Clinical Profile of Patients with Leber Hereditary Optic Neuropathy (LHON): An Ambispective Study of North Indian Cohorts

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Abstract

Background: Leber hereditary optic neuropathy (LHON) is a maternally inherited disease resulting in irreversible visual loss usually in patients belonging to the age group of 15–35 years. Clinically, the patients present with sequential or bilateral, painless, progressive visual loss with central (or ceco-central) scotomas. Although the three mutations, namely, G11778A, T14484C, and G3460A contribute to >95% of LHON cases globally, the relative frequency of each mutation varies. **Aims and Objectives:** We aimed to assess the clinical and genetic profile of patients with mutation-positive LHON at a north Indian tertiary care center. **Materials and Methodology:** One hundred sixty-one patients (61 prospective and 100 retrospective) presenting with the clinical diagnosis of LHON were screened for the three known mitochondrial mutations (G1178A, G3460A, T14448C). Patients were assessed for detailed clinical, ophthalmological, and neurological examinations. Five milliliter of blood sample was taken to assess the three known mutations using DNA isolation and Sanger sequencing. **Results and Discussion:** Clinical profile of 83 patients with both positive and negative mutations was analyzed. Twenty-three out of 161 patients (14.3%) tested positive for either of the three mutations. The majority of the patients harbored G11778A mutation (56.52%) followed by T14484C (34.78%) and G3460A (8.69%). No statistical difference could be noted between the clinical profiles of mutation-negative and -positive patients.

Keywords: Hereditary, painless, progressive, sequential, visual loss

Introduction

Visual loss is one of the highly feared complications of neurological disorders. According to a population-based survey, optic atrophy is one of the five major causes of complete vision loss, constituting a prevalence of 0.8%–5% globally and 11% in India.[1] Evaluation of painless progressive visual loss with ensuing optic atrophy poses a huge challenge. Leber hereditary optic neuropathy (LHON) is a maternally inherited, mitochondrial disorder characterized by bilateral, acute, or sub-acute, painless loss of central vision typically presenting between 15 and 35 years of age.[2,3] Males are predominantly affected but the disorder may also be seen in females, though rarely (males-to-female ratio = 3:1).[3,4] A definitive diagnosis requires meticulous exclusion of acquired inflammatory, infective, compressive, toxic, and nutritional causes supplanted by a genetic study. While the exact prevalence of LHON is not known, it was estimated to be 13.57 per 10,000 patients or 1:737 in a south Indian cohort[5,6] (95% CI 10.23–17.66 per 10,000) and 1 in 50,000 in a study group from northeast England.[4] Although the three mutations G11778A, T14484C, and G3460A contribute to > 95% of LHON cases globally,[4] the relative frequency of each mutation varies.[7,8] The mutations of complex I of the mitochondrial oxidative phosphorylation pathway elevates the oxidative stress and decreases the production of adenosine triphosphate (ATP) production which eventually results in retinal ganglion cell (RGC) apoptosis.[3,9] This disrupts the visual signaling pathways of RGCs, resulting in extreme vision damage that causes blindness. Limited literature is available on the natural profile of LHON. We present the clinical profile of LHON patients from a north Indian tertiary care center.

Materials and Methods

This was a hospital-based ambispective cohort study carried out in a tertiary care hospital in north India. A total of 161 patients presenting with the clinical diagnosis of LHON were screened for the three known mitochondrial mutations (G1178A, G3460A, and T14448C). The data of patients reported to have

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positive and negative mitochondrial mutations was collected and assessed in detail [Table 1]. All the patients (with positive and negative mutations) were underwent workup for secondary causes of optic neuropathy like infectious, inflammatory, or ischemic optic neuropathies. Other differentials like vision loss secondary to raised intracranial pressure, metabolic or toxic or nutritional causes were adequately ruled out before making the diagnosis of LHON.

