Rapid Thinning of Retinal Nerve Fiber Layer is Predictive of a Bad Visual Prognosis of Nd4/11778 Mutation Leber Hereditary Optic Neuropathy

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Abstract

Objective: To investigate the visual outcome predictive factors of 11778G>A/ND4 mutation Leber hereditary optic neuropathy (LHON), and to determine whether the thinning rate of retinal nerve fiber layer (RNFL) thickness is predictive of a good/bad visual prognosis of LHON patients in late chronic phase.

Methods: This was a retrospective analysis, 16 cases of LHON patients diagnosed as 11778 G>A/ND4 mutations by mt-DNA sequencing and met our inclusion criteria were performed. All patients were followed at least 4 times in our hospital, and at least one visit was performed over 30 months after onset. We divided the eyes into two groups based on the last-visit best corrected visual acuity (BCVA) of late chronic phase (over 30 months after symptom onset), Group A with last-visit BCVA ≤ log MAR 0.5 and Group B with last-visit BCVA>log MAR 0.5, then analyzed the relationship between BCVA and parameters above of the two groups.

Results: A total of 11 eyes in group A, 9 of which were from males and 2 from females, and 21 of them were in group B, of which 13 were from males and 8 were from females. The initial age，course，BCVA and RNFL thickness in the initial visit and the last visit have no significant difference between the two groups. The BCVA at the last follow-up of the two groups was 0.18 ± 0.17 and 1.09 ± 0.36, respectively, and the differences were significant between them (P=0.000). The RNFL at the last visit was 57.45 ± 6.23 and 55.52 ± 12.72 microns, respectively, and the differences were significant (P=0.024). At 4th, 8th and 12th months after onset, the number of eyes with RNFL thickness exceeding 100, 80, and 70 microns in group A was 10, 11, and 9 eyes, respectively, while the number of eyes with RNFL values exceeding 100, 80, and 70 microns in group B was only 8, 7, and 5 eyes, respectively, and the differences were significant (p=0.004, 0.000, 0.002, respectively).

Conclusions: This research suggests final visual acuity of BCVA ≤ log MAR 0.5 was not associated with gender, initial age, course, initial BCVA and RNFL thickness. Within 24 months after onset, the thickness of RNFL in eyes with better prognosis were higher than that with worse prognosis, especially at 4, 8 and 12 months, suggesting that Rapid thinning of retinal nerve fiber layer within two year after onset is predictive of a bad visual prognosis of ND4/11778 mutation Leber hereditary optic neuropathy.

Keywords: Leber hereditary optic neuropathy; Visual prognosis; Retinal nerve fiber layer; Mitochondrial DNA mutations; Optical coherence tomography

Background

Leber hereditary optic neuropathy (LHON) is a maternally inherited disease associated with mitochondrial DNA (mtDNA) point mutations, which usually affecting young men and leading to retinal ganglion cells (RGCs) degeneration and optic atrophy within a year of symptoms, resulting in visual acuity decrease, color vision impairment, huge central scotomas, and temporal pallor of the optic disc [1].

Before symptom onset, vascular abnormalities such as microangiopathy and telangiectatic vessels can be detected by fundus examination, optical coherence tomography (OCT) measurement and histopathology studies; we can also observe other preclinical changes such as optic disc hyperemia and retinal nerve fiber layer (RNFL) swelling. In the early symptomatic stage a loss of macular RGCs on OCT may be detected, however, with visual acuity and fields still being normal [2], patients do not conscious about the disease until a rapid visual loss and central scotoma occur. Then rapid RNFL loss will be found especially within 4 months [3,4], which we called sub-acute phase, and visual field loss may ongoing accompanied with RNFL thinning though the visual acuity stabilizes in 4-12 months. In the chronic phase (one year after symptoms) visual acuity and visual field of most patients remain stable, and the poor vision will accompany them for the rest of their lives. Yet there is no proven therapy for LHON despite many purported treatments being tested [5,6] until in June 2015, when the European Medicine Agency (EMA) approved idebenone (Raxone, Santhera Pharmaceuticals, Liestal, Switzerland) based on a sufficient amount of clinical evidence for safety and partial efficacy in a subgroup of treated patients. In this study 85 confirmed LHON patients were observed for a period of 24 weeks. The results showed that there was no statistical difference in the visual acuity, but

the color vision and contrast sensitivity was improved significantly [7-9]. Recently gene therapy in which the defective gene was replaced by the normal gene and expressed, and autologous bone-marrow-derived stem cells technique were tried to apply in LHON treatment and some had provided encouraging results [10-12].

