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Adjunctive Antioxidant Therapy in Neurologic Wilson's Disease Improves the Outcomes

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Abstract

Oxidative stress has been reported in Wilson's disease with neurological manifestation (WDNM), but there is a paucity of studies on the role of adjunctive antioxidant therapy. This study aims to evaluate the efficacy of adjunctive vitamin C and E treatment in reducing oxidative stress and improving clinical outcomes. Forty-nine patients with WDNM were included and their clinical details were noted. Glutathione (GSH), total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured using spectrophotometer at baseline and follow-up. All patients received zinc with or without chelating therapy, and 32 of them prescribed vitamin C (500 mg/day) and E (400 mg/day). Clinical outcomes at 6, 12, and 24 months were categorized as improved, static, or worsened based on improvement in Burke-Fahn-Marsden (BFM) score (≥10%) and/or severity grade (≥ 1). Baseline parameters were similar between two groups; except BFM score was higher in the antioxidant group. At follow-up, the antioxidant group had higher GSH, TAC, and lower MDA levels compared with baseline. Patients on antioxidant treatment experienced improvement more frequently at 6 (53.1% vs. 29.4%), 12 (62.5% vs. 29.4%), and 24 months (68.8% vs. 35.3%) compared with those without antioxidant treatment. In WDNM, adjunctive vitamin C and E treatment reduce oxidative stress and improve clinical outcome.

Keywords Wilson's disease · Oxidative stress · Copper · Vitamin C and E

Introduction

Wilson's disease (WD) is an autosomal recessive copper (Cu) metabolism disorder due to the ATP7B mutation in the chromosome 13q14.3. ATP7B is a hepatic transmembrane Cu-transporting ATPase, and mutation of this gene impairs Cu trafficking in hepatocytes (Thomas et al. 1995). Free Cu in the hepatocytes is initially neutralized by glutathione (GSH) or metallothionein, and excess catalytically active Cu may undergo Fenton redox chemistry resulting in oxidative stress (Jomova and Valko 2011; Nagasaka et al. 2009). Increased oxidative stress has been reported both in the patients with WD (Kalita et al. 2015; Nagasaka et al. 2009) and in animal Cu toxicity model (Kumar et al. 2016; Musacco-Sebio et al. 2014). Impaired excretion of Cu in bile leads to an increase in circulating Cu and deposition in other organs such as the cornea, lens, brain, kidney, and bone marrow (Bull et al. 1993; Tanzi et al. 1993). Free Cu may cross the blood-brain barrier and induce oxidative stress in WD with neurological manifestation (WDNM) (Choi and Zheng 2009). Cu toxicity induces apoptosis and astrocytosis of the hippocampus and frontal cortex through direct, glutamate, and oxidative stress-mediated pathways leading to impaired memory and learning (Kalita et al. 2018). The neurological manifestation in WD usually occurs around 17 years of age with clinical or subclinical hepatic dysfunction (Ferenci 2017; Kalita et al. 2014a). Zinc and penicillamine treatment affects Cu metabolism and improves clinical outcome but does not normalize natural antioxidant capacity parameter in WD (Gromadzka et al. 2014). The use of penicillamine and trientine has been reported to result in worsening in 25–50% patients; many patients do not improve even after discontinuation of chelating drugs (Kalita et al. 2014a; Kim et al. 2019; Litwin et al. 2015). It has been noted that during the worsening, there is an increase in serum-

Highlights
Free copper induces oxidative stress in Wilson's disease.
Vitamin C and E treatment lower oxidative stress.
Adjunctive antioxidant therapy improves outcomes.

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free Cu and oxidative stress compared to those who improve (Brewer et al. 2009; Chen et al. 2012; Gromadzka et al. 2014). In WD patients, lower level of antioxidant status such as vitamin E and GSH and total antioxidant capacity (TAC) have been reported (Dalgic et al. 2005; von Herbay et al. 1994). In isolated copper-overloaded rat hepatocytes, antioxidant therapy ameliorates oxidant injury (Sokol et al. 1996). Oxidative stress plays a key role in WD, which may be aggravated by chelating agents due to the release of tissue Cu resulting in neurological deterioration (Kalita et al. 2019). Dysregulation of cytokines and chemokines was reported, and these were associated with the severity of the neurological symptoms in WD (Kalita et al. 2016; Wu et al. 2019). Vitamin C/E supplementation reduces oxidative stress, neuroinflammation, and neuronal degeneration in an animal model (Bernardo-Colon et al. 2018; Betti et al. 2011; Pansani et al. 2018). Vitamin E supplementation has been associated with neuroprotection and improvement in clinical outcome in patients with seizure and multiple sclerosis (Guan et al. 2018; Mehvari et al. 2016). The use of adjunctive antioxidants such as vitamin E and/or vitamin C may help in preventing the worsening and improving clinical outcomes of the patients with WD. In this study, we report the role of adjunctive vitamin E and vitamin C treatment in reducing oxidative stress and improving the clinical outcome of WD patients with neurological manifestations.

