Small Fiber Peripheral Neuropathy in Wilson Disease: An In Vivo Documentation by Corneal Confocal Microscopy

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Purpose. Wilson disease (WD) is a disorder of hepatic copper metabolism leading to copper accumulation in hepatocytes and in extrahepatic organs, as the brain and cornea. The aim of this study was to investigate central corneal changes and in particular to assess the parameters of corneal subbasal nerve plexus (SBNP) in patients affected by WD, using corneal confocal microscopy (CCM).

Methods. A total of 24 patients affected by WD and 24 healthy control subjects were included in this cross-sectional comparative study. One eye of each subject was examined to quantify different corneal parameters. Mean cell diameter and mean cell density of the epithelium; number of fibers (NF), nerve fiber length density (NFLD), number of branchings (NBr), number of beadings (NBe), and fiber tortuosity (FT) of the SBNP; mean cell density of keratocytes of the anterior, medium, and posterior stroma; and mean cell density, polimegatism, and pleomorphism of the endothelium, and central corneal sensitivity were analyzed.

Results. Wilson disease induced significant alterations in SBNP, and corneal epithelium. The NFLD ($P < 0.0001$), NF ($P = 0.001$), NBe ($P = 0.025$), and NBr ($P < 0.0001$) were significantly lower, whereas FT ($P < 0.0001$) was significantly higher in WD subjects compared to controls. Moreover mean epithelial cell diameter ($P < 0.0001$) and mean epithelial cell density ($P < 0.0001$) were significantly higher and lower compared to controls, respectively.

Conclusions. The CCM showed significant corneal changes in SBNP, with concomitant corneal epithelium changes in WD, demonstrating the presence of small fiber peripheral neuropathy in these patients. The CCM may contribute to diagnosis and monitoring of the peripheral nervous system involvement in WD.

Keywords: corneal confocal microscopy, Wilson disease, subbasal nerve plexus

Wilson disease (WD) is an inherited autosomal recessive disorder of hepatic copper metabolism leading to copper accumulation in hepatocytes and in extrahepatic organs, as the brain and cornea.[1] The WD gene *ATP7B* is localized in the chromosome 13 and encodes a transmembrane copper-transporting P-type ATPase.[2] This ATPase physiologically transports copper into the trans-Golgi compartment, for incorporation into the plasma protein ceruloplasmin, and into the bile, for excretion of excess stores. Defective *ATP7B* function results in impaired biliary excretion of copper.[2] Clinical presentation of WD widely varies, but it commonly consists of either predominantly hepatic or neuropsychiatric symptoms. Asymptomatic patients, however, often are detected by family screening.[3,4] There are two alternative groups of pharmacological agents as first-line therapy: copper chelators (i.e., D-penicillamine, trientine) and zinc salts (i.e., zinc sulfate), effective in treating WD.[5,6] Ophthalmological manifestations of WD include: Kayser-Fleischer (KF) corneal ring, caused by the granular deposition of copper in the corneal Descemet membrane, and sunflower cataract, induced by deposits of copper in the middle of the lens.[2,4] Other less common findings are: night blindness, exotropia, optic neuritis, and optic disc pallor.[2,4] Although many studies reported the involvement of the central nervous system (CNS) in WD, very few studies analyzed the damage of peripheral nerves in this disease.[7–10]

The cornea is one of the most densely innervated tissues of the body, and is richly supplied by sensitive and autonomic peripheral nerve fibers.[11] Corneal fibers are mainly sensitive (A delta and C fibers) and originate from the ophthalmic branch of the trigeminal nerve.[12–15] The best analyzed and quantified neural component of the cornea is the corneal subbasal nerve plexus (SBNP), a monolayer of fine, amyelinated nerve fibers, which has shown discrete changes in different systemic disorders.[16–18] Corneal confocal microscopy (CCM) is a fast reliable and repeatable technique to analyze the human cornea in vivo, allowing a high magnification imaging of different corneal structures, including corneal nerves.[19–21] This diagnostic technique is recognized as the gold standard to quantify, in a noninvasive way, the different corneal layers, in ocular and systemic diseases.[16,19,22] A quantitative analysis of corneal SBNP may be useful to evaluate if subclinical signs of peripheral nerves damage exist in WD.

