Clinical Utility of Longitudinal Measurement of Motor Threshold in Wilson's Disease

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Abstract: This study describes the longitudinal changes of resting motor threshold (RMT) and central motor conduction time (CMCT) in 18 patients with Wilson's disease (WD). The RMT, CMCT, and Global Assessment Scale for Wilson Disease (GAS-WD) were measured at baseline and at follow-up after 12.94 ± 7.23 months. There was a significant decrease in the RMT (72.11 ± 18.62 vs. $63.7 \pm 15.52\%$; p-value $= 0.002$) and GAS-WD scores (14.38 ± 5.35 vs. 9.77 ± 6.47 ms; p-value $= 0.04$). CMCT did not improve despite chelation therapy. Hence, RMT may serve as a marker of chelation efficacy in WD.

RÉSUMÉ: **Utilité clinique des mesures longitudinales du seuil moteur au repos dans le cas de la maladie de Wilson**. Cette étude entend décrire les changements longitudinaux affectant le seuil moteur au repos (SMR) et la vitesse de conduction nerveuse motrice (VCNM) chez 18 patients atteints de la maladie de Wilson. Tant le SMR et la VCNM que la *Global Assessment Scale for Wilson Disease* (GAS-WD) ont été mesurés au départ de l'étude et au moment d'un suivi après $12,94 \pm 7,23$ mois. Une diminution notable du SMR ($72,11 \pm 18,62$ contre $63,7 \pm 15,52$ % ; $p = 0,002$) et de la GAS-WD ($14,38 \pm 5,35$ contre $9,77 \pm 6,47$ ms ; $p = 0,04$) a pu être observée. En revanche, aucune amélioration sensible de la VCNM n'a été notée en dépit d'une thérapie par chélation. Par conséquent, il se pourrait que le SMR, dans le cas de la maladie de Wilson, puisse être utilisé comme marqueur de l'efficacité de la thérapie par chélation.

Transcranial magnetic stimulation (TMS) is a non-invasive technique used to measure the in vivo neurophysiological parameters such as the cortical resting motor threshold (RMT) and central motor conduction time (CMCT). Previous studies have reported poor cortical excitability and prolongation of CMCT in Wilson's disease (WD).[1] However, the longitudinal changes of these measures and the effect of chelation therapy on them are not clear. The existing literature provides conflicting results. Few studies have reported improvement in CMCT with adequate chelation, whereas other studies, including a study from our group, reported no demonstrable improvement in the cortical excitability.[1–4] However, these findings were based on recording from a few subjects with varying duration of disease and chelation status. Furthermore, evidence from clinical and neuroimaging studies support that adequate and long-term chelation improves the clinical and MRI abnormalities.[5,6] Despite the contradictory results from previous studies, we hypothesize that the neurophysiological measures may improve after adequate treatment. In this study of 18 subjects of neurological WD, we investigate the longitudinal changes of RMT and CMCT and also discuss the effect of chelation therapy.

This study was conducted at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. All participants provided informed consent prior to recruitment. The neurophysiological and clinical variables measured included the RMT, CMCT, and Global Assessment Scale for Wilson Disease (GAS-WD) score. All patients were evaluated longitudinally at two occasions; prior to starting of chelation therapy (RMT_1, $CMCT_1$, and $GAS\text{-}WD_1$) and in the subsequent follow-up (RMT_2, $CMCT_2$, and $GAS\text{-}WD_2$). After the baseline measurement of RMT_1, $CMCT_1$, and $GAS\text{-}WD_1$, patients were started on medications. The chelation regimen in subjects consisted of Penicillamine (12–15 mg/kg/day in 2–3 divided doses) and Zinc (150 mg/day of elemental zinc in 3 divided doses).[5] All medications were stopped for at least 24 hours to allow wash-out of the drug. The demographic details and drug profile of patients are provided in Table 1.