Subjects and Methods

Inclusion Criteria

The patients with WDNM attending the neurological service of a tertiary care teaching hospital from 2012 to 2018 were prospectively included. The diagnosis of WDNM was based on characteristic clinical features (cognitive decline, neuropsychiatric symptoms, or movement disorders), Kayser-Fleischer ring on slit lamp examination, high urinary Cu (> 40 μg/24 hour), and low serum ceruloplasmin (< 20 mg/dl) (Czlonkowska et al. 2018; European Association for Study of 2012; Kalita et al. 2014b; Roberts et al. 2008). The study was approved by the Institute Ethics Committee and patients or their relative consented for the treatment. A pedigree chart was prepared for each indexed patient. A detailed clinical history including duration of neurological symptoms, age at neurological manifestation, and history of jaundice was noted. Patients were examined for anemia, jaundice, edema, hepatosplenomegaly, and ascites. Neurological examination included mental status evaluation by Mini-Mental State Examination (MMSE). The patients were considered cognitively impaired if the MMSE score was <29 for 9 years of schooling, < 26 for 5–8 years of schooling, and < 22 for 0–4 years of schooling (Crum et al. 1993). Neurobehavioral abnormality was evaluated by Neuropsychiatric Inventory (Cummings 1997). They were also examined for movement disorders and pyramidal and cerebellar signs. The severity of dystonia was assessed by Burke-Fahn-Marsden (BFM) score (Krystkowiak et al. 2007). The severity of other movement disorders was graded on a 0–4 scale in which 0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = markedly severe. The severity of WDNM was based on the sum score of 5 neurological signs (dysarthria, tremor, ataxia, rigidity/bradykinesia, and dystonia/chorea) (Kalita et al. 2011). Each neurological sign was given a score of 0 to 3 based on severity (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The severity of neurological WD was categorized as grade 0 (sum score 0), grade-I (mild; sum score = 1), grade-II (moderate; sum score = 2–7, patients were independent for activities of daily living), and grade-III (sum score > 7, patients were dependent for activity of daily living) (Grimm et al. 1991; Wiles 1990).

Investigations

Blood counts, hemoglobin, fasting blood sugar, serum bilirubin, ALT, AST, creatinine, calcium, alkaline phosphatase, phosphorus, sodium and potassium, and prothrombin time were done. Serum and 24-hour urinary Cu were measured by atomic absorption spectrophotometer (GBC Avanta Sigma; GBC Scientific Equipment PTY Ltd., Dandenong, Victoria, Australia). Serum ceruloplasmin level was estimated by the method described by Schosinsky et al. (Schosinsky et al. 1974). Serum-free Cu was estimated by subtracting three times of serum ceruloplasmin from the total Cu. Cranial MRI was done using 3 T MRI scanner, and T1, T2, and FLAIR images were obtained. The abnormal signal intensity and their locations were noted. The MRI lesion score was calculated by adding the number of MRI lesions.

Measurement of Oxidative Stress Markers

Plasma glutathione (GSH), total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured using a spectrophotometer. The details are described in our earlier paper (Kalita et al. 2014b).

Treatment

All the patients received penicillamine, zinc, or both. Penicillamine was started in a dose of 250 mg daily then increased by 250 mg every 2–4 weeks to a maximum of 250 mg three times daily. Zinc was prescribed in the form of zinc acetate 50 mg thrice daily. The patients who were treated in the last 2 years also received vitamin E (400 mg once a day in adults and 200 mg in children) and vitamin C 500 mg in addition to Zn and penicillamine. Symptomatic treatment for movement disorder such as trihexyphenidyl, clonazepam,