The aim of this study was to assess corneal changes in subjects affected by WD using CCM, focusing on SBNP to detect early peripheral nerve alteration before any systemic clinical manifestation.

Materials and Methods

Population

This cross-sectional comparative study included 24 patients affected by WD recruited between March and September 2012. The diagnosis of WD was based on copper metabolism and genetic examination, and was supported by international diagnostic criteria, as described previously.[1] Approval was obtained from the local institutional review board and all subjects gave their informed consent before being enrolled in the study, in accordance with the tenets of the Declaration of Helsinki.

The inclusion criterion was an established diagnosis of WD, while the exclusion criteria were corneal dystrophies; previous history of glaucoma, ocular hypertension, ocular surgery, or injury; any form of infectious keratitis; history of contact lens wear; any dry eye diseases; and any other systemic disease that might affect the cornea. All subjects underwent a full and accurate slit-lamp microscopic examination to exclude any clinically manifest corneal disease and to assess the presence of Kayser-Fleischer ring and/or of the sunflower cataract. Of 27 consecutive patients with WD, we included 24 WD patients. One case was excluded for previous photorefractive keratectomy (PRK), one for contact lens wearing, and one for epithelial dystrophy.

Clinical and demographic characteristics were collected, including age, sex, age at the diagnosis, duration of the disease, urinary copper, systemic symptoms, ophthalmic signs, and current therapy. Neurological and hepatic predominant forms of the disease were previously distinguished according to the presence and intensity of individual signs at the time of diagnosis.[1,23] To evaluate the 24-hour urinary copper parameters, we considered the ratio between the value in micromoles and the upper value of normality of each subject. These data were used to calculate the score proposed by Ferenci et al.[1] (score 0, normal cupruria; score 1, 1–2 × upper limit of normal [ULN] cupruria; score 2, >2 × ULN cupruria).

A total of 24 age-matched healthy subjects was studied as controls. They were free of any systemic disease, and had negative history of ocular disease or use of contact lenses.

Corneal Confocal Microscopy (CCM)

We performed CCM using the Confoscan 4.0 device (Nidek, Gamogori, Japan), equipped with an Achroplan nonapplanating ×40 immersion objective lens (Zeiss, Oberkochen, Germany). Each examination was performed according to a standard procedure, described previously, by one experienced examiner.[24] The Z-ring was used for all examinations, and only the central cornea was evaluated. Illumination intensity was kept constant for all cases. The standard dimension of each image produced was 0.132 mm^2, with an optical section thickness of 5.5 μm.

Image Analysis

Images were selected by a single masked operator. Selected images were evaluated by two different masked experienced observers. For each cornea, the last clear and centered frame of all superficial epithelium images was chosen to calculate the mean cell diameter (micrometers), using the analysis software of the instrument, by averaging the diameters of 10 randomly selected cells. Basal epithelium cell density (cells/mm^2) was quantified by manually counting the cells within a region of interest (0.05 mm^2). The corneal SBNP was evaluated, choosing the best focused image of this structure. For each frame of SBNP images, five parameters were analyzed using the "Nerve Tracking V1.3.0" (a semiautomatic image processing tool of the instrument): number of fibers (NF), nerve fiber length density (NFLD), number of branchings (NBr), number of beadings (NBe), and fiber tortuosity (FT). The NF was calculated and defined as the number of principal nerve trunks per image. The NFLD was quantified as the total length of the nerves (micrometers) per square millimeter. The NBr was calculated and defined as the total number of branchings per frame. The NBe was defined as the number of hyperreflective points in 100 μm of one fiber randomly chosen. The same standard magnification was kept for all images during the counting procedure.[18,25] The NT was calculated on one fiber using the tortuosity index of the Nerve Tracking tool. The best focused image was selected from the anterior, medium, and posterior stroma for the assessment of keratocyte density (cells/mm^2), by manually counting the cells in fixed area (0.1 mm^2). All exams were performed using the Z-ring device. To determine anterior, medium, and posterior stroma, the total number of all the images immediately upon the endothelium and beneath the subbasal nerve plexus was divided in three parts and the keratocytes density was calculated by manually counting the cells in fixed area (0.1 mm^2) in the best focused image from these three layers, as described by Erie et al.[26]

