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Predictors of seizure in Wilson disease: A clinico-radiological and biomarkers study

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ABSTRACT

Background: There is paucity of studies on predictors of seizures in Wilson disease with neurological manifestation (WDNM), and none has evaluated the role of copper (Cu) induced oxidative stress, proinflammatory and excitotoxicity in the genesis of seizure.

Objectives: To report frequency, refractoriness, and outcome of seizure in WDNM. We also evaluate role of Cu induced oxidative stress, excitotoxicity and cytokines in predicting seizures.

Methods: The diagnosis of WDNM was based on clinical, MRI, KF ring and 24 h urinary Cu. Detailed clinical examination including severity of WD, occurrence of seizure, seizure semiology, antiepileptic drug (AED) and breakthrough seizures were noted. Cranial MRI and electroencephalography findings were noted. Serum free-Cu, oxidative stress markers (glutathione, total antioxidant capacity, malondialdehyde), glutamate and cytokines (interleukin 6, 8 and 10 and tumour necrosis factor α) were measured by atomic absorption spectrophotometer, spectrophotometer, fluorometer and flow cytometer respectively, and correlated with seizures. Patients were treated with zinc with or without penicillamine, and those with epilepsy received second-generation antiepileptic drugs (AEDs).

Results: Out of 110 patients with WDNM, 16(14.5%) had seizures; focal in 11(68.7%) and generalized in 5(31.3%). Patients with seizure had higher serum free-Cu (35.87 ± 1.34 vs 31.72 ± 0.68; P = 0.02), severe dystonia (P = 0.04), and more frequent cortical (100% vs 6.4%; P < 0.01) and subcortical (81.3% vs 20.2%; P < 0.01) lesions on MRI compared to those without seizure. Oxidative stress markers (glutathione, total antioxidant capacity, malondialdehyde), cytokines and glutamate were elevated in WDNM compared to controls. On multivariate logistic regression analysis, cortical involvement (OR = 105.49; 95%CI = 8.74–1272.39; P < 0.01) and number of MRI lesions (OR = 1.99; 95% CI = 1.11–3.57; P = 0.02) were independent predictors of seizure. The seizures were controlled with single and dual AEDs in seven patients each, and two patients needed three AEDs. All the patients had seizure remission for a median follow up of 66(24–180) months.

Conclusion: About one-sixth WDNM patients have seizures especially in those with cortical and extensive MRI lesions. Seizures are easily controlled by AEDs.

1. Introduction

Wilson disease (WD) is an autosomal recessive metabolic disorder due to the ATP7B mutation in chromosome 13q14.3 (Bull et al., 1993). There is impaired excretion of copper (Cu) from the hepatocytes into bile. Failure to incorporate Cu into ceruloplasmin leads to accumulation of Cu in various organs mainly in liver, cornea, lens and brain (Kluska et al., 2018; Thomas et al., 1995). In normal individuals, circulating free Cu constitute 5–10% which is increased to several folds in WD. Free Cu induces oxidative stress which releases cytokines and glutamate (Brewer, 2008; Goyal et al., 2008; Kalita et al., 2014). In animal studies on Cu toxicity, increase in oxidative stress, and expression of caspase-3 and glutamate excitotoxicity have been reported (Jiang et al., 2015; Kalita et al., 2018a,b; Pal et al., 2013). Reduced Cu is highly reactive

Abbreviations: ADL, Activities of daily living; AED, Antiepileptic drugs; BFM, Burke-Fahn-Marsden; CLD, Chronic liver disease; Cu, Copper; DWI, diffusion weighted imaging; GSH, Glutathione; KF, Kayser-Fleischer; LPO, Lipid peroxidation; MRI, Magnetic resonance imaging; MDA, Malondialdehyde; MMSE, Mini Mental State Examination; ROS, Reactive oxygen species; TAC, Total antioxidant capacity; TNFα, Tumour Necrosis Factor-alpha, IL:Interleukin, WD:Wilson Disease; WDNM, Wilson Disease with neurological manifestation