Sometimes LHON patients can recover spontaneously especially in younger patient and some type of mutation (such as 14484/ND6) even though the mechanisms are not clear [13]. However, 11778G>A/ND4 is the most common mutation type which will happen in over 80% of patients while this type of patients has a very poor prognosis [13]. Visual acuity recovering was seldom happened in 11778G>A/ND4 mutation patients even in young cases, many patients will eventually have a vision of less than Log MAR 0.5.

Loss of best corrected visual acuity (BCVA) to worse Log MAR 0.5 (or 20/70) is an important criterion for judging low vision or visual disability, under this circumstance children may have learning problems and adults may have difficulty making a living. Once been diagnosed 11778G>A/ND4 mutation, patients are eager to know whether low vision will be happened on him/herself or not, while doctors also want to know which indicators have predictive effects on the patient's visual prognosis. Some literatures reviewed clinical factors that were predictive of a good visual recovery such as sex, taking idebenone, mean age at onset, mean lowest visual acuity, the only finding is that patients had less severe reduction of the visual acuity at 1 year after the onset got a better visual prognosis [14].

In this research we want to determine other clinical factors that are predictive of a good/ bad visual recovery of 11778G>A/ND4 mutation LHON patients in late chronic phase. We measured RNFL by OCT to find whether there were some relations between RNFL (especially at sub-acute and dynamic phase) and BCVA in chronic phase of 11778G>A/ND4 mutation cases.

Materials and Methods

Subjects

This was a retrospective study that the charts of a series of patients diagnosed with LHON and available for study with OCT, between May 2008 and July 2017, in the Ophthalmology Department of the Dongfang Hospital (Second Clinical College of Beijing University of Chinese Medicine, Peking, China) were considered for inclusion in the study. The following criteria were required for final patient inclusion: All patients had recent onset of visual symptoms (less than 24 weeks for the first eye involved), Patients were followed at least 4 times every half-year and the last visit should be over 30 months (late chronic phase) since onset, all patients were unrelated, and all carried 11778G>A/ND4 mitochondrial DNA mutations as determined by genetic testing in Beijing Institute of Ophthalmology.

We divided the eyes into two groups based on BCVA in each final visit (late chronic phase, at least 30 months after symptoms). The eyes with better visual acuity at late chronic phase (last visit BCVA <log MAR 0.5) were set up as group A, and the eyes with poor visual acuity at late chronic phase (last visit BCVA>log MAR 0.5) was set as group B, then we compared and analyzed RNFL thickness in each stage and other parameters of the two groups to find whether the change of those parameters were associated with visual prognosis.

Clinical evaluation

Included patients underwent clinical evaluation by an experienced neuro-ophthalmologist that included visual acuity (VA) (Log MAR), automated visual fields (VFs) (OCTOPUS 101; Switzerland) was obtained to quantify the mean defect (MD)/ mean sensitivities (MS), and High-definition optical coherence tomography (OCT) (Cirrus 4000; Carl Zeiss) was obtained to quantify the RNFL.

The time of symptom onset was assessed by the patient's history. Tomographic imaging of both eyes was performed using a high-definition spectral domain OCT (Cirrus 4000; Carl Zeiss). Each subject had both eyes scanned by the same technician using standard acquisition protocol of optic disc cube 200 × 200 for the RNFL analysis. The quality of the obtained images was assessed by evaluation of the signal strength (a value from 0 to 10 in arbitraries units) automatically provided by system. Only scans with signal strength above 6 units were included in the analysis. Subsequently, a recognition algorithm detected the inner and outer borders of the RNFL, from a 1.73 mm diameter circle centered in the optic nerve. The distance between the two lines was measured as RNFL thickness at specific locations around the optic nerve: temporal, superior, nasal, and inferior. The average RNFL thickness values expressed in microns (microns) were used for analysis. Normal RNFL thickness was defined as ranging between 87 and 100 microns. RNFL thicknesses were compared with BCVA in each stage of disease progression for analyses.