For the endothelium, the following parameters were calculated automatically, by the instrument: cell density (cells/mm^2), polimegatism (percentage of oversized cells), and pleomorphism (percentage of hexagonal cells).

Corneal Esthesiometry

Corneal sensitivity of the central cornea was measured by the Cochet-Bonnet esthesiometer. The examination started with a 60-mm length of nylon filament and continued by shortening it by 5 mm until the patient felt the touch of the filament. It was applied perpendicularly to the central cornea.[27] Corneal sensitivity was measured before the corneal confocal microscopy examination.

Statistical Analysis

Only one eye was considered in the statistical analysis and the examiner was blind to this choice. A secretary, not involved in patients' examination, was in charge of applying the randomization procedure to the patients as they were enrolled into the study. The procedure was based on a sequence of 2 × 2 latin squares to guarantee balanced number of right and left eyes in the sample. All analyses were performed using SAS software 9.1.3 (SAS, Inc., Cary, NC, USA). The Student's *t*-test was used to compare quantitative continuous variables between WD patients and controls. Quantitative discrete variables were analyzed using the Mann-Whitney *U* test. Pearson's and Spearman's correlation was used to examine the relationship between continue and noncontinuous variables, respectively. For all tests a *P* value of 0.05 was considered statistically significant. The interclass correlation coefficient (ICC) was calculated between the two masked examiners.

Results

A total of 24 subjects affected by WD (15 men and 9 women, study group) with a mean age of 35.1 ± 8.2 years (range, 20–47 years), and 24 matched healthy subjects (15 men and 9

Table 1. Quantification of Different Parameters in Patients Affected by Wilson Disease and Controls

Parameter	Wilson	Controls	*P*
Superficial epithelium, M ± SD			
Cell Diameter, μm	23.49 ± 1.70	20.78 ± 2.62	<0.0001
Basal epithelium, M ± SD			
Cell Density, cell/mm²	4260 ± 442.10	6885 ± 265.10	<0.0001
SBNP, M ± SD			
NFLD, μm/mm²	9891 ± 3169	13860 ± 3904	<0.0001
NF	3.75 ± 1.51	5.23 ± 1.48	0.001
NBr	1.25 ± 0.96	1.89 ± 0.78	0.025
NBe, n/μm	10.02 ± 2.02	12.25 ± 2.35	<0.0001
FT index	7.17 ± 2.17	5.18 ± 1.20	<0.0001
Anterior stroma, M ± SD			
Cell Density, cell/mm²	716 ± 76.28	679.1 ± 65.84	0.080
Medium stroma, M ± SD			
Cell Density, cell/mm²	556.5 ± 53.12	548.6 ± 55.96	0.618
Posterior stroma, M ± SD			
Cell Density, cell/mm²	546.5 ± 49.52	538.1 ± 58.69	0.593
Endothelium, M ± SD			
Density, cells/mm²	2984 ± 350.9	2821 ± 356.5	0.116
Polimegatism, %	35.46 ± 6.34	38.50 ± 5.88	0.092
Pleomorphism, %	51.44 ± 8.78	48.40 ± 7.07	0.192

M ± SD, mean ± SD.

women, control group) with a mean age of 35.3 ± 9.3 (range, 19–49 years) were included (no significant difference for age; $P = 0.0948$). Of the patients, 10 had predominantly CNS neurological presentation, while 14 had a predominantly hepatic presentation. Considering the study group, 10 patients (6 men and 4 women) were characterized by a previous KF ring (still present in two eyes at the moment of confocal examination). One patient presented with a sunflower cataract, not associated with KF ring. At the moment of the examination, WD patients were receiving different maintenance therapies: 19 (79.2%) were receiving zinc acetate, 2 (8.3%) D-penicillamine, and 3 (12.5%) zinc acetate associated with trientine. Mean age at the diagnosis was 16.7 ± 9.2 years, and the mean duration of the disease was 18.4 ± 9 years.