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and can cross the blood-brain barrier leading to neuronal and glial damage directly. Highly reactive brain tissues are more vulnerable to oxidative injury (Wang and Michaelis, 2010). Cranial imaging reveals the involvement of corpus striatum and substantia nigra more commonly than cortical or subcortical involvement (Ranjan et al., 2015; Sinha et al., 2006). Epidemiological studies have reported epilepsy in 6.2% to 8.3% patients with Wilson disease (Czlonkowska et al., 2018; Dening et al., 1988; Prashanth et al., 2010). A hospital registry revealed 10 times increase in seizure frequency in the patients with WD compared to general population (Dening et al., 1988). Only a few studies have evaluated the role of clinical severity of WD, MRI changes and biochemical findings in the occurrence of seizures (Dening et al., 1988; Machado et al., 2006; Prashanth et al., 2010). In a large cohort of 490 patients with WD, seizure occurred in 8.3% and related to subcortical white matter involvement on MRI (Prashanth et al., 2010). None of the studies has evaluated the relationship between oxidative stress, cytokines, and glutamate in the genesis of seizures in WD. Glutathione (GSH) is a water-soluble antioxidant and present in almost all the cells (Farinati et al., 2003; Sies, 1999). Total antioxidant capacity (TAC) measures the overall antioxidant status of the body (Bruha et al., 2012; Koracevic et al., 2001). Malondialdehyde (MDA) is an end product of lipid peroxidation and suggests the status of oxidative stress (Dalgic et al., 2005; Janero, 1990). Increased oxidative stress may result in the liberation of various pro-inflammatory cytokines and glutamate. All these biochemical changes in the brain may induce cellular damage and alter cell excitability resulting in various clinical manifestations. In this study, we report the frequency of seizures in a cohort of WD with neurological manifestation (WDNM) patients, and evaluate the role of clinical severity, MRI changes, biomarkers of oxidative stress, inflammation, and excitotoxicity in predicting the occurrence of seizure.

2. Subjects and methods

The study was conducted in a tertiary care teaching hospital in Uttar Pradesh, India during 2010 to 2017. This is a retrospective analysis, and the data were extracted from a prospectively maintained WDNM registry. The history and clinical examinations were verified by one of the two senior neurologists (JK or UKM). The diagnosis of WD was based on clinical features, serum ceruloplasmin (< 20 mg/dl), urinary Cu (> 40 μg m/24 h) and Kaiser-Fleisher ring on a slit lamp examination (European Association for Study of, 2012; Ferenci, 2017)

2.1. Clinical evaluation

A detailed medical history including demographic information, pedigree chart, age at onset of neurological symptoms, duration of illness, history of liver dysfunction and haemolytic anaemia was taken. Cognitive functions were evaluated using Mini-Mental State Examination (MMSE) scale and was considered abnormal if the MMSE score was below the education norms (Crum et al., 1993). Presence of movement disorders such as dystonia, chorea, athetosis, myoclonus or tremor was noted. Dystonia severity was scored as per Burke-Fahn-Marsden (BFM) grading scale (Krystkowiak et al., 2007). The neurological severity of WD was graded as grade 0 to grade III based on 5 signs including ataxia, bradykinesia, dysarthria, dystonia and tremors (Grimm et al., 1991). Severity was graded as 0 when no symptoms; grade I when symptoms were mild, grade II when symptoms were moderate, and grade III when patients were dependent and had severe manifestations (Grimm et al., 1991; Kalita et al., 2011; Wiles, 1990). The occurrence of seizures was noted including semiology, frequency, duration, and response to the antiepileptic drugs. Status epilepticus was defined if the generalized convulsion continued for more than 5 min or if there are recurrent seizures without regaining consciousness in between (Trinka et al., 2015; Kalita et al., 2018a,b). Presence of breakthrough seizure was also noted during the follow-up.

2.2. Investigations

Blood counts, haemoglobin, erythrocyte sedimentation rate, liver and kidney function tests, serum albumin, calcium, alkaline phosphatase, sodium, potassium and serum ceruloplasmin were measured. Serum and urinary Cu were measured using atomic absorption spectrophotometer. Free serum Cu was calculated by subtracting three times the serum ceruloplasmin level (mg/dl) from the total serum Cu level (μg/dl). Abdominal ultrasound was done for evidence of chronic liver disease including alteration in liver echotexture, portal vein diameter, splenomegaly or ascites. Cranial MRI was done using a 3T-MRI scanner (Signa GE Medical System, Wisconsin, USA). T1, T2, FLAIR, and DWI sequences were obtained, and the location and extent of abnormal signal changes were noted.