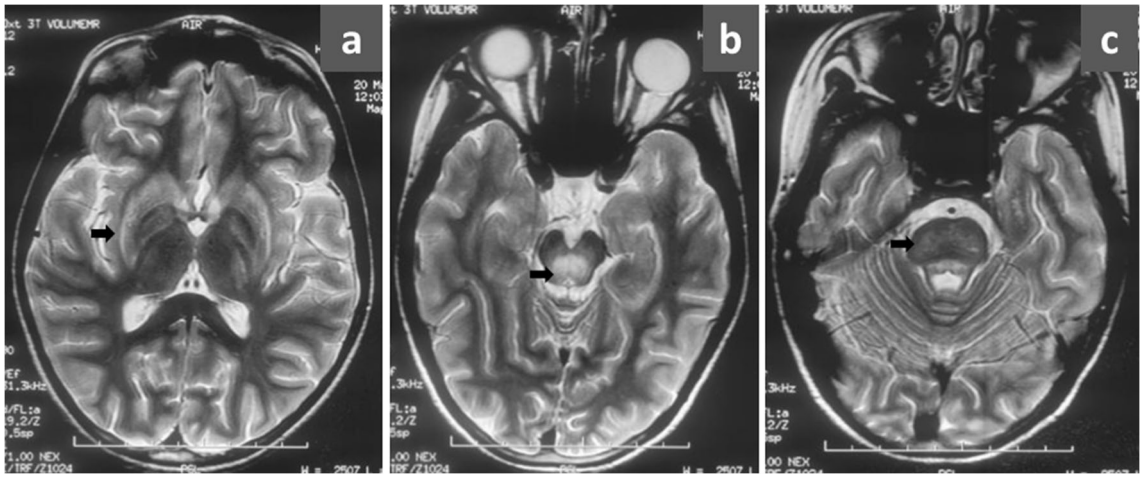

Fig 1. Cranial MRI, axial section on T2 sequence of Wilson's disease patients. He had severe generalized dystonia with grade-III neurological severity. (A) There is hyperintensity of corpus striatum (arrow) and thalamus bilaterally. (B) Midbrain lesion looking like "face of giant panda." (C) Hyperintense lesion in the pons (arrow)

tetrabenazine and diazepam, lorazepam, and baclofen in various combinations was also prescribed (Czlonkowska et al. 2018; European Association for Study of 2012).

Follow-up

Patients were followed up at 6, 12, and 24 months or earlier if needed. Their neurological severity and BFM scores were recorded. Hematological, liver function test and coagulation profile were repeated. GSH, TAC, and MDA level were also measured on follow-up. Clinical worsening on treatment was considered if they had new neurologic symptoms and signs, increase in BFM score by at least 10% from the baseline, and/or one or more grade deterioration in severity grading (Bruha et al. 2012; Kalita et al. 2014a). The patients were considered improved if there was $\geq$ one grade improvement in neurological severity grade of $\geq$10% improvement in BFM score.

Table 1 Baseline demographic, clinical, and MRI finding between the patients with and without adjunctive treatment antioxidant treatment

Parameter		Antioxidant given ($N = 32$)	Antioxidant not given ($N = 17$)	*p*-value
Age (years)		15.97 ± 6.40	15.53 ± 6.46	0.82
Duration of illness (months)		25.5 ± 35.62	27.47 ± 39.85	0.76
Severity grade		2.38 ± 0.71	2.18 ± 0.81	0.38
Mini-Mental State Examination score		23.88 ± 4.67	24.33 ± 2.61	0.73
BFM score		74.11 ± 33.76	49.47 ± 36.97	0.02
S. Bilirubin (mg/dl)		1.0 ± 0.59	0.81 ± 0.41	0.26
Alanine aminotransferase (U/L)		32.94 ± 15.88	67.52 ± 12.45	0.27
S. Ceruloplasmin (mg/dl)		8.8 ± 3.6	7.6 ± 4.2	0.51
Coagulopathy		1.32 ± 0.48	1.40 ± 0.57	0.56
S. Albumin (g/dl)		4.16 ± 0.62	3.87 ± 0.74	0.71
Hemoglobin (mg/dl)		12.6 ± 1.7	11.05 ± 0.9	0.21
TLC/mm^3		5730 ± 2691	6261 ± 2011	0.59
Platelets/mm^3		142 ± 56	116 ± 61	0.51
MRI lesion score		7.41 ± 2.50	6.27 ± 2.19	0.31
Treatment given				
Number of patients	Penicillamine (P)	6 (18.8%)	6 (29.4%)	0.41
	Zinc (Zn)	4 (12.5%)	2 (11.8%)	
	Both (P + Zn)	22 (68.8%)	9 (58.8%)	

Value is in mean ± SD

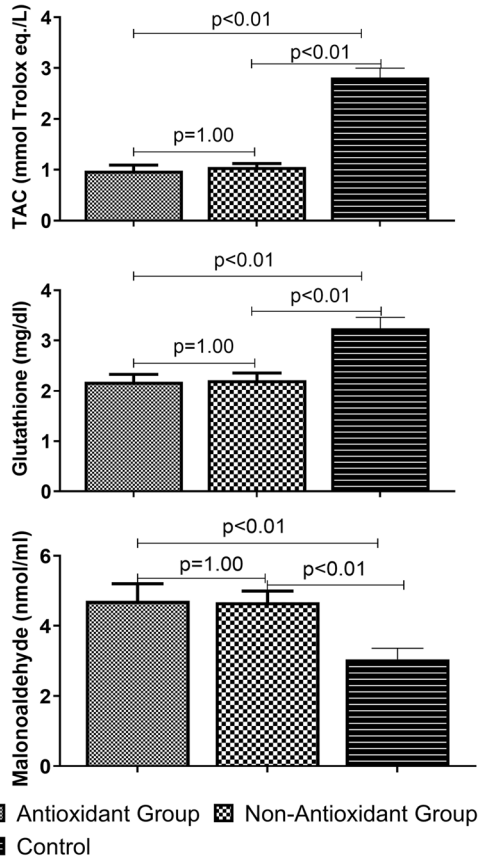

Fig 2. Error bar diagram shows a comparison of (A) total antioxidant activity (TAC), (B) glutathione (GSH), and (C) malondialdehyde (MDA) between control and patients with neurological Wilson's disease who were prescribed adjunctive antioxidant (vitamin C, vitamin E) and who did not. The baseline GSH, TAC, and MDA levels were similar in both the Wilson's disease groups. The control group had higher GSH and TAC levels and lower MDA levels compared with both Wilson's disease groups

