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A hospital-based five-year prospective study on the prevalence of Leber's hereditary optic neuropathy with genetic confirmation

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Purpose: To estimate the prevalence of Leber hereditary optic neuropathy (LHON) along with genetic screening at a tertiary eye care center in southern India.
Methods: Patients with LHON were identified at the Neuro-Ophthalmology Clinic, Aravind Eye Hospital (AEH; Madurai, India) from 2015 to 2019. Clinical data were collected along with blood samples. Genetic testing was performed for the confirmation of LHON using a multiplex PCR restriction fragment length polymorphism (RFLP) approach to detect the primary mutations 3460A, 11778A, and 14484C in mitochondrial DNA (mtDNA).
Results: During the study period, 1,598,441 outpatients attended AEH of whom 40,527 were referred to the Neuro-Ophthalmology Clinic. Among them, 55 patients were diagnosed with LHON. The male to female ratio was 8.2:1.0, and the mean age at onset was 20.95 years (SD 8.940). The estimated prevalence was 1:737 or 13.57 per 10,000 (95% confidence intervals [CI] 10.23–17.66) at the Neuro-Ophthalmology Clinic. The frequency of primary mutations in the patients with LHON was determined as 43.6% (24/55), giving a prevalence of 1:1689 or 5.92 per 10,000 (95% CI 3.78–8.81).
Conclusions: The high prevalence of LHON observed at a single hospital highlights the impact of the disease in southern India. As the epidemiology of LHON remains unexplored in this region, these findings will pave the way to evaluate the national prevalence. Further, screening the whole mitochondrial genome may help to increase the detection of mutations to estimate the accurate prevalence of the disease.

Leber hereditary optic neuropathy (LHON; OMIM 535000) is a rapidly progressing mitochondrial genetic disorder that leads to visual failure, predominantly in young adults. The clinical characterization includes bilateral, painless loss of central vision by virtue of retinal ganglion cell (RGC) death followed by atrophy of the optic nerve [1,2]. Visual failure is either simultaneous or sequential. Both eyes are affected at the same time in the simultaneous vision loss, whereas the second eye is involved with a median interval of 6–8 weeks in the case of sequential vision loss [3]. The peak age at onset of visual deterioration is the second and third decades of life. However, rare cases of late onset LHON have also been reported in older individuals (>50 years) [4]. It is caused by one of the three primary point mutations (m.G3460A, m.G11778A, and m.T14484C) in the mitochondrial genes encoding the NADH dehydrogenase (ND) subunits 1, 4, and 6 (MT-ND1, MT-ND4, and MT-ND6), respectively. These subunit proteins are involved in the formation of mitochondrial oxidative phosphorylation (OXPHOS) complex I otherwise known as the NADH: ubiquinone oxidoreductase [5]. It is a large enzyme complex comprising 45 subunit proteins including 7 core subunits encoded by the mitochondrial DNA (mtDNA). The electron transport chain (ETC) begins from complex I as it transfers electrons from NADH to ubiquinone and so on to the other complexes present in the mitochondrial inner membrane to generate energy in the form of ATP (ATP). Therefore, mutations in these genes affect the structure and assembly of the subunits, engendering a complex I deficiency [6]. The alterations introduced by the primary LHON mutations contribute to decreased ATP production and accreted reactive oxygen species (ROS). This oxidative stress plays a crucial role in activating apoptosis in RGCs, contributing to the pathophysiology of the disease [7]. Approximately 98% of the patients with LHON in Denmark [8], 73% in South Korea [9], 67% in Finland [10], and 35% in China [11] were identified to harbor one of the three primary mutations. Despite harboring a causative mutation, only 50% of men and 10% of women develop optic neuropathy. This incomplete penetrance and male preponderance make the etiology of the condition more complex, indicating the influence of other genetic and environmental factors, such as smoking and heavy alcohol

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consumption [12]. The prevalence of the disease was estimated at 1 in 31,000 in northeast England, 1 in 39,000 in the Netherlands, 1 in 48,000 in Finland, 1 in 54,000 in Denmark, 1 in 113,300 in Australia, and 1 in 526,000 in Serbia [8,10,13–16]. Although these reports provide the prevalence of the disease due to the mutations previously described among different populations, it remains unexplored in India. In addition, the frequency of primary mutations, age at onset, and gender bias have been poorly investigated in Indian patients with LHON. Thus, we conducted the present hospital-based study to explore the prevalence of LHON at a tertiary eye care center in southern India. We also evaluated the age at onset, gender bias, and relative frequency of primary mutations in the study participants.