2.3. Measurement of oxidative stress markers, cytokines, and glutamate

Five ml venous blood was collected in each EDTA and plain vials. Plasma and serum were separated and kept in −80 °C till analysed. GSH, TAC, and MDA were measured by spectrophotometer (Janero, 1990; Koracevic et al., 2001; Tietze, 1969). Serum glutamate was measured using fluorescence microplate reader (Invitrogen, USA) and cytokines (tumour necrosis factor α, IL-6, IL-8 and IL-10) were assayed by flow cytometer with the bead-based immunoassay (BD Biosciences, USA). These biochemical parameters were also measured in age and gender-matched controls; 64 controls were included for oxidative stress markers and glutamate, and 44 for cytokines (Supplementary Table S1).

2.4. Treatment

Patients were treated with penicillamine in a dose of 250 mg daily and the dose was increased to 250 mg every 2 weeks up to thrice daily. If there was any deterioration, penicillamine was stopped. Elemental zinc in a dose of 50 mg thrice a day was prescribed (Czlonkowska and Litwin, 2017; European Association for Study of, 2012; Litwin et al., 2017). Vitamin C and E were also used in some patients. Patients on penicillamine also received pyridoxine 20 mg daily. Vitamin D and calcium supplementation were given in those with low serum calcium and high alkaline phosphatase. The patients with seizures were treated with the antiepileptic drugs (AED) (carbamazepine, gabapentin, phenytoin, levetiracetam or clobazam). Dystonia was treated with trihexyphenidyl, baclofen and tetrabenazine in isolation or in various combinations.

2.5. Statistical analysis

Normality of data was tested by Shapiro-Wilk test. The demographic, clinical, biochemical and MRI findings between the patients with and without seizures were compared using the chi-square test/Fisher's exact test for categorical and independent t or Mann-Whitney U test for continuous variables. The oxidative stress markers, glutamate and cytokines among WD with/without seizure and controls were compared using one-way analysis of variance using Tuckey post Hoc correction. The univariate binary logistic regression analysis was used to identify the variables responsible for the seizure occurrence. All the significant variables found in the univariate analysis have been included in the multivariate binary logistic regression model. Multivariate analysis was done using backward Wald method with the confidence interval of 95% to find out the final model. A variable having a p-value of < 0.05 was considered statistically significant. The statistical analysis was done using Statistical Package for Social Sciences, IBM SPSS Statistics, Version 23.0. Armonk, NY: IBM Corp.

3. Results

There were 110 patients with WDNM whose age ranged between

8–40 (median 14) years, and 25 (22.7%) were females. The age of onset of neurologic symptoms ranged between 5 and 40 (median 12.25) years. The family history of WD was present in 33 (30%) patients. All had movement disorders, which include dystonia in 97(89%), myoclonus in 6 (5.5%), choreoathetosis in 8 (7%) and tremor in 2 (25%) patients. History of jaundice was present in 35 (32%) patients but evidence of liver dysfunction was detected in 60 (55%) patients by ultrasound of abdomen and 39 (35.5%) by liver function tests. Serum bilirubin was raised in 21(18.5%) and SGPT in 40 (36%) patients. Coagulation (INR) abnormality was detected in 39 (35.5%) patients. Cranial MRI was done in 82/110 (74.5%) patients and was abnormal in 77 (94%). The abnormal signal changes were located in caudate in 63(77%), putamen in 76 (92%), globus pallidus in 72(87%), brainstem in 49(60%), cerebellum in 9 (11%), subcortical white matter in 27(33%) and cerebral cortex in 22 (26%) patients.

3.1. Seizures

Sixteen (14.5%) patients had seizures, which were focal in 11(68.7%) and generalized in 5 (31.3%) patients. Five (31.3%) of these patients had status epilepticus, and it was the presenting symptom in one patient. Six patients had seizure at presentation, and 10 had while on treatment for WD. Interictal electroencephalogram (EEG) revealed epileptiform discharges in 10 out of 16 patients. The seizures were controlled with single AED in seven; dual AEDs in seven and two patients needed three AEDs. All the patients had seizure remission for a median of 66 (24–180) months. Five patients on single AED had breakthrough seizures, in whom seizures were controlled after increasing the dose of AED. The details of AEDs are summarized in Table 1.