Statistical analysis

Statistical analysis was performed in SPSS version 19 software (IBM Corp., Armonk, NY)., and the following primary parameters of descriptive statistics were selected: arithmetic mean (M), median (Me), standard deviation (SD), and the minimum (Min) and maximum (Max) values. The assumed distributions of variables compatibility with normal distribution were tested by the Shapiro-Wilk. The statistical hypotheses were verified using the t-Student test, Mann-Whitney U test and Wilcoxon test. The results were quantified statistically significant in cases where the calculated probability satisfied the inequality test, $p<0.05$.
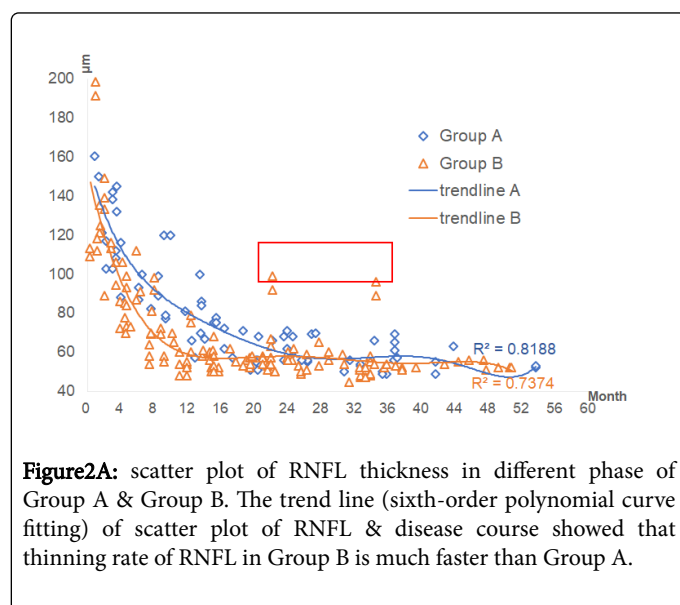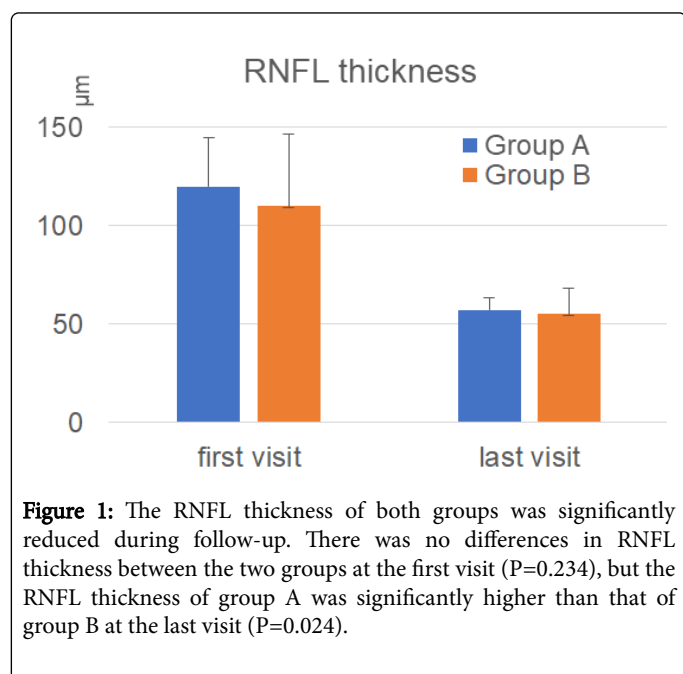
Results

Thirty-two eyes of eleven male and five female patients were met the inclusion criteria and BCVA, RNFL were evaluated. However, some patients with too poor vision acuity to cooperate with the Visual field measurement thus we got not enough credible data for further analysis. Eleven eyes with BCVA better than log MAR 0.5 in last visit were set as group A and twenty-one eyes with BCVA worse than log MAR 0.5 in last visit were set as group B. Summary data including gender, onset age, cause from symptoms and BCVA in first and last visit, for these subjects are shown in Table 1. We can see that there were no significant differences of all the data between the two groups accept only the BCVA in the late chronic phase (last visit). Though the onset age and first visit BCVA in Group A were slightly better than Group B.