The mean value of the urinary copper ratio was 5.2 ± 6.1 (range, 0.29–24.4) at diagnosis and 5.4 ± 6.3 (range, 1.1–22.7) at the moment of CCM examination. Using the score proposed by Ferenci et al.[1], 6 (25%) of WD subjects had score 0, 5 (20.8%) score 1, and 13 (54.2%) score 2 at the moment of the diagnosis, while 10 (41.7%) had score 1 and 14 (58.3%) score 2 at the moment of the confocal microscopy examination.

No statistically significant differences were found with respect to corneal sensitivity ($P = 0.096$). The WD patients shows a central corneal sensitivity of 54.1± 6.0 while the controls 56.6 ± 4.0.

A statistically significant difference between WD patients and controls for corneal subbasal nerve plexus and epithelium parameters was found. All parameters of the subbasal nerve plexus were significantly different between the two groups: NFLD ($P < 0.0001$), NF ($P = 0.001$), NBe ($P = 0.025$), and NBr ($P < 0.0001$) were significantly lower, whereas FT was significantly higher ($P < 0.0001$) in WD subjects versus controls (Table 1; Fig. 1). Considering the epithelium, mean cell diameter at the superficial level was significantly higher ($P < 0.0001$), and mean cell density at the deep basal layer significantly lower ($P < 0.0001$) in WD patients versus controls (Table 1; Fig. 2). No significant differences in stroma and

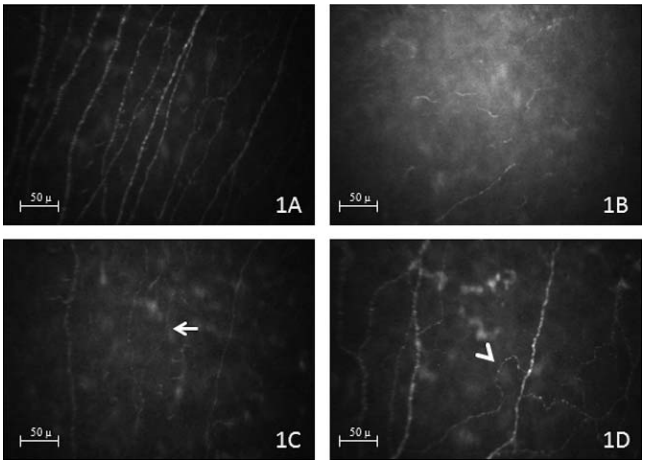

Figure 1. The CCM images of subbasal nerve plexus. Morphological CCM features of subbasal nerve plexus in a control subject (**A**) and three patients affected by Wilson's disease (**B–D**). (**B**) The paucity of nerve fibers is evident when compared to (**A**). (**C**) The *white arrow* points to a nerve where reduced beadings are evident. (**D**) The increased tortuosity (*white arrowhead*) compared to (**A**) also is evident.

endothelium were observed between the two groups (Table 1). The ICC was excellent for all examined parameters, therefore the data of only one examiner are reported. No correlations was found between corneal SBNP (NFLD, NF, NBe, NBr, FT) parameters and epithelial parameters. Similarly, no positive or negative correlations were found between corneal sensitivity results and morphological CCM parameters.