Statistical Analysis

The BFM score, neurological severity grade, oxidative stress markers, and serum and urinary Cu at baseline, 6, 12, and 24 months, were compared by ANOVA using Tukey multiple comparisons. These parameters were also compared between the groups who received adjunctive antioxidants and those who did not, using the independent t-test or Mann-Whitney U test. The clinical outcome at 6, 12, and 24 months was compared using the chi-square test. The statistical analysis was done using GraphPad Prism 5 and SPSS 20 version. The variable having a two-tailed *p*-value of <0.05 was considered significant.

Results

There were 49 patients with WDNM, and of them 8 were females. Their ages ranged between 9 and 34 (median 13) years; 27 children were below 15 years of age. The median age at onset of neurological symptoms was 12 (range 5–34) years. History of jaundice was present in 18, and 14 had a family history of WD. Nine (18.4%) patients had seizures. Cognitive decline was reported by 9 patients, and MMSE score ranged between 16 and 30 (median 25). KF ring was present in 48 (98%) patients. Thirty-one (63.3%) WDNM patients have behavioral abnormalities and included irritability, aggressiveness, depression, and/or anxiety. All the patients had some form of movement disorders; dystonia in 46 (93.9%), tremor in 25 (93.8%), and myoclonus in 4 (8.3%) patients. The median BFM score was 67 (range 3–120). The neurologic severity was grade-III in 23 (46.9%), grade-II in 18 (36.7%), and grade-I in 8 (16.3%) patients. MRI was done in 44 patients and was abnormal in all. MRI revealed thalamic lesion in 32 (69.6%), caudate in 39 (84.8%), putamen in 40

Table 2 Comparison of glutathione (GSH), total antioxidant capacity (TAC), and malondialdehyde (MDA) at baseline, 6, 12, and 24 months in the WD patients with adjunctive antioxidant treatment (Group-I) and without adjunctive antioxidant treatment (Group-II)

Group	Baseline [A]	6 months [B]	12 months [C]	24 months [D]	*p*-value		
					[A]Vs[B]	[A]Vs[C]	[A]Vs[D]
Total antioxidant capacity (mmol Trolox Eq./L)							
[Group-I]	0.98 ± 0.11	1.26 ± 0.14	1.53 ± 0.10	2.16 ± 0.16	0.04	<0.01	<0.01
[Group-II]	1.05 ± 0.07	1.21 ± 0.17	1.42 ± 0.18	1.74 ± 0.10	1.00	0.67	0.01
Glutathione (mg/dl)							
[Group-I]	2.18 ± 0.15	2.27 ± 0.11	2.35 ± 0.15	2.79 ± 0.12	0.01	<0.01	<0.01
[Group-II]	2.21 ± 0.15	2.30 ± 0.08	2.37 ± 0.11	2.46 ± 0.14	1.00	0.21	0.04
Malondialdehyde (MDA) (nmol/ml)							
[Group-I]	4.71 ± 0.49	4.46 ± 0.20	4.06 ± 0.30	3.84 ± 0.16	0.01	<0.01	<0.01
[Group-II]	4.67 ± 0.32	4.70 ± 0.19	4.63 ± 0.19	4.05 ± 0.13	1.00	0.74	<0.01

Value in (Mean ± SEM)

Table 3 The outcome of the patients with neurologic Wilson's disease receiving antioxidant (vitamin C and vitamin E) treatment compared with those without antioxidant treatment

Outcome		Antioxidant group $N = 32$ (%)	Non-antioxidant group $n = 17$(%)	p
6 months	Improved	17(53.1%)	5(29.4%)	0.03
	Static	13 (40.6%)	6(35.3%)	
	Worsened	2 (6.3%)	6(35.3%)	
12 months	Improved	20(62.5%)	5(29.4%)	0.001
	Static	12(37.5%)	6(35.3%)	
	Worsened	0	6(35.3%)	
24 months	Improved	22(68.8%)	6(35.3%)	0.001
	Static	10(31.3%)	5(29.4%)	
	Worsened	0	6(35.3%)	
The difference of BFM score at baseline with 6, 12, and 24 months; median(IQR*)				
Baseline – 6 month		12 (21.50)	3(19.25)	0.07
Baseline – 12 month		19(24.50)	4(23.75)	0.01
Baseline – 24 month		24(36.63)	0(32.00)	0.02

*IQR = Interquartile range

(87%), globus pallidus in 35 (76.1%), brain stem in 30 (65.2%), subcortical white matter in 9 (19.6%), cortical in 9 (19.6%), and cerebellar in 6 (13%) patients (Fig. 1). Ultrasound abdomen revealed evidence of chronic liver disease in 28 (57.1%), splenomegaly in 21(43.8%), and ascites in 6 (12.5%) patients.