METHODS

Study population and prevalence estimation: The present study was performed at the Aravind Eye Hospital (AEH), a tertiary eye care center in Madurai, Tamil Nadu, India. The study adhered to the guidelines of the Declaration of Helsinki as well as the ARVO statement on human subjects. The protocol was approved by the Institutional Ethics Committee of AEH. With verbal informed consent, the data on the new outpatients including the Neuro-Ophthalmology clinic were retrieved from the medical records department for the five-year period from January 2015 to December 2019. Individuals diagnosed with LHON were identified at the Neuro-Ophthalmology clinic.

Prevalence was estimated based on the ratio or proportion of the number of patients with newly developed, clinically ascertained LHON to the number of new patients in the Neuro-Ophthalmology Clinic. In addition, the prevalence was calculated for the total number of new outpatients in the hospital.

Inclusion criteria: Individuals presenting with sudden onset, bilateral (either simultaneous or sequential), and painless reduction of visual acuity were identified in regular clinical practice by the Neuro-Ophthalmologists as well as the patient's history was also considered during the diagnosis. Individuals suspected to have LHON underwent dilated fundus evaluation and optical coherence tomography (OCT) examination. The fundus evaluation included one of the characteristic features: circumpapillary telangiectatic microangiopathy, swelling of the retinal nerve fiber layer (RNFL), absence of leakage on fluorescein angiography (to distinguish LHON from true disc edema), and atrophy of the optic nerve at the late stage. Patients were excluded from the study if they had been exposed to a known optic nerve toxin and presented with biochemical evidence of multiple sclerosis or another systemic inflammatory disease.

Clinical and demographic information: The medical record of each LHON patient was screened manually to collect the clinical data such as Best-corrected visual acuity (BCVA), central field, color vision, and optic disc evaluation. With written informed consent of the patients or legal guardians, the demographic information was collected including the age at disease onset, gender, consanguinity in parents and family history of visual failure.

Molecular genetic analysis: With written informed consent, 5 ml of peripheral blood sample and pedigree details were collected from each patient. Genomic DNA was extracted using the modified salting-out method [17]. Genetic analysis was performed to detect the primary mutations by using an end-point, multiplex PCR with restriction fragment length polymorphism (RFLP) approach according to a previous study [18]. The PCR primers and reaction conditions were given in Table 1. In brief, PCR primers were designed to introduce a *Mae*III (Roche, Mannheim, Germany, Catalogue Number 10,822,230,001) restriction site (↓GTnAC) in the presence of 3460A and 14484C mutations as the site occurs naturally in the 11778A mutation. The PCR products were digested with the *Mae*III enzyme at 55 °C for 3 h followed by visualization on a 3% agarose gel. The results were further validated with Sanger sequencing for the target regions as described previously [19].

Statistical analysis: The 95% confidence interval (CI) was determined appropriately using Stata software version 14 (College Station, TX). Normality of the data was checked using the Shapiro–Wilk test. The p value was based on two independent sample *t* tests, and a p value less than or equal to 0.05 was considered statistically significant.

RESULTS

Prevalence of LHON: A total of 1,598,441 outpatients attended AEH during the 5-year period from January 2015 to December 2019. Among the outpatients, 40,527 cases were referred to the Neuro-Ophthalmology Clinic, where 55 LHON probands were identified from genealogically unrelated families (Table 2). According to the patient statistics above, the prevalence of LHON was estimated at 1:737, or 13.57 per 10,000 patients (95% CI 10.23–17.66 per 10,000) in the Neuro-Ophthalmology Clinic. In addition, the prevalence was calculated at 1:29063 or 0.344 per 10,000 (95% CI 0.26–0.45 per 10,000) with respect to the outpatients (1,598,441).

Clinical and demographic information: With reference to the BCVA, patients showed mild to profound visual impairment.