Detailed history and findings on clinical, ophthalmological, and neurological examinations were assessed. Special attention was given to best-corrected visual acuity (BCVA) using Snellen charts, color vision testing using Ishihara plates, direct or indirect ophthalmoscopy, automated perimetry (using Humphery field analyzer), and fundus fluorescein angiography (FFA). The diagnosis of LHON was based on the typical history of painless progressive sequential or bilateral loss of vision, central (or ceco-central) visual field loss, primary optic atrophy (optic disc edema may be present in acute stages), peripapillary telangiectatic vessels with or without tortuosity of retinal arterioles and absence of leak at optic disc on FFA and/or presence of one of the three known LHON mutations.

Table 1: Clinical characteristics of Leber hereditary optic neuropathy (LHON)

Clinical characteristics (*n*=73)	Patients with Positive Mutations (%)	Patients with Negative Mutations (%)
Median (IQR) age in years	20 (17, 32)	32 (24,45)
Mean (SD) age in years	23.82 (10.12)	34.8 (12.87)
Age group (years) at presentation		
<15	01 (4.35)	01 (2)
15-20	12 (52.17)	05 (10)
21-25	04 (17.39)	08 (16)
26-30	00 (00)	05 (10)
31-35	02 (8.70)	10 (20)
36-40	01 (4.35)	05 (10)
41-45	02 (8.7)	04 (8)
>45	01 (4.35)	12 (24)
Sex		
Male	17 (73.91)	40 (80)
Female	06 (26.09)	10 (20)
Marital status		
Unmarried	18 (78.26)	15 (30)
Married	05 (21.74)	35 (70)
Age group (years) at onset		
10-15	07 (30.43)	09 (18)
16-20	09 (39.13)	05 (10)
21-25	03 (13.04)	09 (18)
26-30	02 (8.70)	06 (12)
31-35	01 (4.35)	07 (14)
36-40	00 (00)	03 (6)
41-45	00 (00)	02 (4)
>45	01 (4.35)	09 (18)
Nature of onset		
Sub-acute	17 (73.91)	42 (84)
Acute	06 (26.09)	08 (16)
Sequential		
Sequential	14 (60.87)	21 (42)
Simultaneous	9 (39.13)	29 (58)
Family history		
Present	09 (39.13)	08 (16)
Visual acuity		
Normal	01 (2.17)	02 (2)
Mild (6/6-6/18)	01 (2.17)	10 (10)
Moderate (6/24-6/60)	14 (30.44)	41 (41)
Severe (worse than 6/60)	30 (65.22)	47 (47)
Type of mutation		
G11778A/ND4	13 (56.52)	00
T14484C/ND6	08 (34.78)	00
G3460A/ND1	02 (8.7)	00

Compressive, infiltrative, toxic, nutritional, and inflammatory causes of visual loss that may phenotypically mimic LHON were meticulously excluded. Visual loss was graded as mild (6/6 to 6/18), moderate (6/24 to 6/60), and severe (worse than 6/60) for ease of interpretation of data. The collected 5 ml of blood sample from patients was stored in an EDTA vacutainer at −20°C degrees. The blood sample was collected during the first visit of the patient. Blood samples were mixed with 30 ml of erythrocyte lysis buffer (ELB) and incubated for 30 minutes followed by centrifugation at 3000 rpm for 10 minutes. After discarding the supernatant, the process was repeated. After discarding the resulting supernatant, the DNA pellet was mixed with 5 ml ELB + 530 ml 20% SDS + 60 ml proteinase K 28 ml (20mg/ml). This was followed by the addition of 0.1 vol. 5 M NaCl (1060 µl) + equal vol. of isopropanol. DNA thus obtained was transferred in Eppendorf with 70% alcohol followed by incubation, centrifugation at 13,000 rpm, repeat washes, and incubations. The DNA thus extracted was subjected to detection of mutations using namely 3460 G to A, 11778 G to A, and 14484 T to C in the ND4, ND1, and ND6 subunit genes, respectively.