Treatment

Adjunctive antioxidants were prescribed to 32 patients, and 17 patients did not receive antioxidants. In the adjunctive antioxidant group, 22 patients received penicillamine and zinc, 6 only penicillamine, and 4 only zinc, whereas in non-antioxidant group 10 patients received penicillamine and zinc, 5 only penicillamine, and 2 only zinc. The demographic, baseline clinical, biochemical, and MRI of the patients receiving antioxidants were similar to the non-antioxidant group, but BFM score was higher in the patients on the antioxidant arm (Table 1).

Effect of Adjunctive Antioxidants on Oxidative Stress Markers

Baseline comparison of biomarkers revealed reduced MDA and elevated GSH and TAC levels in the controls compared to WD patients (Fig. 2). At 6, 12, and 24 months follow-up, the patients on adjunctive antioxidants had higher GSH and TAC levels and reduced MDA levels compared to baseline. Whereas the patients without adjunctive antioxidant treatment had no change in GSH, TAC, and MDA until 12 months, but at 24 months, they also had an increase in GSH and TAC and reduction in MDA levels (Table 2).

Clinical Outcome

The baseline BFM score was although higher in the adjunctive antioxidant group compared to the non-antioxidant group, but the change in BFM score at 12 and 24 months was more marked in the antioxidant group. Taking BFM and clinical severity grade together, the outcome of the patients in the antioxidant group was better compared to the non-antioxidant group at 6, 12, and 24 months (Table 3). At 24 months, 28 (57.1%) patients improved [22 (68.8%) in antioxidant group and 6 (35.3%) in non-antioxidant group]. Fifteen patients remained static [10 (31.3%) in the antioxidant and 5 (29.4%) in the non-antioxidant group], and 6 worsened [none in antioxidant group and 6 (35.3%) in non-antioxidant group; $p = 0.001$]. At 24 months, the patients who improved or remained static had higher GSH and TAC and lower MDA levels compared with those who worsened (Fig 3).

Discussion

In the present study, adjunctive antioxidant treatment in WDNM reduced oxidative stress and improved clinical outcome until 24 months. This is the first human study reporting the effect of vitamin C and E in addition to the standard treatment of WD. Vitamin E and C were used because of their antioxidant properties and evidence of higher oxidative stress in WDNM (Kalita et al. 2014b). Moreover, the lower level of α-tocopherol has been reported in WD (Ogihara et al. 1995). The role of antioxidants has been evaluated in cell culture and animal studies on Cu toxicity. Incubation of Cu-overloaded hepatocytes with D-α-tocopheryl succinate resulted in complete amelioration of Cu-induced changes in viability and lipid peroxidation (Sokol et al. 1996). In Iberian pigs, dietary α-

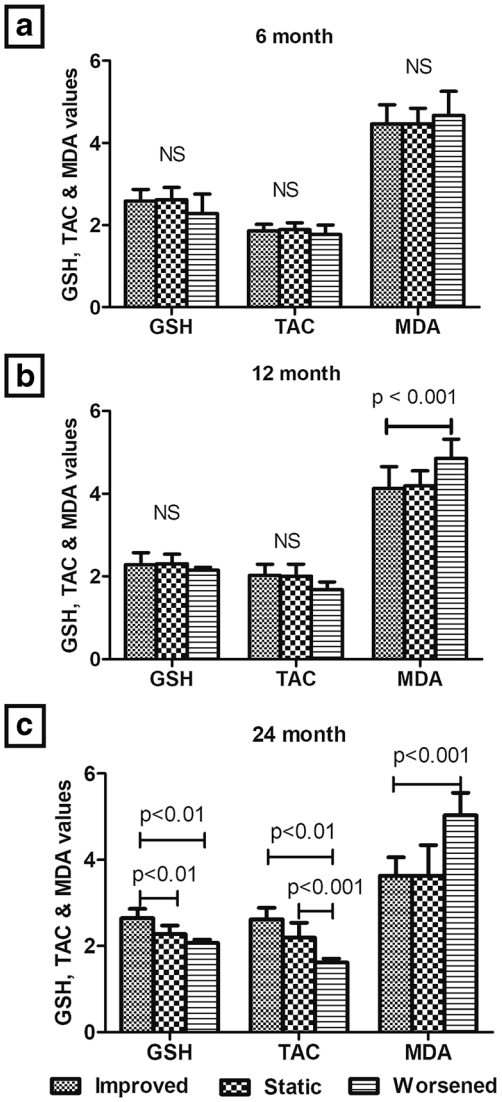

Fig 3. Error bar diagram shows the relationship of glutathione (GSH mg/dl), total antioxidant capacity (TAC nmol Trolox Eq/l), and malondialdehyde (MDA nmol/ml) with the outcome at (A) 6 months, (B) 12 months, and (C) 24 months. At 24 months, the patients who improved had higher GSH and TAC and lower MDA levels compared with those who worsened