The comparison between predominantly neurological and predominantly hepatic presentation did not show any statistical differences between the two subgroups (data not shown). No significant differences were documented among different treatment groups (data not shown). No correlations were found between the urinary copper ratio at diagnosis or at the moment of examination and corneal SBNP parameters (NFLD, NF, NBe, NBr, FT) or corneal epithelial parameters. No statistical differences were observed between the group with Ferenci's score 1 and 2 at the moment of examination (data not

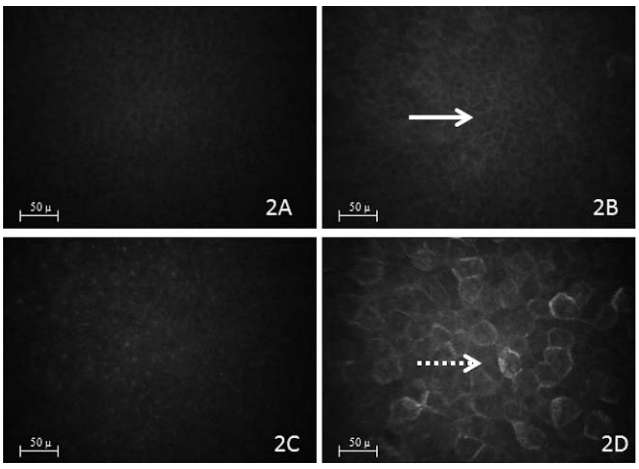

Figure 2. The CCM images of epithelium. Images of corneal basal epithelium (**A**) and superficial epithelium (**C**) in a control subject (**B**) and in a patient affected by Wilson's disease (**D**). (**B**) The reduction of corneal basal epithelium cells density is shown with *white arrow*. (**D**) An increased size of superficial epithelial cells also is evident with respect to control subject (*dotted arrow*).

Table 2. Quantification of Different Parameters in Patients Affected by Wilson Disease With Previous or Persistent Copper Deposits (Cu+), Versus Patients Who Never Presented Copper Deposits (Cu–) and Controls

Parameter	Cu+	Cu–	*P*
Superficial epithelium, M ± SD			
Cell diameter, μm	23.53 ± 1.84	23.46 ± 1.64	n.s.
Basal epithelium, M ± SD			
Cell density, cell/mm^2	4266 ± 367.6	4254 ± 522.9	n.s.
SBNP, M ± SD			
NFLD, μm/mm^2	9054 ± 2851	10730 ± 3368	n.s.
NF	3.33 ± 1.29	4.17 ± 1.47	0.002
NBr	1.08 ± 0.67	1.42 ± 1.18	0.036
NBe, n/μm	9.83 ± 2.39	10.21 ± 1.66	n.s.
FT index	8.04 ± 2.63	6.29 ± 1.14	<0.0001

n.s., not significant.

shown). The comparison between patients with previous or persistent ocular copper deposits (Cu+) and patients who never presented any copper deposit at ocular level (Cu–) is shown in Table 2. A statistically significant difference was found in FT between the Cu+ and Cu– groups ($P < 0.0001$). Only Cu+ group had a statistical difference in NF and NBr parameters versus controls ($P = 0.002$ and $P = 0.036$, respectively).

Discussion

Corneal confocal microscopy is an in vivo, noninvasive, and reproducible diagnostic technique that allows the examination of the living human cornea in healthy and in pathological situations.[16,22,28] Considering that the human cornea is the most innervated tissue of the body, CCM also is used to study peripheral nerve damage in different systemic diseases, including diabetes, neurological disorders, storage diseases, and toxic neuropathies.[16,19,22]

Wilson disease is a rare inherited autosomal recessive disorder of copper metabolism whose hallmarks are liver damage, neuropsychiatric symptoms, and KF corneal ring. The most common ophthalmological signs are KF ring and sunflower cataract, while less common findings are night blindness, exotropia, optic neuritis, and optic disc pallor.[2,4] The presence of KF ring correlates with CNS involvement, being detectable in nearly 100% of subjects with CNS involvement, and in approximately 50% of those with hepatic and presymptomatic WD.[29] Wilson disease is due to numerous mutations of the gene encoding a copper-transporting P-type ATPase, the *ATP7B* gene, that has a crucial role in the copper excretion into the bile.[30–32] The progressive accumulation within the hepatocytes of copper induces liver dysfunction with storage capacity impairment. Therefore, the unbound copper is delivered into the blood stream and is deposited in other extrahepatic tissues, including the brain and cornea, which are eventually damaged.[33] Although many studies reported the involvement of the CNS in WD, peripheral neuropathy has been described rarely.[7–10]

