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ORIGINAL ARTICLE

Optical coherence tomography in patients with Wilson's disease

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Objectives: Wilson disease (WD) is an autosomal recessive disorder that leads to copper accumulation and deposition in different organs, frequently affecting visual pathways. Recent studies have detected morphological changes of the retina in patients with WD using optical coherence tomography (OCT). Measuring the thickness of the retinal nerve fibre layer (RNFL) with OCT provides an objective assessment of integrity and morphological abnormalities of the retina. The aim of this study was to evaluate the relationship between OCT parameters and form of the disease, therapy and symptoms duration, as well as severity of neurological impairment.

Methods: The study comprised of 52 patients with WD and 52 healthy controls (HC). All the patients were on a regular and stable chelation therapy and/or zinc salts. Patients were divided into two groups, with neurological (NWD) or hepatic form of the disease (HWD). OCT was performed to assess the RNFL thickness.

Results: The WD patients had significantly lower intraocular pressure in both eyes and lower RNFL thickness than the HC. There were no differences between NWD and HWD in any of the ophthalmologically tested parameters. No significant correlations were found between clinical features and retinal thickness parameters. Stratification of the cohort according to the disease duration showed that disease duration did not influence the RNFL thickness.

Conclusion: We found that involvement of the retina represented a subclinical finding in neurologically intact patients in the HWD group. Nevertheless, the value of OCT as a biomarker for the assessment of the clinical course and progression of WD still remains uncertain.

KEYWORDS
optical coherence tomography, retinal nerve fibre layer, Wilson's disease

1 | INTRODUCTION

Wilson's disease (WD) is an autosomal recessive disorder caused by mutations of the copper transporter *ATP7B* gene on chromosome 13, in which impaired biliary copper excretion leads to its accumulation and deposition in different parts of the body, particularly in the liver and brain.[1,2]

Eyes (cornea, lens and retina), as well as visual pathways, are also frequently affected in WD patients.[3] Besides the Kayser-Fleischer (KF) ring that was present in almost all WD patients with neurological

form of the disease,[4,5] other rare ophthalmological manifestations in WD included sunflower cataracts,[6,7] optic neuritis, optic disc pallor and exotropia.[8-10] Functional disturbances in visual pathways were also reported using electroretinography and visually evoked potentials.[11-13] More recent studies have detected morphological changes of the retina in patients with WD using optical coherence tomography (OCT),[12,14,15] a non-invasive imaging technique that uses illumination with optical beams.[16] Anterior segment optical coherence tomography (AS-OCT) could provide useful, accurate and objective diagnostic valuation of copper deposits creating KF ring in WD patients.[17] Measuring the thickness of the retinal nerve fibre layer (RNFL), which is mainly composed of unmyelinated axons with OCT, provides an objective assessment of integrity and morphological abnormalities of the retina.

Thinning of the RNFL as a result of axonal loss has been previously suggested to be a marker of neurodegeneration in various diseases, including WD,[14,18,19] particularly in those WD patients with more prominent MRI changes[12] or more severe neurological impairment.[15]

The aim of this study was to evaluate the relationship between OCT parameters and form of the disease, therapy and symptoms duration, as well as severity of neurological impairment.

2 | MATERIALS AND METHODS

This cross-sectional, non-interventional, observational study has been approved by the Ethics Committee of the Medical School University of Belgrade (Serbia). The study comprised 52 patients with WD consecutively recruited at the Movement Disorders Department, Clinic of Neurology CCS from January 2019 to December 2019 and 52 age- and sex-matched healthy controls (HC).

The diagnosis of WD was established according to the international criteria[9] and afterwards, genetically confirmed. All the patients were on a regular and stable chelation therapy (d-penicillamine and trientine) and/or zinc salts (Table 1).

Neurological evaluation was based on a detailed neurological interview and examination. According to the main affected system, the patients were divided in two groups, with neurological (NWD) or hepatic forms of the disease (HWD). For evaluation of the neurological dysfunction in NWD, Tier 2 of the *Global Assessment Scale (GAS) for WD*[20] was used. For the assessment of ophthalmological parameters in patients according to disease duration, we stratified our cohort into four groups: disease duration shorter than 5 years, 5–10 years, 10–15 years and longer than 15 years. The patients were also examined by an experienced ophthalmologist (Ophthalmology Clinic CCS) who besides the best-corrected visual acuity, slit-lamp anterior segment examination, as well as a slit lamp with a calibrated Goldmann tonometer used for Goldmann applanation tonometry (GAT), the central corneal thickness measurement, indirect fundoscopy, automated visual field examination (only reliable fields with a fixation loss rate ≤33% and false-positive and false-negative rates ≤20% were included) and an ultrasonographic axial length measurements, also conducted the Optical Coherence Tomography (OCT) examination (Optovue iVue SD-OCT; Optovue Inc,).