tocopherol in a dose of 100 mg/kg significantly reduced lipid peroxidation, but dietary Cu did not modify the oxidative susceptibility of lipids (Rey and Lopez-Bote 2001). In an experimental study of Cu toxicity in rats, the oxidative stress markers were evaluated following different antioxidants such as selenium, α-tocopherol, and reduced glutathione. The concentration of Cu in the liver and kidney was reduced maximally by the use of α-tocopherol followed by selenium and GSH. Lipid peroxidation in the liver and kidney was suppressed maximally by α-tocopherol followed by selenium improved reduced glutathione (Rana and Verma 1997). It has been postulated that vitamin E acts as a lipid-soluble antioxidant on the membrane and selenium as cytosolic GSH peroxidase (Chen et al. 1993). α-lipoic acid protects hepatic cells from copper toxicity and may be useful in WD (Smirnova et al. 2018).

Cellular mechanisms to combat oxidative stress are critical for cellular homeostasis. The enzyme system including superoxide dismutase, catalase, glutathione peroxidase, and a number of thiol-reductase is linked to either NADH or NADPH, which are the source of reducing equivalent. Vitamin E, vitamin C, and glutathione are nonenzymatic electron receptors and play a major role in neutralizing oxidative stress. Vitamin E acts as an antioxidant by stabilizing unpaired electron and induces synthesis of metallothionein I and II, which prevent lipid peroxidation. Vitamin E has been associated with neuroprotection and control of seizures (Betti et al. 2011; Mehvari et al. 2016; Pansani et al. 2018). Vitamin E supplementation in multiple sclerosis patients reduces lipid peroxidation and improves clinical outcomes (Guan et al. 2018). Vitamin C serves as an electron donor and thus can terminate a free radical chain reaction. There is concern about the use of vitamin C in the presence of excess metal ion. The majority of in vitro cell culture studies have shown aggravation of oxidative stress by vitamin C in the presence of excess Cu, but the animal study has not observed this finding (Chen et al. 1993; Rey and Lopez-Bote 2001). High dietary ascorbic acid lowers Cu level in the blood and liver in Guinea pigs (Tsuchiya and Bates 1997). A significant decrease (37%) in vitamin E to lipid ratio has been reported in the patients with WD with high free serum Cu (> 10 μg/dl) (von Herbay et al. 1994). We have found a reduction in oxidative stress in the patients with WDNM who were on adjunctive vitamin C and vitamin E. The use of Zn may also reduce oxidative stress. Zinc, iron, molybdenum, calcium, phosphorus, and vitamin C impair absorption of Cu. Zinc also removes Cu from its binding sites where it may generate free radicals. The β-carotene, α-lipoic acid, and polyphenols have shown to attenuate Cu-induced oxidative stress (Chen et al. 1993; Shay et al. 2009; Smirnova et al. 2018). In a study, the use of Zn with interferon in chronic hepatitis C has resulted in a reduction in hepatic fibrosis, ferritin, and oxidative stress leading to improvement in hepatic encephalopathy (Nagamine et al. 1997). In our study, adjunctive vitamin C and E in WDNM reduced oxidative stress and improved neurological outcome.

Limitation

The limitation of the study is a non-randomized design, and we have not done oxidative stress markers in the cerebrospinal fluid. The Wilson's disease is a rare inherited disorder; therefore, a multicenter study may be able to answer some of these questions, which may result in a better outcome of this disabling disease. We have not studied the variations in the ATP7B mutation or the presence of genetic modifier gene such as the apolipoprotein E (APOE) allele or polymorphisms

in the copper metabolism domain-containing 1 (COMMD1), antioxidant 1 copper chaperone (ATOX1), X-linked inhibitor of apoptosis (XIAP), and methylenetetrahydrofolate reductase (MTHFR) gene.

Conclusion

Adjunctive vitamin C and vitamin E therapy in WDNM reduce oxidative stress and improve clinical outcome.

Funding None

Compliance with Ethical Standards

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

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

Abbreviations BFM Burke-Fahn-MarsdenCNS Central nervous systemCu CopperGSH GlutathioneLPO Lipid peroxidation MRI Magnetic resonance imagingMDA MalondialdehydeWD Wilson's diseaseTAC Total antioxidant capacity

References

Bernardo-Colon A, Vest V, Clark A, Cooper ML, Calkins DJ, Harrison FE, Rex TS (2018) Antioxidants prevent inflammation and preserve the optic projection and visual function in experimental neurotrauma. Cell Death Dis 9:1097. https://doi.org/10.1038/s41419-018-1061-4