3.2. Correlation of seizure with clinical, laboratory and MRI parameters

The patients with seizures had higher BFM score (70.06 ± 6.63 vs 54.50 ± 2.39; P = 0.04) and serum Cu level (35.87 ± 1.34 vs 31.72 ± 0.68 mg/dl; P = 0.02) compared to those without seizure.

Table 2
Demographic, clinical and biochemical parameters of patients with Wilson disease who had seizures compared to those without seizures.

Wilson disease (N = 110)		Seizure Present (n = 16)	Seizure Absent (n = 94)	p value
Gender (Female/Male)		2/14	23/71	0.52
Age (years)		14.94 ± 1.11	16.34 ± 0.72	0.44
Duration of illness (months)		31.53 ± 11.41	18.77 ± 2.40	0.09
Severity grade				p value
	I	–	16	–
	II	11	51	0.41
	III	5	27	1.00
SGOT(mg/dl)		54.19 ± 8.71	51.89 ± 3.96	0.81
SGPT (mg/dl)		49.25 ± 8.43	42.79 ± 4.38	0.50
S. Bilirubin (mg/dl)		0.79 ± 0.06	0.76 ± 0.03	0.85
S. Creatinine (mg/dl)		0.82 ± 0.04	0.90 ± 0.03	0.06
Ceruloplasmin (mg/dl)		7.54 ± 0.83	8.61 ± 0.30	0.19
Hemoglobin (gm/dl)		11.89 ± 0.04	11.99 ± 0.19	0.81
S. Cu (mg/dl)		35.87 ± 1.34	31.72 ± 0.68	0.02
Urinary Cu (μg/24 hr)		144.44 ± 10.64	127.39 ± 4.02	0.15
BFM Score		70.06 ± 6.63	54.50 ± 2.39	0.04

Mean ± SEM; BFM = Burke–Fahn–Marsden; Cu = copper, S = serum; SGOT = Serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase.

The age, gender, global severity, neurological severity, liver and kidney function tests and electrolytes were not different in the patients with and without seizures (Table 2). Cranial MRI revealed higher frequency of cortical (100% vs 6.4%; P < 0.005), subcortical white matter (81.3% vs 20.2%; P < 0.05) and cerebellar (25%) involvement, and number of MRI lesions (8.88 ± 1.03 vs 7.08 ± 2.36; P < 0.01) in the patients with seizures compared with no seizure (Table 3 & Fig.1).

In WD patients, oxidative stress markers (GSH, TAC, and MDA), cytokines (IL6, IL8, IL10 and TNFα) and glutamates levels were higher compared to healthy controls (Table 4). However, these markers were not different between the patients with and without seizures (Table 4). On multivariate analysis, cortical involvement (OR = 105.49; 95%

Table 1
Clinical radiological and antiepileptic drugs in the patients with seizures.

Case. No	Age (years)	Sex	Severity Grade	MRI Findings	AED (Total Dose/day)
1	18	M	III	B/L T2 hyperintensity in caudate, globus pallidus and thalamus	Gabapentin (900 mg)
2	20	M	II	B/L T2 hyperintensity in putamen, Globus pallidus with frontal cortex involvement	Levetiracetam (1000 mg), Clobazam(40 mg)
3	20	M	III	B/L T2 hyperintensity in putamen and globus pallidus with bilateral involvement of frontal lobe	Clobazam (10 mg) Levetiracetam (1500 mg) Carbamazepine (600 mg)
4	10	F	II	B/L T2 hyperintensity in putamen with frontal cortex involvement	Sodium Valproate (600 mg)
5	28	M	II	B/L T2 hyperintensity in putamen thalamus with frontal cortex involvement	Gabapentin (600 mg) Phenytoin (300 mg)
6	16	M	III	B/L T2 hyperintensity in midbrain, thalami putamen and large high frontal cortical lesion	Levetiracetam (1500 mg) Clobazam (20 mg)
7	31	M	II	B/LT2 hyperintensity in putamen thalamus with frontal cortex involvement	Phenytoin(300 mg)
8	12	F	II	B/L T2 hyperintensity in Putamen thalamus, midbrain pons and white matter with frontal cortex involvement	Sodium Valproate (600 mg)
9	20	M	II	B/L T2 hyperintensity in Putamen thalamus with frontal cortex involvement	Levetiracetam (1500 mg) Carbamazepine(600 mg) Clobazam (10 mg)
10	23	M	II	B/L T2 hyperintensity in Putamen thalamus with frontal cortex involvement	Carbamazepine (600 mg)
11	26	M	III	B/L T2 hyperintensity in midbrain thalami putamen and large high frontal, parieto-occipital cortical lesion	Levetiracetam (1500 mg) Clonazepam(1 mg)
12	16	M	III	B/L T2 Hyperintensity in Putamen thalamus with frontal cortex involvement	Levetiracetam (1500 mg)
13	20	M	II	B/L T2 hyperintensity in putamen thalamus with frontal cortex involvement	Oxcarbazepine (900 mg) Clonazepam (1 mg)
14	20	M	II	B/L T2 hyperintensity in Putamen thalamus with frontal cortex involvement	Levetiracetam (1000 mg) Clobazam(15 mg)
15	21	M	II	B/L T2 hyperintensity in putamen, thalamus with frontal cortex involvement	Levetiracetam (1000 mg)
16	15	M	II	B/L T2 hyperintensity in Putamen, thalamus midbrain with frontal cortex involvement	Gabapentin(600 mg) Sodium valproate(750 mg