The recommendations of the IFCN committee were followed for all TMS measurements.[7] Measurements were obtained using a Magstim 200 stimulator and a hand-held, figure-of-eight coil while the subject relaxed in a comfortable chair in a quiet room. Two Ag–AgCl electrodes, arranged in a belly-tendon montage

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Table 1: Demography and treatment profile of patients with WD

Characteristic	(n = 18)
Gender distribution (M:F)	11:7
Mean age (in years)	18.9 ± 8.69
Duration of disease (in months)	12.9 ± 7.23
Compliance to chelation	
Regular treatment	16 (88.8%)
Treatment default	2 (11.1%)
Medications[a]	
Penicillamine	18 (100%)
Zinc	18 (100%)
Trihexyphenidyl	10 (55.5%)
Baclofen	6 (33.3%)
Levodopa + Carbidopa	3 (16.6%)

[a]Daily dose of medicines (minimum–maximum) – Penicillamine: 500–750 mg; Zinc: 100–150 mg; Trihexyphenidyl: 4–12 mg; Baclofen: 10–20 mg; Levodopa: 100–300 mg; Carbidopa: 25–75 mg. Recording during the follow-up was performed after withholding medication for at least 24 hours.

Table 2: Clinical and neurophysiological measures at baseline and at follow-up visit in WD

Clinical symptom	Pre-chelation	At follow-up	p-value
Dystonia	7 (38.8%)	4 (22.2%)	NS
Bradykinesia	4 (22.2%)	2 (11.1%)	NS
Postural tremor	6 (33.3%)	4 (22.2%)	NS
Cerebellar signs	2 (11.1%)	1 (9.0%)	NS
Chorea	1 (9.0%)	0	NS
KF ring	18 (100.0%)	18 (100.0%)	NS
Pyramidal signs	8 (44.4%)	7 (38.8%)	NS
Dysarthria	8 (44.4%)	5 (27.7%)	NS
Drooling of saliva	4 (22.2%)	2 (11.1%)	NS
Psychosis	2 (11.1%)	1 (9.0%)	NS
Dysphagia	0	0	
Intellectual impairment	4 (22.2%)	4 (22.2%)	NS
GAS-WD score	14.38 ± 5.35	9.77 ± 6.47	0.04*
Neurophysiological measures			
Motor threshold (%)	72.11 ± 18.62	63.7 ± 15.52	0.002*
Central motor conduction time (millisec)	7.81 ± 2.79	8.60 ± 1.42	NS

NS = Not significant. Data are based on the presence or absence of symptom. "*" denotes significant p-values after correction for multiple comparison.

with the active electrode placed over the belly of the first dorsal interosseus (FDI) and the reference electrode placed on the metacarpophalangeal joint of the index finger, were used to record the motor evoked potentials (MEP).

The optimal "hotspot" was identified by placing the coil at a 45° angle over the left motor cortex. Stimulation of this "hotspot" elicited motor responses in the contralateral FDI muscle. Relative frequency method was followed for measuring the RMT. From a subthreshold stimulus of 30% of the maximal stimulator output (MSO), the intensity of the stimulus was increased in increments of 5% until the TMS consistently evoked MEPs with a peak-to-peak amplitude of more than 50 μV.[7] The stimulus intensity was sequentially decreased by 1% of MSO until <5 unsatisfactory MEPs were obtained in a trial of 10 recordings. This stimulus intensity plus one was regarded as the RMT. CMCT was determined by motor root stimulation at a suprathreshold stimulus (120% of RMT). The latency between cortical and motor root stimulation was reported as CMCT (in milliseconds).[7]

Statistical analysis for quantifying significant differences in the RMT, CMCT, and GAS-WD score was performed using Wilcoxon signed rank test for non-parametric data. In subjects with unrecordable RMT, we used the 100% (MSO) as the imputed RMT for statistical analysis. Improvement in specific clinical symptoms was evaluated using McNemar's test for paired proportions. Correlation (Spearman's correlation) was performed to identify significant associations between the neurophysiological measures and clinical severity score. A threshold of $p < 0.05$ after Bonferroni correction for multiple comparisons was considered statistically significant.