OCT was performed to assess the RNFL thickness. Both eyes of each participant were examined. A signal strength index (SSI) >40 was accepted. An image was finalized for analysis purpose only if the full extent and depth of the retina was distinguishable clearly. No blinking or eye movement artefacts were accepted. RNFL thickness was measured within an area of diameter 3.45 mm around optic disc. The innermost circle was divided into superior and inferior halves. The outer circle was divided into four sectors—temporal, superior, nasal and inferior at 45°, 135°, 225° and 315°, respectively. The outermost circle was divided into 8 sectors at 0°, 45°, 90°, 135°, 180°, 225°, 270° and 315°, respectively.

The exclusion criteria comprised the presence of any type of preceding ophthalmic surgery or disease, pregnancy, as well as contraindications to apply 1% tropicamide, but none of the patients was excluded due to the presence of any exclusion criteria.

TABLE 1 Clinical and demographic features of patients with neurological and hepatic for of Wilson's disease

Feature	Hepatic form of Wilson's disease	Neurologic form of Wilson's disease	*p*
Number[b]	23 (44%)	29 (56%)	
Gender (male/female)	14/9	21/8	.378
Age (years)[a]	35.1 ± 10.9 (13–55)	42.6 ± 11.2 (28–66)	.021[c]
Age at onset (years)[a]	21.4 ± 10.1 (7–42)	27.7 ± 8.0 (14–45)	.016[c]
Age at diagnosis (years)[a]	22.4 ± 10.3 (7–42)	29.5 ± 7.9 (17–48)	.007[c]
Disease duration (years)[a]	13.6 ± 9.1 (1.5–44)	14.4 ± 9.7 (1–43)	.744
Latency from diagnosis to treatment (years)[a]	0.7 ± 0.9 (0–3)	1.9 ± 2.7 (0–14)	.076
Treatment duration[a]	12.8 ± 9.0 (1–43)	13.2 ± 10.1 (1–45)	.88

[a]values presented as means ± SDs with range in brackets.

[b]values presented as number of patients with percentage in brackets.

[c]$p < .05$.

TABLE 2 Ophthalmic parameters of tested subjects

Parameter	Patients with Wilson's disease (*n* = 52)	Controls (*n* = 52)	OR (95%CI)	*p*
VA R	0.97 ± 0.10	0.97 ± 0.08	3.1 (0.4–7.8)	.623
IOP R	13.25 ± 2.56	14.94 ± 2.01	1.4 (1.1–1.7)	.001
CCT R	557.22 ± 30.11	552.37 ± 31.34	1.0 (0.9–1.1)	.417
LAX R	23.45 ± 0.76	23.37 ± 0.96	0.9 (0.6–1.4)	.661
AVRG RNFL R	97.97 ± 8.72	110.78 ± 11.91	1.2 (1.1–1.3)	.000
RNFL S R	100.08 ± 10.33	114.69 ± 11.26	1.2 (1.1–1.3)	.000
RNFL I R	95.85 ± 9.42	114.51 ± 15.21	1.2 (1.1–1.3)	.000
CD AR R	0.28 ± 0.16	0.26 ± 0.17	0.4 (0.1–4.7)	.481
CV R	0.16 ± 0.19	0.12 ± 0.09	0.2 (0.1–2.5)	.183
VA L	0.98 ± 011	0.98 ± 0.05	1.3 (0.1–12.2)	.905
IOP L	13.23 ± 2.50	15.04 ± 2.33	1.4 (1.1–1.6)	.001
CCT L	559.60 ± 30.08	552.44 ± 31.24	1.0 (0.9–1.1)	.243
LAX L	23.40 ± 0.76	23.40 ± 0.98	1.0 (0.6–1.6)	.999
AVRG RNFL L	97.77 ± 6.35	111.05 ± 10.71	1.2 (1.1–1.4)	.000
RNFL S L	97.87 ± 13.62	113.67 ± 10.91	1.2 (1.1–1.3)	.000
RNFL I L	95.22 ± 8.09	114.57 ± 14.43	1.2 (1.1–1.3)	.000
CD AR L	0.27 ± 0.15	0.22 ± 0.14	0.1 (0.01–1.7)	.110
CV L	0.12 ± 0.16	0.09 ± 0.10	0.2 (0.01–4.1)	.298

Note: Values presented as means ± SDs.