Betti M et al (2011) Dietary supplementation with alpha-tocopherol reduces neuroinflammation and neuronal degeneration in the rat brain after kainic acid-induced status epilepticus. Free Radic Res 45:1136–1142. https://doi.org/10.3109/10715762.2011.597750

Brewer GJ et al (2009) Treatment of Wilson's disease with tetrathiomolybdate: V. control of free copper by tetrathiomolybdate and a comparison with trientine translational research. J Lab Clin Med 154:70–77. https://doi.org/10.1016/j.trsl.2009.05.002

Bruha R et al (2012) Decreased serum antioxidant capacity in patients with Wilson disease is associated with neurological symptoms. J Inherit Metab Dis 35:541–548. https://doi.org/10.1007/s10545-011-9422-5

Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW (1993) The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet 5:327–337. https://doi.org/10.1038/ng1293-327

Chen DB, Feng L, Lin XP, Zhang W, Li FR, Liang XL, Li XH (2012) Penicillamine increases free copper and enhances oxidative stress in the brain of toxic milk mice. 7:e37709–PloS one. https://doi.org/10.1371/journal.pone.0037709

Chen H, Pellett LJ, Andersen HJ, Tappel AL (1993) Protection by vitamin E, selenium, and beta-carotene against oxidative damage in rat liver slices and homogenate. Free Radic Biol Med 14:473–482

Choi BS, Zheng W (2009) Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. Brain Res 1248:14–21. https://doi.org/10.1016/j.brainres.2008.10.056

Crum RM, Anthony JC, Bassett SS, Folstein MF (1993) Population-based norms for the mini-mental state examination by age and educational level. JAMA 269:2386–2391

Cummings JL (1997) The neuropsychiatric inventory: assessing psychopathology in dementia patients. Neurology 48:S10–S16

Czlonkowska A et al (2018) Wilson disease. Nature reviews Disease primers 4:21. https://doi.org/10.1038/s41572-018-0018-3

Dalgic B, Sonmez N, Biberoglu G, Hasanoglu A, Erbas D (2005) Evaluation of oxidant stress in Wsilson's disease and non-Wilsonian chronic liver disease in childhood. Turk J Gastroenterol 16:7–11

European Association for Study of Liver (2012) EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol 56:671–685. https://doi.org/10.1016/j.jhep.2011.11.007

Ferenci P (2017) Diagnosis of Wilson disease. Handbook of clinical neurology 142:171–180. https://doi.org/10.1016/B978-0-444-63625-6.00014-8

Grimm G et al (1991) Comparison of functional and structural brain disturbances in Wilson's disease. Neurology 41:272–276

Gromadzka G et al (2014) Treatment with D-penicillamine or zinc sulphate affects copper metabolism and improves but not normalizes antioxidant capacity parameters in Wilson disease. Biometals 27:207–215. https://doi.org/10.1007/s10534-013-9694-3

Guan JZ, Guan WP, Maeda T (2018) Vitamin E administration erases an enhanced oxidation in multiple sclerosis. Can J Physiol Pharmacol 96:1181–1183. https://doi.org/10.1139/cjpp-2018-0246

Jomova K, Valko M (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283:65–87. https://doi.org/10.1016/j.tox.2011.03.001

Kalita J, Kumar V, Chandra S, Kumar B, Misra UK (2014a) Worsening of Wilson disease following penicillamine therapy. Eur Neurol 71:126–131. https://doi.org/10.1159/000355276

Kalita J, Kumar V, Misra UK (2016) A study on apoptosis and anti-apoptotic status in Wilson disease. Mol Neurobiol 53:6659–6667. https://doi.org/10.1007/s12035-015-9570-y

Kalita J, Kumar V, Misra UK, Bora HK (2018) Memory and learning dysfunction following copper toxicity: biochemical and immunohistochemical basis. Mol Neurobiol 55:3800–3811. https://doi.org/10.1007/s12035-017-0619-y

Kalita J, Kumar V, Misra UK, Ranjan A, Khan H, Konwar R (2014b) A study of oxidative stress, cytokines and glutamate in Wilson disease and their asymptomatic siblings. J Neuroimmunol 274:141–148. https://doi.org/10.1016/j.jneuroim.2014.06.013

Kalita J, Kumar V, Ranjan A, Misra UK (2015) Role of oxidative stress in the worsening of neurologic Wilson disease following chelating therapy. NeuroMolecular Med 17:364–372. https://doi.org/10.1007/s12017-015-8364-8

Kalita J, Misra UK, Kumar V, Parashar V (2019) Predictors of seizure in Wilson disease: a clinico-radiological and biomarkers study. Neurotoxicology 71:87–92. https://doi.org/10.1016/j.neuro.2018.12.005

Kalita J, Misra UK, Pradhan PK (2011) Oromandibular dystonia in encephalitis. J Neurol Sci 304:107–110. https://doi.org/10.1016/j.jns.2011.02.001