Table 1. Primer sequences used for the end-point multiplex polymerase chain reaction*

S.No	Name	Primer Sequence (5′-3′)	Product size (bp)
1	MaeIII 3460 F	CCCCTACGGGCTACTACAACCCTTCGCTGTC	333
	MaeIII 3460 R	GATAGTAGAATGATGGCTAG	
2	MaeIII 11,778 F	AGCAAACTCAAACTACGAACG	164
	MaeIII 11,778 R	TTACTAGCACAGAGAGTTCTC	
3	MaeIII 14,484 F	AATAGCCATCGCTGTAGTATATCCAAAGACAGTCA	236
	MaeIII 14,484 R	GTGCGAGAATAATGATGTATGC	

*PCR conditions include 95 °C for 5 min followed by 35 cycles at 95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Table 2. Number of new patients attended the Aravind Eye Hospital (AEH), Madurai

Year	No. of outpatients attended AEH	No. of patients referred to the Neuro-Ophthalmology clinic	No. of clinically confirmed LHON cases
2015	325,730	9112	11
2016	349,356	9580	10
2017	326,297	8141	9
2018	309,344	6902	14
2019	287,714	6792	11
Total (2015–2019)	**1598441**	**40527**	**55**

In some patients, the impairment was near blindness (Table 3). Fundus evaluation showed optic disc pallor and hyperemic disc changes (Figure 1A, Table 3). Features such as pseudo-edema, RNFL gliosis, and telangiectatic vessels along with disc hyperemia were also observed in some patients (Table 3). Loss of the ganglion cell layer was detected through OCT examination (Figure 1B).

Central field and color vision could not be assessed in some patients due to poor visual acuity. The available data showed that 78% and 50% of the patients developed defective color vision (28/36 patients) and defective central field (15/30 patients), respectively. Fifteen patients had consanguineous parents, and a family history of vision loss was found in nine probands. Around 52.7% of the patients (29 cases) were natives of Tamil Nadu. The remaining patients came from neighboring states: Kerala (23.6%), Andhra Pradesh (18.2%), and Karnataka (1.8%). In addition, 3.6% of the patients were from the northeast region of India.

Gender bias and age at onset: Of the 55 patients with LHON, 49 were men, and six were women, giving a sex ratio of 8.2:1.0. A statistically significant difference (p=0.03) was noted in the mean age at onset between men and women. The age at onset was 11–20 years in the women with a mean of 13.67 years (SD 2.340), whereas the age at onset ranged from

Table 3. Ophthalmic findings in LHON patients.

Ophthalmic findings		No. of patients
Visual impairment with reference to BCVA	Mild (6/10 – 6/18)	7
	Moderate (6/24 – 6/48)	12
	Severe (6/60 – 3/60)	22
	Profound (2/60)	7
	Near blindness (1/60 or less)	7
Optic disc pallor		43
Hyperemic disc		5
Pseudoedema		4
Telangiectatic vessels		1
RNFL gliosis		2

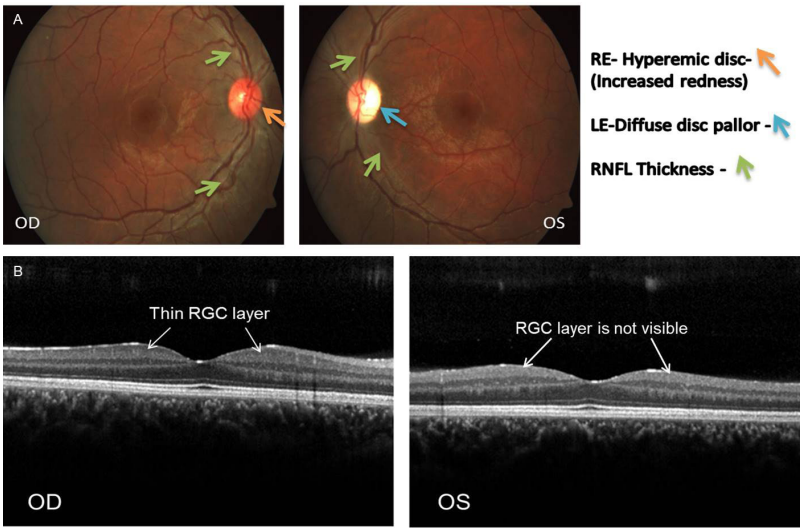

Figure 1. Fundus and OCT examination of patient with LHON. **A**: Evaluation of the fundus shows swelling of the retinal nerve fiber layer (RNFL) in both eyes, hyperemic disc in the right eye (OD), and diffuse disc pallor in the left eye (OS). **B**: Optical coherence tomography (OCT) examination displays loss of the ganglion cell layer.