Miyakawa et al.[5] were the first to focus the attention on peripheral nerves in WD. Examining the biopsy of the sural nerve in a case of WD, they demonstrated the loss and the irregular shape of myelinated nerve fibers, suggesting that the pathological changes in peripheral nerves consist of a primary demyelination, and a secondary involvement of the axon. These investigators hypothesized that these changes are due to the disturbances of copper metabolism, affecting the Schwann cells and the myelin sheat.[5] Using electrodiagnostic tests, Leven et al.[7] demonstrated, in three patients affected by WD, the decrease of motor nerve and sensory nerve conduction velocity, and the impairment of the sensory action potentials, indicating the damage of major peripheral nerves. Some years later, Madden et al.[8] studied a case of a 61-year-old man affected by WD. They described small areas of demyelination in the peripheral nerves and, considering nerve conduction findings, they suggested that axonal degeneration was the main pathologic change.[8] Jung et al.[10] reported one case whose initial manifestation of WD was polyneuropathy. This subject presented with impairment of distal nerve conduction and limb electromyography quantification. Nerve biopsy showed destroyed myelin sheath associated with axonal damage. Hyperesthesia of the hands and feet developed one year later. Moreover, progression of the earlier electrophysiological abnormalities was documented, demonstrating worsening of the earlier mild demyelinating features in all examined peripheral nerves with the addition of axonal changes. The long-term electromyographic findings suggested axonal involvement, and the pathological examination confirmed the loss of myelin sheath and axon, suggesting a mixed type peripheral neuropathy.[10] These literature reports were consistent with Schwann cells as primary target in WD. However, a recent study by von Giesen et al.[9] seems to contradict this hypothesis. These investigators, using functional quantitative sensory tests (thermal, pain, and vibratory sensation), detected a dysfunction of unmyelinated warm-specific C fibers in WD and a relative preservation of myelinated A-β fibers.[9] No pathologic examination or morphologic tests were performed by these investigators.

Accordingly to our data, the entire corneal SBNP is uniformly altered in WD. Therefore, we hypothesize that fibers in the SBNP are damaged in WD, as in other peripheral neuropathies.[16,19,22] The pathogenesis of peripheral fiber damage in WD is poorly known and reported.[7–10] The role of copper metabolism in these fibers must be reconsidered. Copper acts as a cofactor of many important enzymes in cell metabolism, and the reaction of copper ions with poorly reactive oxygen species produces highly reactive radicals. Copper toxicity is probably secondary to the formation of reactive oxygen species (ROS), whose production is tightly controlled in healthy cells. When reducing agents are present, copper catalyzes the formation of hydroxyl radicals via the Haber-Weiss reaction.[34] The hydroxyl radical is the most powerful oxidizing radical in biological systems, and it can react with almost any biological molecule.[35] The overproduction of oxidizing radicals leads to the impairment of essential molecules, such as lipids, proteins, and DNA.[36] In particular, lipid peroxidation may damage cells by changing the permeability of cell membranes, or by directly modifying DNA and other intracellular molecules, such as proteins.[37] Axonal transport may be reduced leading to decreased delivery of growth factors and intermediate products from the synapse to the cell body, resulting in cell apoptosis.[38] Even nondividing neurons are high-energy requiring cells, and their mitochondria are extremely susceptible to oxidative stress, explaining the reason why neuronal function is compromised by radical species.[39]

