symptom onset to diagnosis might be associated with MNDs.

In conclusion, from the results of our study, ALS is the most common MND variant with a decade early age of onset compared to the western world. MRI imaging is necessary to identify other treatable mimics of MNDs and in establishing the diagnosis of BMMA. Electrophysiological evidence of split and reverse split hand sign plays a pivotal role in identifying ALS and BMMA variants.

The role of metal exposures in the pathogenesis of sporadic ALS is still unclear. It is more likely that all these metals interact with one another with an additive or synergistic effect along with epigenome of patients. A further heavy metal analysis must be carried out in a large population-based multicenter study, along with assay in common exposure sources like drinking water, air, soil, occupational areas for an in-depth investigation on their possible involvement in the pathogenesis and in course of the MNDs that can open a door to new therapeutic targets.

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