5 to 56 years in men with the mean at 21.84 years (SD 9.050). Among the 49 men, 21 (42.9%), 18 (36.7%), six (12.2%), three (6.1%), and one patient (2%) developed the optic neuropathy during their second, third, fourth, fifth, and sixth decade of life, respectively (Figure 2). Overall, the mean age at disease onset was 20.95 years (SD 8.940) for all 55 patients.

Prevalence of primary mutations: The primers used in this study amplified three gene products, MT-ND1 (333 bp), MT-ND4 (164 bp), and MT-ND6 (236 bp), in a single polymerase chain reaction (Figure 3-lane: undigested). Digestion with *Mae*III resulted in the detection of internal control in the MT- ND1 product as well as the m.G11778A, m.T14484C mutations in the *MT-ND4* (Gene ID: 4538, OMIM: 516003) and *MT-ND6* (Gene ID: 4541, OMIM: 516006) products respectively (Figure 3). Of the 55 patients, 23 (41.82%) were detected to harbor the 11778A mutation, and one (1.82%) was identified as positive for the 14484C mutation. Another primary mutation, 3460A was absent in the study patients (Figure 3). Together, the primary mutations accounted for 43.64% of patients with LHON. The findings were further validated with Sanger sequencing of the *MT-ND4* and *MT-ND6* genes (Figure 4). Thus, the prevalence of LHON

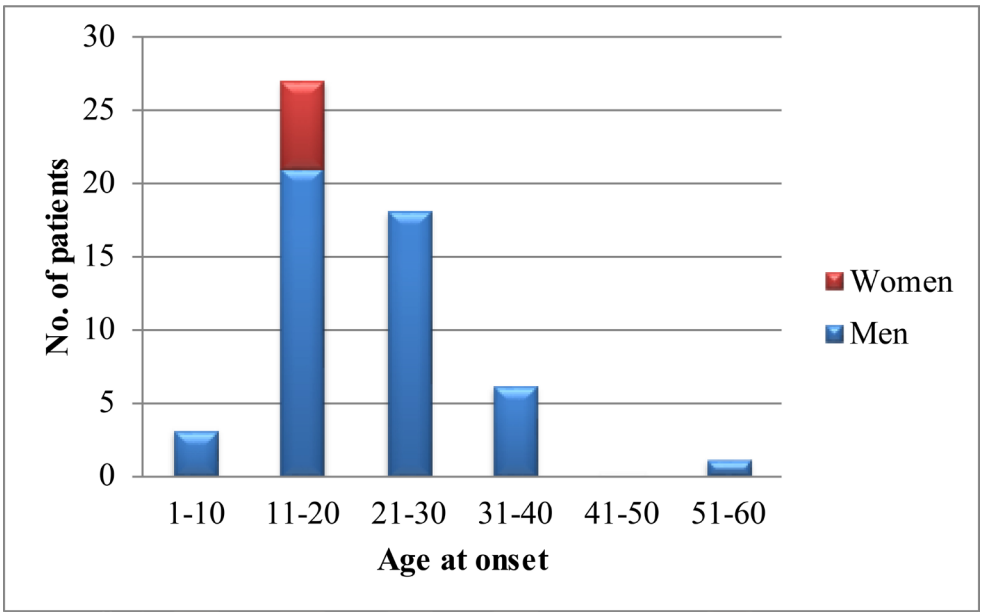

Figure 2. Age at onset of 55 patients with newly developed LHON during 2015–2019. Disease onset was found to be relatively lower in the case of women than in men. A rare case of late onset Leber hereditary optic neuropathy (LHON) was observed in a 56-year-old man.