The mean age of patients at recruitment was 18.94 ± 8.69 years and the mean duration between the first and second recordings was 12.94 ± 7.23 months. Among the subjects, 16 (88.8%) patients were on regular chelation therapy, whereas 2 subjects were treatment defaulters. At pre-chelation measurement, MEP was not recordable at 100% of MSO in three subjects. However, during the follow-up measurement, MEP was recordable in all subjects. On qualitative analysis, 14 subjects had decrease in the RMT and GAS-WD scores. The RMT and GAS-WD score worsened in two subjects who were also treatment defaulters. In yet another two subjects, the RMT and GAS-WD score worsened despite regular therapy. The CMCT value worsened in 11 subjects and improved in 7 subjects. The RMT, CMCT, GAS-WD score, and specific clinical signs in patients prior to chelation therapy and at the follow-up assessment are provided in Tables 2 and 3.

Statistical analysis revealed a significant decrease in the mean RMT (72.11 ± 18.62 vs. 63.7 ± 15.52%; corrected p-value = 0.002) between the first and second assessments. Although statistically significant improvement was not obtained on comparisons of specific clinical features, the overall GAS-WD scores improved significantly (14.38 ± 5.35 vs. 9.77 ± 6.47; corrected p-value = 0.04). The CMCT, however, did not show any significant improvement (7.81 ± 2.79 vs. 8.60 ± 1.42; p-value = 0.2).

There were no significant correlations identified between the RMT, CMCT, and GAS-WD scores at any time point of measurement. However, a significant correlation was obtained between the change in the RMT (RMT_{1-2}) and GAS-WD score ($GAS-WD_{1-2}$) ($r = 0.7$, p-value = 0.002) after correction for the duration of chelation. There was no significant association of the change in CMCT values and GAS-WD scores.

This study demonstrates the longitudinal improvement in the RMT and GAS-WD scores in patients with WD while on

Table 3: Longitudinal measurement of resting motor threshold and central motor conduction time in patients with WD

Patient code/Age/Gender	Duration of chelation	Pre-chelation measurement			Measurement during follow-up		
		RMT_1	$CMCT_1$	$GAS\text{-}WD_1$	RMT_2	$CMCT_2$	$GAS\text{-}WD_2$
Patient 1, 27 years, Male	12 months	90	7.11	18.00	75	7.50	10.00
Patient 2, 12 years, Male	12 months	NR	–	10.00	80	6.90	10.00
[a]Patient 3, 19 years, Female	12 months	50	7.81	10.00	85	6.11	21.00
Patient 4, 8 years, Female	12 months	NR	–	28.00	71	9.57	14.00
Patient 5, 33 years, Male	12 months	73	6.25	17.00	62	6.80	6.00
Patient 6, 18 years, Male	15 months	55	6.03	16.00	51	8.08	11.00
Patient 7, 18 years, Female	26 months	95	13.5	14.00	85	8.60	4.00
Patient 8, 31 years, Male	6 months	70	8.02	14.00	62	7.95	10.00
[b]Patient 9, 18 years, Female	9 months	55	12.8	14.00	65	10.23	14.00
Patient 10, 12 years, Male	36 months	80	8.51	14.00	55	11.2	14.00
Patient 11, 14 years, Male	6 months	67	10.5	8.00	59	10.1	3.00
Patient 12, 26 years, Male	9 months	NR	–	10.00	79	8.11	2.00
[a]Patient 13, 22 years, Female	12 months	67	7.60	16.00	77	7.60	25.00
Patient 14, 19 years, Male	12 months	42	7.90	4.00	42	9.96	2.00
Patient 15, 17 years, Female	12 months	68	8.40	17.00	39	7.82	8.00
Patient 16, 16 years, Male	12 months	48	6.75	15.00	42	8.57	3.00
Patient 17, 17 years, Male	12 months	70	8.60	22.00	44	10.3	11.00
[b]Patient 18, 14 years, Female	6 months	68	8.15	12.00	75	9.56	16.00

CMCT = Central motor conduction time; GAS-WD = Global Assessment Scale for Wilson Disease score; NR = Non recordable; RMT = Resting motor threshold.