Results

Out of 161 patients in the study, 23 (14.3%) tested positive for LHON mutations. Among the remaining patients with negative LHON mutations, complete clinical data of only 50 patients could be retrieved. The comparison of the clinical profile of 73 patients with both positive and negative mutations is presented in Table 1.

Mutation-positive patients

The majority of patients harbored G11778A mutation (56.52%) of ND4 subunit followed by T14484C (34.78%) of ND6 subunit of complex I. G3460A mutation of ND1 was seen in only 2 patients (8.69%) in our study cohort. The mean age at presentation was 23.82 ± 10.12 years and the mean age at onset of symptoms was 20.6 ± 7.69 years [Table 1]. The male-to-female ratio was 3:1 with 17 males and 6 females. No difference in clinical features such as age at onset, duration since diagnosis, nature of onset (acute/sub-acute), visual acuity, and pain at presentation was noted between either group. In this cohort, 17 patients (73.91%) had sub-acute onset of symptoms. All patients had bilateral progressive and painless visual loss. A total of 46 eyes of 23 patients were studied. Of these, 30 eyes (65.22%) had severe visual loss, 14 eyes (30.44%) had moderate and 1 eye (2.17%) had mild visual loss. Only one eye (2.17%) had normal vision. The sequential loss of vision was seen in 14 patients (60.87%) and 9 patients (39.13%) presented with simultaneous visual loss in both eyes [Table 1]. Most of the patients presented with central, ceco-central or whole-field visual field loss. Only 9 patients (39.13%) had a positive family history [Table 1].

Mutation negative patients

The patients in this cohort were older than the patients with positive mutations, with mean age of presentation being 34.8 ± 12.87 years and mean age of onset being 29 ± 13.27 years. The male-to-female ratio was 4:1 which was lower than that of the cohort with positive mutations. Onset of symptoms was sub-acute in 42 patients (84%). Similar to the cohort with positive mutations, visual loss was bilateral in all the patients. A total of 100 eyes of 50 mutation-negative LHON patients were studied. The severity of visual loss in this group was also similar to that of the mutation-positive group. Forty-seven eyes (47%) had severe visual loss and 41 (41%) had moderate visual loss [Table 1]. It was noted that the majority of the eyes (29, 58%) were simultaneously involved in the mutation-negative group [Table 1]. The visual field deficits were similar in mutation-positive patients.

Mean serum lactate levels in patients was 0.89 ± 0.39 (mmol/l) (normal range: 0.25–1.1 mmol/l).

Discussion

Patients in mutation-positive cohort were younger in age at presentation when compared to mutation-negative patients (23.82 ± 10.12 vs. 34.8 ± 12.87).[10,11] While the data on the three commonly known mutations is abundant, limited literature is available on other uncommonly found mutations. Delayed age at presentation can be a feature of patients with mutations other than the ones carried out in our study. In a study by Poincenot *et al*.,[4] the median age at presentation in the cohort from northeast England was 20 years for males and 30 years for females, and the male-to-female ratio was 3:1. Similarly, the age at presentation in a Danish and an Australian study group was 25 years (median) and 26 years (mean), respectively.[5,11,12]

The male preponderance in cohorts with positive and negative mutations (73.91% and 80%, respectively) and with a male-to-female ratio of 3:1 and 4:1, respectively, depicts the well-known gender bias for the development of LHON. Mitochondrial inheritance and the role of an X-linked modifier gene have been postulated for the existent gender bias in LHON.[13–16] It is interesting to note that 6 (26.09%) and 10 (20%) patients were female in the cohorts with positive and negative mutations, respectively. This indicates that both genetic and environmental factors are crucial for the phenotypic expression of the disease. The inheritance of the nuclear modifier locus which regulates the expression of mitochondrial genes may play a role in the development of the disease in female patients.[13,15]

Khanh Vu *et al*.[17] noted that a small subgroup of patients can present with acute visual loss and LHON should be considered as a differential diagnosis in patients presenting with acute-to-subacute optic neuropathy who are refractory to conventional treatment like steroids. The majority of the patients (73.91% and 84%, respectively) in our study presented with sub-acute vision loss in both the cohorts with positive and negative mutations.