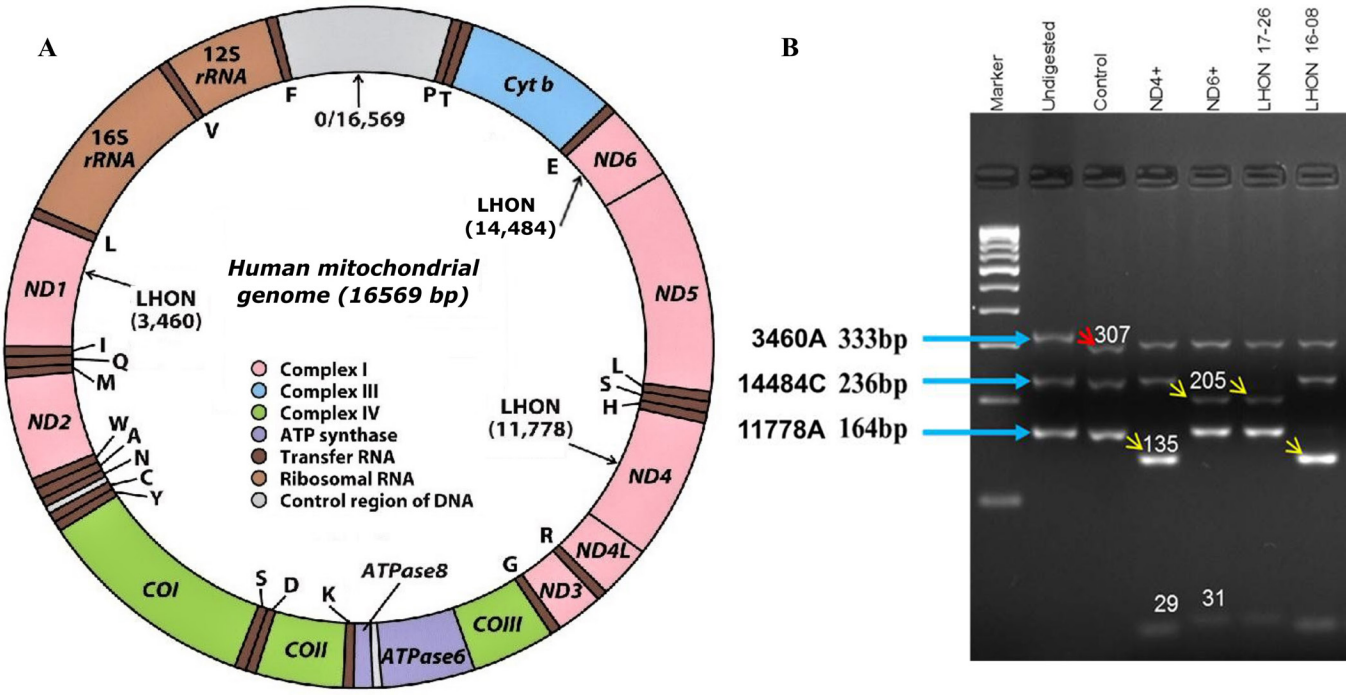

Figure 3. Human mitochondrial genome and detection of primary mutations in LHON samples. **A**: Schematic representation of the human mtDNA map indicating 37 genes including MT-ND1, MT-ND4 and MT-ND6 as well as the positions of primary mutations. Figure adapted from chimerasthebooks. **B**: The red arrow demonstrates the internal control of digestion in the *MT-ND1 gene* product. The yellow arrows indicate the *Mae*III restriction, detecting the corresponding mutations marked in blue arrows.