Then we compared the average RNFL thickness (microns) of the two groups (Figure 1), we found that there was no significant difference in RNFL thickness between the two groups at the first visit (116 (quartiles 103, 142) *vs.* 106 (quartiles 83.5, 129) microns, Z=1.191, P=0.234, Mann-Whitney U test). While the RNFL thickness of group A was significantly higher than that of group B at the last visit (56 (quartiles 53, 63) *vs.* 52 (quartiles 50, 54.5) microns, Z=2.256, P=0.024, Mann-Whitney U test) though the exceeded value is not big.

	Gender		Onset age		Months from onset		BCVA / log MAR	
	M	F	First visit	Last visit	First visit	Last visit	Last visit	
Group A (n=11, BCVA ≤ log MAR 0.5)	9	2	11.45 ± 6.41	3.80 ± 2.50	39.65 ± 7.47	1.16 ± 0.47	0.18 ± 0.17	
Group A (n=21, BCVA >log MAR 0.5)	13	8	13.52 ± 5.13	3.46 ± 2.59	36.68±7.07	1.58 ± 0.75	1.09 ± 0.36	
Chi-Square / Z Mann-Whitney	1.332		1.236	0.478	1.313	1.259	4.596	
P value	0.248		0.216	0.633	0.189	0.208	0	

Table 1: Demographic data and follow-up details of the two groups; BCVA best correct visual acuity; Eyes were divided into group A and group B According to whether BCVA better than log MAR 0.5 (low vision) or not at the last follow-up. There were no significant differences in gender, age, first-time and last follow-up of the course of the disease and the first-time visual acuity so that they can be compared statistically.

Figure 1: The RNFL thickness of both groups was significantly reduced during follow-up. There was no differences in RNFL thickness between the two groups at the first visit (P=0.234), but the RNFL thickness of group A was significantly higher than that of group B at the last visit (P=0.024).

Figure2A: scatter plot of RNFL thickness in different phase of Group A & Group B. The trend line (sixth-order polynomial curve fitting) of scatter plot of RNFL & disease course showed that thinning rate of RNFL in Group B is much faster than Group A.

	4 m from onset		8 m from onset		12 m from onset	
	RNFL ≥ 100	RNF<100	RNFL ≥ 80	RNF<80	RNFL ≥ 70	RNF<70
Group A (n=11, BCVA <log MAR 0.5)	10	1	11	0	9	2
Group A (n=21, BCVA <log MAR 0.5)	8	13	7	14	5	16
Chi-Square	8.182		13.037		9.871	
P value	0.004		0.000		0.002	

Table 2: Distribution of RNFL thickness in different groups of patients with different course of disease; BCVA best correct visual acuity, RNFL retina nerve fiber layer; The thickness of RNFL in group A was significantly higher than that in group B when the course of disease was 4 months, 8 months, and 12 months.

At 4th, 8th and 12th months after onset, the number of eyes with RNFL values exceeding 100, 80, and 70 microns in group A was 10, 11, and 9 eyes, respectively, while the number of eyes with RNFL values exceeding 100, 80, and 70 microns in group B was 8, 7, and 5 eyes, respectively. There were statistical differences between the two groups in the 4th, 8th, and 12th months (Pearson Chi-Square values were 8.182, 13.037, 9.871, P values were 0.004, 0.000, 0.002, respectively). More details were given in Table 2.