While a sequential visual loss is a rule in patients presenting with LHON, patients may present with simultaneous loss of

vision from both eyes.[18] Only 9 patients (39.13%) presented with simultaneous involvement of the eyes in our cohort with positive mutations. However, in the cohort with negative mutations, 29 patients (58%) presented with simultaneous involvement of the eyes.

Of all LHON patients in our study 14.3% patients tested positive for the three commonly known LHON mutations. Of these, 56.52% were homoplasmic for G11778A/ND4 mutation, 34.78% for T14484C/ND6 mutation, and 8.7% for G3460A/ND1 mutation. This data is comparable to the available literature.[4] Poincenot *et al*.[4] reported that G11778A has a higher frequency as compared to the other two mutations. Amongst the patients who had a positive family history, G1778A and T14484C mutations were found to be common. Mackey *et al*.[12] suggested that up to 60%–70% of patients of northern European descent harbor G11778A/ND4 mutation. In a study by Puomila *et al*.,[19] 67% patients were found to harbor G11778A/ND4 mutation in the Finland population followed by T14484C/ND1 (11%) and G3460A (3%).

According to Mashima *et al*.[20] and Jia *et al*.,[21] T14484C/ND6 is present in about 90% of patients with Asian descent. T14484C/ND6 is predominant in patients of French-Canadian descent also.[8] In a study by Sundaresan *et al*.[22] in the south Indian population, G11778A mutation was found in 9 patients (10%) followed by T14484C mutation in three patients (25.6%). Similarly, Mishra *et al*. noted that the most commonly found mutation was G11778aA (followed by T14484C) in their north Indian study cohort.[23,24] In our data, the most common mutation was G11778A followed by T11778A. G3460A was the least common and was found in only two patients.

The low frequency (14.3%) of positive LHON mutations in the Indian population questions the utility of this test to rule out LHON in patients who present with a typical history and clinical features. The reason for the low frequency of primary mutations in our study could be multi-factorial. The mitochondrial abnormalities may be more concentrated in the optic nerves with the presence of low levels in peripheral blood due to tissue mosaicism.[25–27] These low levels of mutational load could escape detection with polymerase chain reaction (PCR) amplification on peripheral blood. Alternatively, one of the rare mitochondrial mutations described in the literature may be responsible for the disease in the Indian population.[8,28] Detecting these mutations would entail whole mitochondrial genome sequencing of LHON patients comprising a larger cohort. Even when such rare or non-synonymous mutations are detected, determining causation versus association is highly challenging.[29,30] Moreover, mechanisms other than mitochondrial etiology may be responsible for the visual loss in patients who tested negative for the primary LHON mutations. A different set of mutations may be responsible for LHON in patients from the Indian subcontinent. Existing literature also recognizes other less common mutations associated with LHON. Testing of these mutations is usually done in research settings.

The present study had limitations in the form of being a small ambispective cohort. Testing for only three commonly known mitochondrial mutations may have missed less frequent mutations responsible for LHON. Whole mitochondrial genome sequencing may aid in recognizing the lesser-known mitochondrial mutations in these patients. Keeping in mind the limited positivity rate of various mutations in LHON, clinical criteria need to be evolved.

Summary and Conclusions

The present study conducted in a tertiary care center in north India was a hospital-based ambispective cohort study to characterize the clinical profile of LHON patients and to detect the frequency of well-known LHON mutations in the study population. The clinical characteristics of our cohort of LHON patients were similar to the published literature from India and across the globally. Our cohort conspicuously stood out due to its low frequency of the well-known LHON mutations that are frequently detected in studies published from Caucasian populations. The low frequency of these well-known mutations in the Indian population questions its utility to rule out LHON in Indian patients with typical clinical presentation.

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Conflicts of interest

There are no conflicts of interest.

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