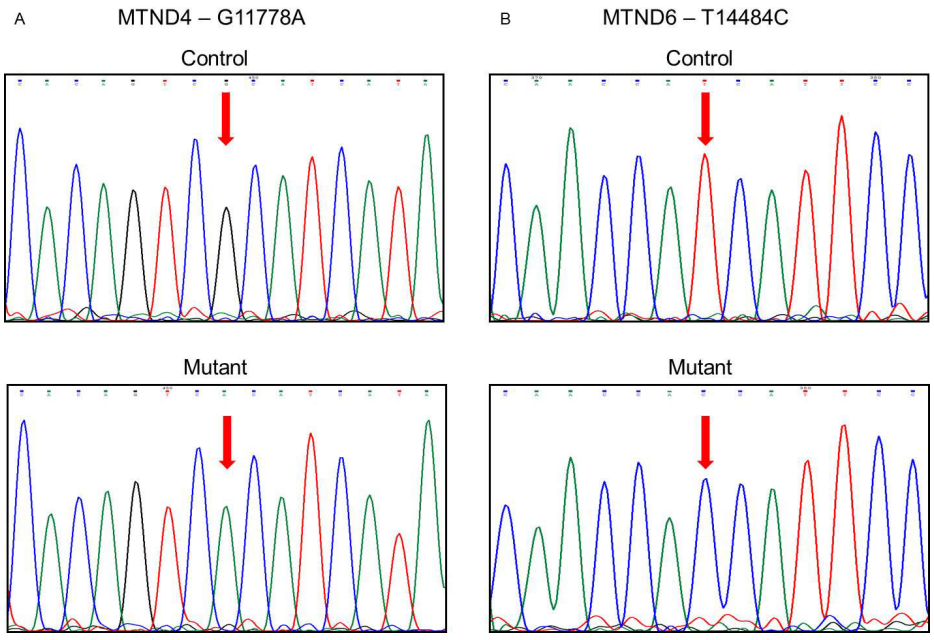

Figure 4. Sanger sequencing validation of restriction fragment length polymorphism. Chromatograms showing the presence of G11778A (left-A) and T14484C (right-B) mtDNA mutations in the same samples confirm the restriction fragment length polymorphism (RFLP) findings.

cases presented with primary mutations was estimated at 1:1689 or 5.92 per 10,000 patients (95% CI 3.78–8.81 per 10,000) in the Neuro-Ophthalmology Clinic. Concerning the total number of new outpatients (1,598,441), the mutation prevalence was 1:66602 or 0.15 per 10,000 outpatients (95% CI 0.096–0.22 per 10,000).

DISCUSSION

Being a rare disease, the prevalence of LHON is not explored till date in India. To estimate the prevalence of the disease, we performed the current hospital-based study along with molecular genetic testing to determine the frequency of the primary mutations. Based on the five-year data, approximately 1 in 40 outpatients was referred to the Neuro-Ophthalmology clinic of AEH. The study showed an average appearance of 11 newly developed LHON cases per year at our hospital.

The present molecular genetic study determined that 43.64% of the patients harbored one of the primary mutations. This frequency is higher than that reported in previous studies from India, which was determined as 27.5% [20] and 36.0% [21]. A possible reason could be the stringent case inclusion criteria followed in the present study. The 11778A mutation in the *MT-ND4 gene* was found to be more common (41.82%) in the study cohort and was consistent with existing reports. Six of the nine patients with a family history of visual failure harbored the 11778A mutation. The patients who were negative for the primary mutations might harbor other rare mutations or synergistic variants in mtDNA, which can be addressed by performing whole mitochondrial genome sequencing [22]. Apart from the three primary mutations, 16 other mutations have been reported thus far to cause LHON. Additionally, 18 candidate mutations were found in singleton cases or in a single family (Foswiki). Estimating the prevalence of newly developed LHON cases harboring the primary mtDNA mutations resulted in 1:1689 patients at the Neuro-Ophthalmology Clinic as well as 1:66602 for the total new outpatients.

The age at onset was much earlier in women. All female patients developed the disease during the second decade of their life. However, most of the male patients developed the disease during their second (42.9%) and third (36.7%) decades of life. The mean age at onset (13.67 years in women, 21.84 years in men) was considerably lower than that observed in Denmark and Japan [8,16]. The unknown reason must be explored to understand the early onset of the disease in women. The overall mean age at onset including men and women (20.95 years) corresponded to that in northeast England (22 years). A singleton case of late onset, genetically confirmed (11778A) optic neuropathy was observed in a 56-year-old man, indicating the low frequency (1.82%) of late onset LHON in this cohort.