Kim JJ, Kim YS, Kumar V (2019) Heavy metal toxicity: An update of chelating therapeutic strategies. J Trace Elem Med Biol 54:226–231. https://doi.org/10.1016/j.jtemb.2019.05.003

Krystkowiak P et al (2007) Reliability of the Burke-Fahn-Marsden scale in a multicenter trial for dystonia. Mov Disord 22:685–689. https://doi.org/10.1002/mds.21392

Kumar V, Kalita J, Bora HK, Misra UK (2016) Relationship of antioxidant and oxidative stress markers in different organs following copper toxicity in a rat model. Toxicol Appl Pharmacol 293:37–43. https://doi.org/10.1016/j.taap.2016.01.007

Litwin T, Dziezyc K, Karlinski M, Chabik G, Czepiel W, Czlonkowska A (2015) Early neurological worsening in patients with Wilson's

disease. J Neurol Sci 355:162–167. https://doi.org/10.1016/j.jns.2015.06.010

Mehvari J, Motlagh FG, Najafi M, Ghazvini MR, Naeini AA, Zare M (2016) Effects of vitamin E on seizure frequency, electroencephalogram findings, and oxidative stress status of refractory epileptic patients. Adv Biomed Res 5:36. https://doi.org/10.4103/2277-9175.178780

Musacco-Sebio R et al (2014) Oxidative damage to rat brain in iron and copper overloads. Metallomics 6:1410–1416. https://doi.org/10.1039/c3mt00378g

Nagamine T et al (1997) The possible role of zinc and metallothionein in the liver on the therapeutic effect of IFN-alpha to hepatitis C patients. Biol Trace Elem Res 58:65–76

Nagasaka H, Takayanagi M, Tsukahara H (2009) Children's toxicology from bench to bed–Liver Injury (3): oxidative stress and anti-oxidant systems in liver of patients with Wilson disease. J Toxicol Sci 34(Suppl 2):SP229–SP236

Ogihara H, Ogihara T, Miki M, Yasuda H, Mino M (1995) Plasma copper and antioxidant status in Wilson's disease. Pediatr Res 37:219–226. https://doi.org/10.1203/00006450-199502000-00016

Pansani AP et al (2018) Long-term monotherapy treatment with vitamin E reduces oxidative stress, but not seizure frequency in rats submitted to the pilocarpine model of epilepsy. Epilepsy Behav 88:301–307. https://doi.org/10.1016/j.yebeh.2018.09.027

Rana SV, Verma S (1997) Protective effects of GSH, alpha-tocopherol, and selenium on lipid-peroxidation in liver and kidney of copper fed rats. Bull Environ Contam Toxicol 59:152–158. https://doi.org/10.1007/s001289900458

Rey AI, Lopez-Bote CJ (2001) Effect of dietary copper and vitamin E supplementation, and extensive feeding with acorn and grass on longissimus muscle composition and susceptibility to oxidation in Iberian pigs. J Anim Physiol Anim Nutr 85:281–292

Roberts EA, Schilsky ML, American Association for Study of Liver D (2008) Diagnosis and treatment of Wilson disease: an update. Hepatology 47:2089–2111. https://doi.org/10.1002/hep.22261

Schosinsky KH, Lehmann HP, Beeler MF (1974) Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. Clin Chem 20:1556–1563

Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM (2009) Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta 1790:1149–1160. https://doi.org/10.1016/j.bbagen.2009.07.026

Smirnova J, Kabin E, Järving I, Bragina O, Tõugu V, Plitz T, Palumaa P (2018) Copper(I)-binding properties of de-coppering drugs for the treatment of Wilson disease. α-Lipoic acid as a potential anti-copper agent. Sci Rep 8:1463–1463. https://doi.org/10.1038/s41598-018-19873-2

Sokol RJ, McKim JM Jr, Devereaux MW (1996) alpha-tocopherol ameliorates oxidant injury in isolated copper-overloaded rat hepatocytes. Pediatr Res 39:259–263. https://doi.org/10.1203/00006450-199604001-01565

Tanzi RE et al (1993) The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat Genet 5:344–350. https://doi.org/10.1038/ng1293-344

Thomas GR, Forbes JR, Roberts EA, Walshe JM, Cox DW (1995) The Wilson disease gene: spectrum of mutations and their consequences. Nat Genet 9:210–217. https://doi.org/10.1038/ng0295-210

Tsuchiya H, Bates CJ (1997) Vitamin C and copper interactions in guinea-pigs and a study of collagen cross-links. Br J Nutr 77:315–325

von Herbay A, de Groot H, Hegi U, Stremmel W, Strohmeyer G, Sies H (1994) Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease. J Hepatol 20:41–46

Wiles C (1990) Quantification of neurologic deficit. Journal of Neurology Neurosurgery & Psychiatry 53:628

Wu P, Dong J, Cheng N, Yang R, Han Y, Han Y (2019) Inflammatory cytokines expression in Wilson's disease. Neurol Sci 40:1059-1066. https://doi.org/10.1007/s10072-018-3680-z