Abbreviations: AVRG RNFL L, average RNFL thickness left eye; AVRG RNFL R, average RNFL thickness right eye; CCT L, central corneal thickness left eye; CCT R, central corneal thickness right eye; CD L, cup disc left eye; CD R, cup disc right eye; CV L, cup volume left eye; CV R, cup volume right eye; IOP R, intraocular pressure right eye; LAX L, axial length left eye; LAX R, axial length right eye; RNFL I L, RNFL thickness inferior segment left eye; RNFL I R, RNFL thickness inferior segment right eye; RNFL S L, RNFL thickness superior segment left eye; RNFL S R, RNFL thickness superior segment right eye; VA L, visual acuity left eye; VA R, visual acuity right eye.

2.1 | Statistical analysis

Comparisons of the OCT and clinical and demographic features were performed using the Student's *t* test. Univariate correlations between each of the OCT variables and clinical features were evaluated using the Spearman correlation coefficients. Statistical analysis was performed using SPSS, and *p*-values below .05 were considered statistically significant. Logistic regression analysis was used for the examination of groups of WD patients stratified according to the disease duration and its influence on examined ophthalmological parameters.

3 | RESULTS

Fifty-two patients with WD (35 females and 17 males; mean age 39.3 0± 11.6 years) were divided in two groups: NWD (21 females and 8 males; mean age 42.6 ± 11.2 years) and HWD (14 females and nine males; mean age 35.1 ± 10.9 years). HC comprised 35 females and 17 males, with a mean age of 39.8 ± 11.8 years). Sex distribution and age at the examination were not statistically different between the groups.

Clinical and demographic features of both groups of patients are presented in Table 1. Patients in HWD when compared to NWD group were younger, had onset of WD at an earlier age, and were also younger at the time of diagnosis establishment. Thirty patients were treated with D-penicillamine, and 26 also received zinc-sulphate.

Patients with WD had significantly lower intraocular pressure in both eyes and lower RNFL thickness when compared to HC (e.g. the RNFL thickness in WD patients was lower both in the superior and inferior segments) (Table 2; Figure 1). No significant differences were found in any of the ophthalmologically tested parameters between NWD and HWD groups (Table S1). OCT findings of some of our patients are presented as Figures S1-S6. Also, no significant correlations were found between clinical features (age, duration of disease, duration of treatment, dosage of D-penicillamine, GAS Neurological assessment) and retinal thickness parameters (AVRG RNFL, RNFL S, RNFL I).

When patients were stratified according to disease duration no association was found between disease duration and different ophthalmological parameters between hepatic and neurological form of WD (Table S2).

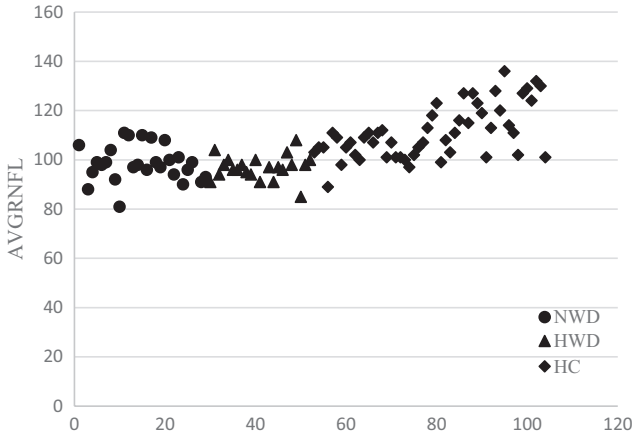

FIGURE 1 Values of average RNFL thickness in the superior and inferior segments in NWD and HWD patients and healthy control subjects. HC, Values of average RNFLT in HC subject; HWD, Values of average RNFLT in NWD patients; NWD, Values of average RNFLT in NWD patients

4 | DISCUSSION

Our findings confirmed abnormalities in the morphological status of the retina in WD patients when compared to matched HC (eg significant difference in the RNFL thickness), but without difference in the RNFL thickness between the two main forms of WD (NWD and HWD).