The male to female ratio was approximately 8.2:1.0. This proportion was higher than that described in the other populations, which is 6:1 in Serbia [15], 5.4:1.0 in the Netherlands [16], 3.7:1.0 in Denmark [8], 3.4:1.0 in Finland [10], and 3.3:1.0 in England [13] but similar to that in Japan (8:1) [16]. The factors speculated for this gender bias are the nuclear modifier genes on the X-chromosome [23] and circulating estrogen in women [24].

The strength of the present study is the large patient volume at a tertiary eye care center in India. As LHON is a rare genetic disease, a hospital-based study requires a large study population to estimate the prevalence. Therefore, the high patient number at the hospital provided adequate support to conduct this study. While analyzing preceding reports [8,10,13,15,16], we identified a comparatively higher number of patients with LHON at a single center within a short time period (Table 4). Further, the stringent case inclusion criteria enhanced the quality of the study by increasing the mutation detection rate compared to previous reports from India

Table 4. Previous studies on LHON prevalence.

Country	Study design	Duration (years)	Number of patients identified
Finland	Population based, Clinical follow-up [10]	34 (1970–2004)	108
Denmark	Population based, Tertiary national referral center [8]	~32 (1980–2012)	104
North East England	Population based, Prospective, Referral based [13]	12 (1990–2002)	70
Serbia	Population based, Prospective [15]	~12 (2000–2013)	14
Japan	Population based, Multiple centers (1397 facilities), Questionnaire based survey [16]	1 (2014)	72
Present study	**Hospital based, prospective, Single center**	**5 (2015–2019)**	**55**

[20,21]. Nevertheless, the entire mitochondrial genome should be screened to identify other mutations associated with LHON in this cohort. Moreover, due to poor visual acuity, a group of patients could not be tested for color vision and central fields. As a result, the data on these parameters could not be evaluated for this patient group.

To conclude, this study demonstrated the prevalence of the rare mitochondrial genetic disease, LHON, at a tertiary eye care center in southern India. To the best of our knowledge, this is the first attempt from India to estimate the prevalence of LHON and it is difficult to compare with the existing reports from other countries at this stage, since all the studies are population-based. The gap between the diagnosis and detection of mutations insist on the necessity of whole mitochondrial genome sequencing for the suspected LHON samples to further ascertain the condition and to improve the treatment options based on the genetic testing results.

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REFERENCES

1. Newman NJ, Wallace DC. Mitochondria and Leber's hereditary optic neuropathy. Am J Ophthalmol 1990; 109:726-30. [PMID: 2346203].

2. Danielson SR, Wong A, Carelli V, Martinuzzi A, Schapira AHV, Cortopassi GA. Cells bearing mutations causing Leber's hereditary optic neuropathy are sensitized to Fas-Induced apoptosis. J Biol Chem 2002; 277:5810-5. [PMID: 11741983].

3. Yu-Wai-Man P, Griffiths PG, Hudson G, Chinnery PF. Inherited mitochondrial optic neuropathies. J Med Genet 2009; 46:145-58. [PMID: 19001017].

4. Dimitriadis K, Leonhardt M, Yu-Wai-Man P, Kirkman MA, Korsten A, De Coo IF, Chinnery PF, Klopstock T. Leber's hereditary optic neuropathy with late disease onset: clinical and molecular characteristics of 20 patients. Orphanet J Rare Dis 2014; 9:158-[PMID: 25338955].

5. Mackey DA, Oostra RJ, Rosenberg T, Nikoskelainen E, Bronte-Stewart J, Poulton J, Harding AE, Govan G, Bolhuis PA, Norby S. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 1996; 59:481-5. [PMID: 8755941].

6. Mckenzie M, Ryan MT. Assembly factors of human mitochondrial complex I and their defects in disease. IUBMB Life 2010; 62:497-502. [PMID: 20552642].

7. Kogachi K, Ter-Zakarian A, Tian J, Karanjia R, Sadun A. The Elusive Pathophysiology of Leber's Hereditary Optic Neuropathy. Vis. Pan-Am J 2016; 15:102-5. .

8. Rosenberg T, Nørby S, Schwartz M, Saillard J, Magalhães PJ, Leroy D, Kann EC, Duno M. Prevalence and Genetics of Leber Hereditary Optic Neuropathy in the Danish Population. Invest Ophthalmol Vis Sci 2016; 57:1370-5. [PMID: 27007794].