We included all the scatter plots of all RNFL values measured during the follow-up of both groups, and fitted the curves using exponential, linear, logarithmic, power, and polynomial [2-6], respectively. The sixth-order polynomial has the highest R2 value for curve fitting, so the sixth-order polynomial is used to fit and obtain the trend line. The result is shown in Figure 2A below. It can be seen from the figure that the RNFL value of group A patients decreased to about 60 microns after 24 months, while that of group B decreased to 60 microns after about 12 months. It can also be seen from the trend line that the RNFL value of group A is higher than that of group B within 24 months from the onset of the disease, and the RNFL values of the two groups tend to be equal after 24 months. The thinning trend of RNFL in group A was slower. Note that there was an exception on the way to case14 (red box). BCVA of case14 OS and OD were log MAR 0.7 and log MAR 0.6 at the last follow-up, thus case 14 OS and OD were classified into Group B, but further study found that visual field of both the two eyes were very good at the last visit and the MD values (OCTOPUS 101, tG2 procedure) were 0.4 dB and 1.3 dB, respectively. The patient had no obvious difficulty in daily life. The reason for poor BCVA was the presence of moderate central dark spot of the visual field in both eyes. The six-in-one chart of the visual field test was showed as follows in Figure 2B.

Discussion

LHON has been categorized into 4 clinical groups: asymptomatic mutation carriers, subacute phase with disease duration less than 6 months, dynamic phase with duration between 6-12 months and chronic LHON with disease duration more than 1 year [15]. High-definition optical coherence tomography (OCT) has shed some light on the events occurring at the optic nerve head in LHON [16], Recent studies with OCT revealed that the RNFL thickening involves asynchronously the 4 quadrants through a 3-month period, followed by progressive thinning, furthermore, OCT clearly detects atrophy of the RNFL in the chronic stage [3].

In the present study, LHON patients were followed up for more than 30 months and OCT measurement was performed. We found that although RNFL decreased to less than 60 microns in almost all patients, the rate of RNFL thinning was not consistent among patients with different visual prognosis. We found the RNFL thickness of group A was significantly higher than that of group B at the last visit (56 (quartiles 53, 63) *vs.* 52 (quartiles 50, 54.5) microns, Z=2.256, P=0.024, Mann-Whitney U test),, but the exceeded value is not big, partly because of the case 14 OD and OS had over 80 microns in last visit, If case 14 who had an almost normal visual field was excluded from the B group, then the recalculated average of the remaining 19 eyes RNFL thickness should be only 51.63 ± 3.25 microns. Some researchers [17] have pointed out that there is no significant correlation between the visual acuity of LHON and the thickness of RNFL. The results of this study were different. The main reason may be that eyes in the above literature were not compared in different stages of the disease, because in the early phase RNFL changed rapidly and BCVA were not stable

too. Therefore, we found that patients with better BCVA did not have thicker RNFL in initial visit, while eyes with thicker RNFL have better vision in late chronic phase when RNFL and visual acuity were relatively stable.
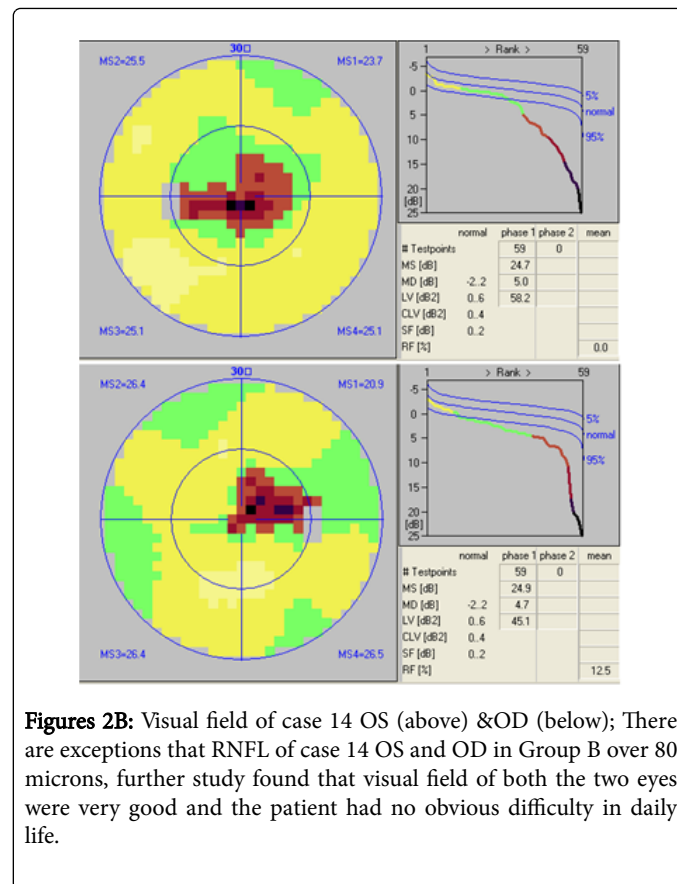
Figures 2B: Visual field of case 14 OS (above) &OD (below); There are exceptions that RNFL of case 14 OS and OD in Group B over 80 microns, further study found that visual field of both the two eyes were very good and the patient had no obvious difficulty in daily life.