Our results were in line with previous findings. Albrecht et al[14] reported OCT changes in WD patients, although they were not divided according to the predominant clinical expression (NWD or HWD), treating them as a uniform group. Langwinska et al[12] measured macular and retinal nerve fibres in WD patients with (all patients with NWD) and without MRI changes (majority of patients with HWD had normal imaging findings). They found that macular and retinal nerve fibres were thinner and markedly different in those with MRI changes, although subtle abnormalities were also evident in cases without visible pathology on brain imaging. In continuation, the same authors concluded that retinal thinning occurred in more advanced stages of WD or predominantly in patients with a more severe brain pathology. A significant negative correlation was found between OCT parameters and severity of neurological impairment.[15]

Using a slightly different approach, we separated patients into NWD and HWD groups and found no differences between them (eg patients with hepatic form of WD patients also had statistically significant lower RNFL thickness, as observed in those with NWD). Retrograde thinning of the RNFL has been previously proposed to be an early marker of neurodegeneration. Thus, in patients with Alzheimer's disease, it was considered to be one of the earliest signs, appearing even earlier than the hippocampal damage.[21] Moreover, it has also been suggested that RNFL thickness changes could represent a marker of disease progression. In patients with Parkinson's disease it correlated with its duration and severity,[22,23] while in patients with Huntington's disease (HD) temporal RNFL thickness was inversely correlated with disease duration.[18] Interestingly enough, our patients with isolated HWD also had thinner RNFL.

The retina consists of axons and glia without myelin and maybe a good structure for visualizing the degree of neurodegeneration,[24] including neurodegeneration in WD due to the copper accumulation that also involved the retina. Sturniolo et al[25] investigated parameters of the corneal sub-basal nerve plexus in patients with WD using corneal confocal microscopy. They found that the nerve fibre length density, number of fibres, number of beadings, and number of branching were significantly lower and fibre tortuosity was significantly higher in patients with WD when compared with HC. The main pathways of copper toxicity in WD were direct oxidative stress and apoptosis, resulting in cell death in the affected tissues.[26] To a certain extent, the excess copper was neutralized by antioxidants, but an elevated level of reactive oxygen species beyond a certain limit might induce undesirable oxidative damage in the cells.[27,28] Therefore, the observation of thinner RNFL supported prior observations that chronic copper-related degeneration affected unmyelinated fibers.[21]

Data obtained in this study are in line with our previous publication,[29] which found abnormal brain MRI findings in a group of 16 neurologically asymptomatic and untreated patients with purely hepatic form of WD.

However, lesions in brain parenchyma were detected in 44% of treated patients. The lower frequency of lesions in the group of treated in comparison with untreated patients indicated that they might be reversible in the course of chronic chelating therapy. Interestingly enough, RNFL thickness in our cohort was stable and changes were irreversible despite the continual treatment.

Remarkably, it was shown that disease duration does not influence the RNFL thickness. Albeit, when patients with WD were stratified according to the disease duration, RNFL thickness remained unchanged. Although we have not performed a follow-up study, we calculated that this stratification might be helpful in conclusion that disease duration does not affect the RNFL thickness.

It was not clear whether the treatment of our patients might have an effect on the visual system or the retinal morphology. There was no correlation between the dosage of d-penicillamine and retinal thickness in our study, although previous investigation reported changes of the retinal pigment epithelium under d-penicillamine therapy in one case report.[30] However, even though d-penicillamine was the most common treatment for WD, no other similar case with retinopathy induced by d-penicillamine or other chelating agent has been reported.

Langwińska-Wośko et al[12,15] suggested the promising role of OCT as a potential non-invasive technique that could be used together with brain MRI and disease severity scores to assess progression or monitor treatment in WD patients. However, our data revealed that the retina was involved even in patients without overt neurological symptomatology who were on stable, long-lasting therapy.

We have also shown that WD patients had lower IOP pressures than the matched HC, but this issue deserves further studies.

The main disadvantage of our study is the lack of follow up data. We plan to continue examinations in the future. Also, we have tried to overcome this drawback by stratification of the cohort according to the disease duration. Our analysis suggested that disease duration did not influence RNFL thickness.

In conclusion, our WD patients with and without neurological symptoms tended to perform worse on some ophthalmological tests than the matched HC. According to our findings, the retinal involvement represented a subclinical, but definitive finding in neurologically intact patients in the HWD group, as well as in neurologically affected WD patients. RNFL thickness does not correlate with the disease and treatment duration, and it is persistently present early at disease onset, and even after extended disease duration. However, controlled longitudinal studies are needed to assess the value of OCT as a biomarker of the clinical course and progression of WD, as well as the effects of treatment.

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CONFLICT OF INTEREST

Marina Svetel has received speaker's honoraria from Actavis. Vladimir Kostić has received research grants from the Ministry of Education, Science, and Technological Development, Republic of Serbia and the Serbian Academy of Science and Arts; and speaker honoraria from Actavis and Salveo. Nataša Dragašević, Nikola Kresojević, Čarna Jovanović, Jelena Vitković, Marija Božić, Marko Svetel and Tatjana Pekmezović declare no confict of interest.

ETHICAL STATEMENT

The study was approved by the Institutional Review Board of the Clinic of Neurology and have, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

INFORMED CONSENT

All patients gave informed consent.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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