9. Kim JY, Hwang J-M, Chang B-L, Park SS. Spectrum of the mitochondrial DNA mutations of Leber's hereditary optic neuropathy in Koreans. J Neurol 2003; 250:278-81. [PMID: 12638016].

10. Puomila A, Hämäläinen P, Kivioja S, Savontaus M-L, Koivumäki S, Huoponen K, Nikoskelainen E. Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur J Hum Genet 2007; 15:1079-89. [PMID: 17406640].

11. Jiang P, Liang M, Zhang J, Gao Y, He Z, Yu H, Zhao F, Ji Y, Liu X, Zhang M, Fu Q. Prevalence of Mitochondrial ND4 Mutations in 1281 Han Chinese Subjects With Leber's Hereditary Optic Neuropathy. Invest Ophthalmol Vis Sci 2015; 56:4778-88. [PMID: 26218905].

12. Kirkman MA, Yu-Wai-Man P, Korsten A, Leonhardt M, Dimitriadis K, De Coo IF, Klopstock T, Chinnery PF. Gene-environment interactions in Leber hereditary optic neuropathy. Brain 2009; 132:2317-26. [PMID: 19525327].

13. Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 2003; 72:333-9. [PMID: 12518276].

14. Spruijt L, Kolbach DN, de Coo RF, Plomp AS, Bauer NJ, Smeets HJ, de Die-Smulders CE. Influence of mutation type on clinical expression of Leber hereditary optic neuropathy. Am J Ophthalmol 2006; 141:676-82. [PMID: 16564802].

15. Jančić J, Dejanović I, Samardžić J, Radovanović S, Pepić A, Kosanović-Jaković N, Ćetković M, Kostić V. Leber hereditary optic neuropathy in the population of Serbia. Eur J Paediatr Neurol 2014; 18:354-9. [PMID: 24508359].

16. Ueda K, Morizane Y, Shiraga F, Shikishima K, Ishikawa H, Wakakura M, Nakamura M. Nationwide epidemiological survey of Leber hereditary optic neuropathy in Japan. J Epidemiol 2017; 27:447-50. [PMID: 28392196].

17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215-[PMID: 3344216].

18. Eustace Ryan S, Ryan F, Barton D, O'Dwyer V, Neylan D. Development and validation of a novel PCR-RFLP based method for the detection of 3 primary mitochondrial mutations in Leber's hereditary optic neuropathy patients. Eye Vis (Lond) 2015; 2:18-[PMID: 26605371].

19. Rieder MJ, Taylor SL, Tobe VO, Nickerson DA. Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. Nucleic Acids Res 1998; 26:967-73. [PMID: 9461455].

20. Mishra A, Devi S, Saxena R, Gupta N, Kabra M, Chowdhury MR. Frequency of primary mutations of Leber's hereditary optic neuropathy patients in North Indian population. Indian J Ophthalmol 2017; 65:1156-60. [PMID: 29133642].

21. Saikia BB, Dubey SK, Shanmugam MK, Sundaresan P. Whole mitochondrial genome analysis in South Indian patients with Leber's hereditary optic neuropathy. Mitochondrion 2017; 36:21-8. [PMID: 27989883].

22. Achilli A, Iommarini L, Olivieri A, Pala M, Hooshiar Kashani BH, Reynier P, La Morgia C, Valentino ML, Liguori R, Pizza F, Barboni P. Rare primary mitochondrial DNA mutations and probable synergistic variants in Leber's hereditary optic neuropathy. PLoS One 2012; 7:e42242-[PMID: 22879922].

23. Ji Y, Jia X, Li S, Xiao X, Guo X, Zhang Q. Evaluation of the X-linked modifier loci for Leber hereditary optic neuropathy with the G11778A mutation in Chinese. Mol Vis 2010; 16:416-24. [PMID: 20300564].

24. Giordano C, Montopoli M, Perli E, Orlandi M, Fantin M, Ross-Cisneros FN, Caparrotta L, Martinuzzi A, Ragazzi E, Ghelli A, Sadun AA. Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. Brain 2011; 134:220-34. [PMID: 20943885].

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