The RNFL thickness between the two groups was significant differences in the 4th, 8th, and 12th months since symptom onset. Thinning of RNFL may suggest RGC apoptosis and axonal disintegration since the health of RGC cells and their axons is the basis for the RNFL to maintain its normal structure. It is suggested that the poor visual acuity of Group B may result from more RGC damage or more severe mitochondrial dysfunction in the acute phase.

LHON involves mitochondrial dysfunction. After the metabolic defect, compensation will typical occur just like many other mitochondrial disorders such as mitochondrial myopathy, lactic acidosis, and stroke like episodes [18], the compensatory mechanisms including increasing mitochondrial biogenesis may lead to RNFL thickening which may also beneficial to RGCs and their axons survive and saving more visual function. The balance of the see-sawing between the metabolic injuries produced by complex I dysfunction and the compensatory response by the RGCs and their axons may be an explanation for our findings, that eyes with slower RNFL thinning may have higher compensation activities.

In addition, there is controversy over whether treatment of idebenone is still needed after 1 year of onset of LHON. Most experts believe that there is insufficient evidence to continue treatment with idebenone after 1 year of onset [15]. This study believes that in some cases (Group A) RNFL were still undergoing thinning changes during the period 12-24 months after onset, suggesting that mitochondria

dysfunction and compensation are still active and idebenone treatment should be continued to improve the patient's visual prognosis after 1 year of onset, though further high-level clinical evidence should be performance to confirm it.

It should be noted that if we can predict final BCVA of LHON patients earlier, physicians and patients will anticipate the risk of low vision beforehand so that early treatment and training of low vision can be performed, which may improve the patient's ability to adapt under low vision conditions.

Our study has some limitations and further investigation is warranted. First, only 16 patients and 32 eyes were included for analyze, the sample size was small. Second, Because of the limitations of previous detection conditions, this study did not obtain follow-up data on the thickness of patients with ganglion cell complex (GCC). However, some literature reported that GCC may change earlier than RNFL, and GCC originated from the optic disc macular bundle of nerve fibers, which is is more closely related to central vision [18]. Thus, GCC may be more suitable for BCVA prediction indicators theoretically. In addition, this study did not explore the relationship between RNFL and visual field. In fact, visual field is also an important indicator of low vision, and has a significant impact on patients' quality of life. The main reason is that some patients have too poor vision acuity to cooperate with the measurement, while on the other hand, some patients were tested using different procedures (LVC, N2, or tG2) during the follow-up process, which causing more difficulties to statistics. Future studies will address these issues.

Conclusion

In this study, we divided the 11778 G>A/ND4 mutation LHON eyes into two groups based on whether the BCVA reached the low vision standard (log MAR 0.5) in the late chronic phase (over 30 months after onset). This research suggests final visual acuity of BCVA ≤ log MAR 0.5 was not associated with gender, initial age, courseinitial BCVA and RNFL thickness. However, the thickness of RNFL in Group A was significantly higher in the last visit, indicating that the BCVA were positively correlated with RNFL thickness in the late chronic phase. The thickness of RNFL in eyes with better final (late chronic phase) BCVA were higher than that of lower BCVA within 24 months after onset, especially at 4, 8 and 12 months, suggesting that the thinning rate of RNFL may be a predictor of low vision outcome in ND4/11778 mutation LHON.

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