AED = Antiepileptic drug; BL = bilateral; F = female; M = male; MRI = magnetic resonance imaging.

Table 3
Comparison of cranial MRI findings between the patients with Wilson disease with and without seizures.

Brain region Involved	Seizure Present (n = 16)	Seizure Absent (n = 94)	P value
Thalamus	16 (100%)	57 (60.6%)	0.197
Globus Pallidus	16 (100%)	57(60.6%)	0.197
Putamen	16 (100%)	61(64.9%)	0.591
Caudate	11 (68.8%)	53(56.4%)	0.51
Brain stem	9(56.3%)	40(42.6%)	1.00
Cerebellar	4(25%)	5(5.3%)	1.00
White matter	13(81.3%)	19(20.2%)	< 0.01
Cortex	16(100%)	6(6.4%)	< 0.01
MRI Lesion load (Mean ± SEM)	8.88 ± 0.26	7.08 ± 0.24	< 0.01

CI = 8.74–1272.39; P < 0.01) and number of MRI lesions (OR = 1.99; 95% CI = 1.11–3.57; P = 0.02) were independent predictors of seizure after adjusting for serum Cu, BFM score, subcortical white matter and cerebellar lesions on MRI.

4. Discussion

In our study, 14.5% patients with WDNM had seizures, and it was related to the severity of disease as evidenced by BFM score, serum Cu, and MRI evidence of cortical, subcortical and cerebellar involvement. The prevalence of seizure in WD although higher than the general population (Dening et al., 1988; Prashanth et al., 2010), but is less common than encephalitis, cerebral venous sinus thrombosis, intracranial haemorrhage, infections and head injury (Misra and Kalita, 2011; Rajshekhar, 2016; Silverman et al., 2002). Involvement of the cortical region was seen in almost all of our patients with seizure. The lower frequency of seizure in WD compared to CNS infection and cerebral venous sinus thrombosis may be due to chronicity of WD, and location of brain pathology in deep grey and white matter. The MRI studies in WDNM have revealed involvement of putamen in 64–86%, caudate in 33–67%, midbrain 41–77%, pons in 4–26%, globus pallidus in 38–81%, thalamus 54–60%, cerebral cortex in 12–59%, subcortical white matter in 30–59% and cerebellum in 12–50% patients (Kim et al., 2006; King et al., 1996; Litwin et al., 2013; Ranjan et al., 2015; Sinha et al., 2007).

Nearly all the patients in our cohort with seizure had the cortical involvement especially frontal region (93.7%), whereas only 6.4% without seizures had cortical involvement. Subcortical (81.3%) and cerebellar (25%) involvements were also more common in seizure than the non-seizure group. The cortical neuronal excitability may be enhanced by disruption of subcortical ascending inhibitory projections from brain stem and cerebellum. Cerebellar stimulation has been shown to reduce seizure frequency in animals as well as in humans (Krook-Magnuson et al., 2014). Cortical neuronal excitability may also be affected by Cu presence or Cu mediated oxidative stress, pro-inflammatory cytokines and glutamate release. In our study, serum Cu