To the best of our knowledge, only one previous study analyzed corneal features in patients affected by WD using CCM. In this work, Ceresara et al.[40] demonstrated that corneal copper deposits in the peripheral cornea were detectable in 75% of WD cases using CCM compared to 25% using slit-lamp biomicroscopic (clinical) examination. Copper was (at least theoretically) identified as peripheral hyperreflective granular microdeposits at the level of Descemet membrane. The same investigators did not find any alteration of SBNP, epithelium,

stroma, and endothelium; however, they highlighted that copper accumulation in the peripheral cornea is more frequent than currently reported.[40] Conversely, in the present study, we focused only on the central cornea, which is the area where individual corneal layers are better seen and more precisely quantified, with special attention to the SBNP. The presence of previous or persistent biomicroscopically visible corneal copper deposits seems to be strictly related in the reduction of the number of the fibers and branchings, and in increased tortuosity. Our data clearly show that all parameters of SBNP are altered, documenting, for the first time to our knowledge, a (corneal) peripheral nerve damage in WD. The decrease of major SBNP parameters confirms the damage (and death) of a significant number of small nerve fibers, whereas the increase of FT is a sign of tentative nerve regeneration.[41] A similar involvement of all parameters of SBNP has been documented in other systemic disorders (i.e., dry eye and diabetes mellitus, which were excluded in this study),[17,42–44] with increased number of Langherans cells.[45] However, in the present study, we did not find any sign of local inflammation, such as dendritic cells and/or keratocytes activation. Whether the morphological alteration of the SBNP in WD could lead to a functional impairment also was investigated using the Cochet-Bonnet (C-BE) esthesiometer, the most frequent used method to evaluate the corneal sensitivity. We did not find any statistical differences between WD patients and controls. This result could be explained with the several drawbacks of this procedure and its inconsistent results, as previously reported in the literature. The limited range of stimulus intensities allowed by the C-BE could be considered inadequate in our population. The C-BE has a mostly supra-threshold range of stimulus intensities[46,47] and in 60.9% of previously reported subjects, it was unable to measure the true corneal sensation threshold.[48]

Regarding the corneal epithelium, we found an increase in the diameter of the superficial cell layer with (compensatory) increase in the basal layer. This phenomenon already has been described associated with SBNP alterations. In fact, corneal nerve fibers of the SBNP are essential for the trophism of corneal epithelium, and contribute to the maintenance of the integrity of the entire ocular surface.[44] In fact, it has been demonstrated that corneal nerves produce different neuropeptides and neurotransmitters which have a neurotrophic influence and promote the action of diffusible factors that stimulate epithelial growth, proliferation, and differentiation.[49]

A reason for SBNP changes may be theoretically attributed to corneal nerves toxicity secondary to WD chronic therapy. Peripheral neuropathy and optic atrophy secondary to penicillamine therapy has been reported.[6,10] All patients included in this study were receiving therapy for WD (zinc acetate, D-penicillamine or zinc acetate associated with trientine), but none of them had optic nerve atrophy (or retinal nerve fiber layer changes quantified by optical coherence tomography, data not shown), and the different treated groups showed no differences in corneal neuropathy. The limited number of patients treated with D-penicillamine and zinc acetate associated with trientine may influence these results. Another limitation of this study is the lack of electrophysiological data related to the peripheral nervous system. However, previous studies, mainly performed in diabetes and toxic neuropathies, clearly showed that small fiber peripheral neuropathies are better identified by corneal confocal microscopy and skin biopsy, than any electrophysiological test.[50,51] Skin biopsy data were unavailable in this population, even because data about small nerve fiber involvement was previously unknown.

In conclusion, this study demonstrated, using CCM, the alteration of the corneal subbasal nerve plexus with associated corneal epithelial damage in WD, suggesting the presence of small fiber peripheral neuropathy caused by copper toxicity in WD. These data also confirmed that CCM is becoming an essential parameter to study the small fibers in peripheral neuropathies and should be requested more extensively not only by ophthalmologists, but also by gastroenterologists, diabetologists, and neurologists. Moreover, when a reliable diagnostic instrument devoted to exactly quantify corneal sensitivity threshold, corneal sensitivity studies may be warranted to identify if the morphological changes of SBNP are related to changes in fibers function.

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