[a]Treatment defaulters; [b]Patient on regular treatment with worsening of RMT and GAS-WD score.

chelation therapy. There was a significant association between the improvement in RMT and GAS-WD scores denoting the potential of RMT measurement as a neurophysiological biomarker in assessing chelation efficacy.

TMS enables the study of the functional properties of the motor cortex and corticospinal tract (CST). The factors affecting the RMT, CMCT, and their mechanism are not well understood. It is believed that RMT is a measure of the excitability of the cortico-cortical fibers projecting to the corticospinal neurons, whereas CMCT depicts the electrical conduction through the CST.[1–4,8] The RMT changes observed in WD, hence, is a reflection of the cortico-motoneuronal dysfunction causing the decrease in the motoneuron excitability. The motoneuron excitability is influenced by the basal ganglia dysfunction and primary intracortical damage which are further dependent on copper toxicity in WD.[1–4,8,9] Therefore, chelation therapy may decrease the concentration of circulating copper and improve the clinical abnormalities and basal ganglia dysfunction. This is further supported by a previous study that has demonstrated an inverse relationship between copper concentration and cortical excitability.[10] It is likely that adequate chelation may improve the RMT by decreasing the copper content and its detrimental sequelae, at least when adequately and timely treated. Furthermore, the changes in the RMT paralleled the changes in GAS-WD score both in treatment defaulters and in subjects who adhered to the chelation regimen. The worsening of RMT and GAS-WD score observed in two subjects while on regular treatment may be due to penicillamine-induced worsening.[5] Hence, our study

demonstrates that longitudinal measurement of RMT may reflect the clinical status of the subject and may have clinical relevance in the evaluation of chelation efficacy in individual subjects. In contrast to our findings, few studies have not observed improvement in RMT following chelation therapy.[1–3] However, their conclusions were based on the follow-up measurements of one patient in Jhunjhunwala et al. and three subjects in Perretti et al.[2,3] Nonetheless, it is possible that the timing and adherence to therapy may be key factors influencing improvement in RMT.

In contrast to RMT, lack of improvement in CMCT in our study may suggest irreversible damage to the CST. Previous neurophysiological studies using TMS in different diseases have demonstrated the marked prolongation of CMCT to indicate the loss of neurons or demyelination changes.[1–4,8] However, mildly prolonged CMCT in our cohort probably may suggest a limited neuronal loss than demyelination.[8,11,12] The involvement of CST in WD has been established by previous clinical, neurophysiological, and neuroimaging studies.[1,4–6] However, reversibility of CST involvement is not well known. Longitudinal TMS studies by Perretti et al. (three subjects) and Hefter et al. (six subjects) have reported improvement in CMCT with adequate chelation.[3,4] The causes for this apparent disparity in our results are uncertain. However, it should be noted that, although not statistically significant, a majority of patients had an improvement of CMCT value from the baseline measurement in this study. It is possible that reversibility of CST involvement may be dependent on how early chelation therapy is instituted.

To conclude, this study evaluated longitudinal TMS changes in WD and demonstrated the reversible nature of copper-induced neurophysiological changes. Our results imply the reversible nature of cortico-motoneuronal dysfunction of the motor cortex and potentially irreversible nature of CST involvement in WD. However, comprehensive and prospectively designed studies on larger cohorts of patients are necessary to validate our findings.

STATEMENT OF AUTHORSHIP

AS: Conception, execution, organization of the study, statistical analysis, and writing of first draft.

NK: Conception, execution of study, and writing of first draft.

KJ: Conception,organization, and execution of the study.

SP: Organization and critical review.

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DISCLOSURES

AS, KJ, NK, SP, and PKP have nothing to disclose.

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