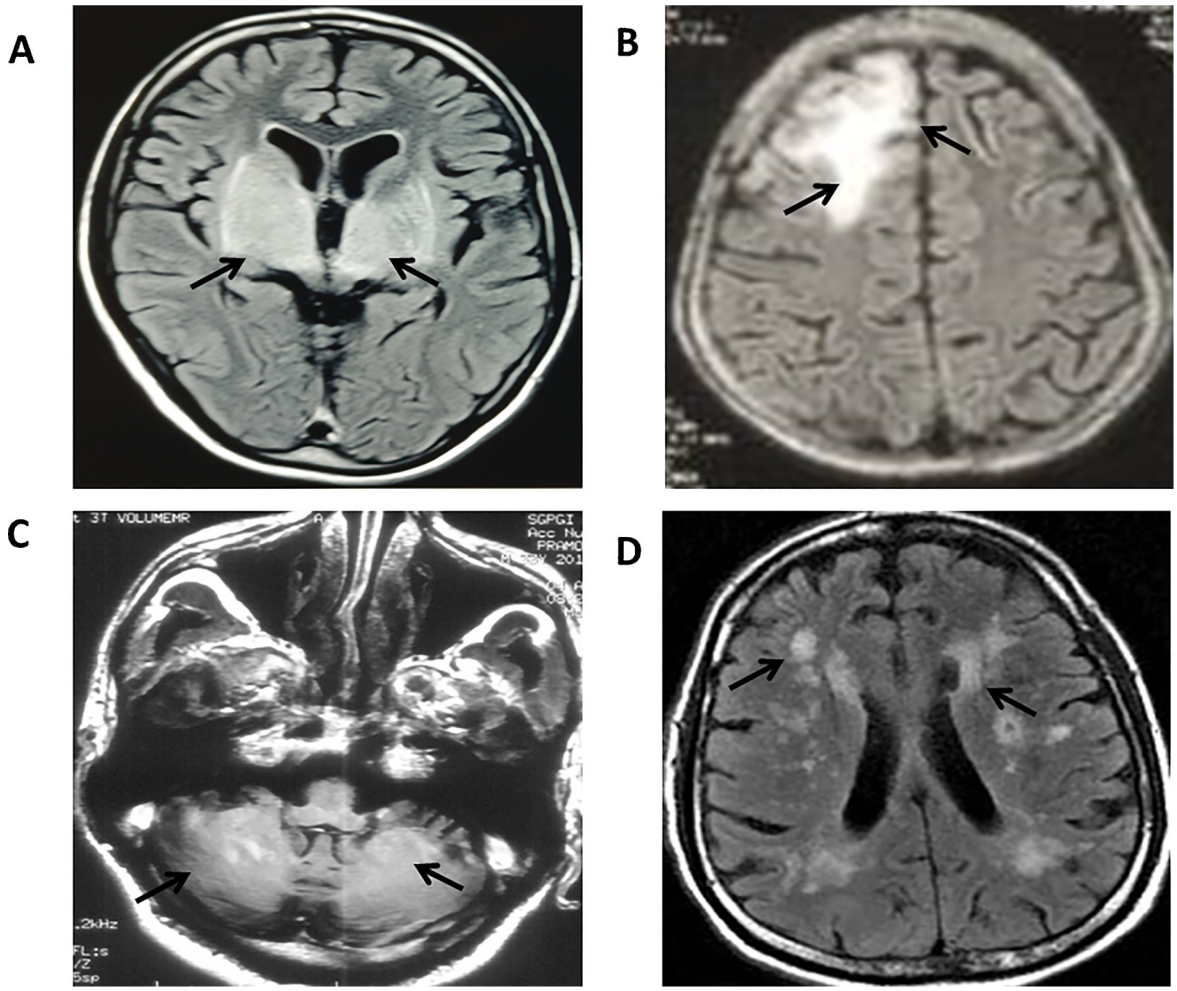

Fig. 1. Cranial MRI of a Wilson disease patient presenting with cognitive decline, dystonia and seizures. His cranial MRI, axial section in FLAIR sequence shows hyperintensity in (A) putamen and globus pallidus and (B) right frontal cortical and subcortical white matter. Cranial MRI of another patient with seizure shows hyperintensity in (C) cerebellum, and (D) subcortical white matter.

Table 4
Comparison of oxidative stress, glutamate and cytokine level between control and patients of Wilson disease with and without seizures.

Oxidative Stress*	Control (n = 64) [A]	Seizure present (n = 16) [B]	Seizure absent (n = 80) [C]	P value [A vs B]	P value [A vs C]	P value [B vs C]
GSH (mg/dl)	2.73 ± 0.04	2.11 ± 0.12	2.20 ± 0.08	< 0.01	< 0.01	1.00
MDA (nmol/ml)	3.03 ± 0.17	4.78 ± 0.11	4.95 ± 0.19	< 0.01	< 0.01	1.00
TAC (Trolox_Eq_ mmol/L)	2.29 ± 0.02	1.67 ± 0.07	1.59 ± 0.03	< 0.01	< 0.01	1.00

Glutamate (μmol/L)*	Control (n = 64)	Seizure Present (n = 12)	Seizure Absent (n = 51)	P value [A vs B]	P value [A vs C]	P value [B vs C]
	19.96 ± 0.27	25.13 ± 0.52	23.51 ± 0.64	< 0.01	< 0.01	1.00

Cytokines (pg/ml)#	Control (n = 44)	Seizure Present (n = 5)	Seizure Absent (n = 23)	P value [A vs B]	P value [A vs C]	P value [B vs C]
IL-6	5.2(5.34)	117.45 (5593.92)	14.41(189.96)	< 0.01	< 0.01	0.16
IL-8	5.63(5.52)	3884.77(9596.07)	373.40(3903.04)	< 0.01	< 0.01	0.25
IL-10	2.05(1.37)	1.80(4.48)	1.69(1.01)	0.31	0.12	1.00
TNFα	3.61(3.58)	58.42(157.69)	15.97(28.46)	< 0.01	0.02	0.45

GSH = Glutathione; MDA = malondialdehyde; TAC = total antioxidant capacity; IL = interleukin; TNFα = tumour necrosis factor alpha.
* Data were presented as Mean ± SEM, # Data were presented as medians (IQRs- interquartile ranges).

was higher in the patients with seizure than those without seizure were. Oxidative stress markers, cytokines, and glutamate levels were although higher than the healthy controls, but were not different between the WD patients with and without seizures. This may be due to the expression of these markers nearly in all the WD patients, and the blood was collected during the interictal period. Moreover, measurement of these biomarkers in the cerebrospinal fluid would have provided better information. Earlier studies have reported higher MDA and cytokines levels, and lower TAC and GSH levels in WD patients (Kalita et al., 2014). Excessive release of Cu and oxidative stress also played a major role in worsening of WD following penicillamine treatment, which occurs in 20–30% patients (Brewer et al., 1987; Kalita et al., 2015; Sinha and Taly, 2008). However, these studies did not correlate the biomarkers with seizures.

Seizure as a presenting feature has been reported in 1.6–5.6% patients with WD, and during the treatment in 2.5–4.5% patients (Dening et al., 1988; Prashanth et al., 2010; Taly et al., 2007). In the pre penicillamine era, seizure and status epilepticus were the terminal events in the majority of patients with WD (Dening et al., 1988; Prashanth et al., 2010; Sinha et al., 2010). In our study, 5.5% of patients had seizures at presentation and 14.5% developed seizures during a median 5years duration of treatment. Higher frequency of seizures in our study may be due to the referral bias, as majority of our patients had severe illness. In the study by Denning et.al, 53.8% patients had remission of seizure, 30.7% had reduction in seizure frequency, and 15.3% did not respond to AED (Dening et al., 1988). In the study by Prashanth et al., 68.3% had seizure remission, 17.1% had reasonable control, and 9.7% remained uncontrolled (Prashanth et al., 2010). In our study, all the patients had seizure remission; 43.7% with single AED, 43.7% with two AEDs and 12.5% needed three AEDs for seizure control. Better seizure control in our study may be due to second-line AEDs, which are safer in liver disease, as nearly all the WDNM are likely to have clinical or subclinical hepatic dysfunction.

The limitation of the study is retrospective design, heterogeneous AED protocol and biomarkers were not measured in the cerebrospinal fluid, which might have given better status of these biomarkers in central nervous system.

It can be concluded that seizures occur in 14.5% of patients with WDNM especially in those with cortical, subcortical and cerebellar involvement on MRI. Seizure in WDNM responds well to treatment.

A conflict of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Ethics approval

The Institutional Ethics Committee, SGPGIMS, Lucknow has approved the research (Ethic No., A-03: PGI/IMP/IEC/56/19.08.2011).

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neuro.2018.12.005.

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