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An analytical longitudinal observational study on the association of Vitamin D insufficiency in subjects with primary (idiopathic) demyelinating optic neuritis using visual evoked potential and optical coherence tomography

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ABSTRACT

Background: Optic neuritis (ON) is an acute and often immune-mediated inflammatory condition of the optic nerve. Vitamin D acts as an anti-inflammatory agent and may confer neuroprotection. Visual evoked potential (VEP) and optical coherence tomography (OCT) are emerging tools for demyelinating diseases. **Aims and Objectives:** We tried to correlate between Vitamin D insufficiency and acute demyelinating ON using different parameters such as VEP, ganglion cell layer (GCL) thickness, and retinal nerve fiber layer (RNFL) thickness. **Materials and Methods:** This observational longitudinal analytical study included thirty non-consecutive patients with primary ON and 30 healthy controls. All patients with ON underwent detailed clinical and ophthalmological examination, and detailed blood workup, including serum 25 (OH) Vitamin D. VEP P100 latency, amplitude, OCT, RNFL thickness, and GCL thickness at presentation and after 3 months from May 2019 to November 2020. **Results:** Vitamin D insufficiency (below 30 ng/mL) was present in 60% of cases of ON. The baseline VEP showed significantly prolonged P100 latency in affected eyes in the Vitamin D insufficient group (mean 129.78 ± 7.97 ms vs. 121.0 ± 4.99 ms) whereas the P100 amplitude was not significantly altered between the two groups (5.5 ± 3.13 μV vs. 7.08 ± 3.01 μV). The baseline RNFL thickness (132.21 ± 10.69 μm vs. 118.01 ± 10.4 μm) and GCL thickness (76.82 ± 2.04 μm vs. 73.06 ± 3.2 μm) were greater in affected eyes of vitamin D insufficiency ON. There was greater RNFL thinning (79.93 ± 3.42 μm vs. 74.80 ± 3.5 μm) and GCL thinning (64.78 ± 1.9μm vs. 69.02 ± 2.22 μm) in affected eyes of ON with Vitamin D insufficiency at 3 months. **Conclusion:** Vitamin D insufficiency was found in most cases of ON. Insufficient Vitamin D positively correlated with optic nerve affection severity as evidenced by significantly increased baseline thickness of RNFL and GCL and more thinning of RNFL and GCL at the end of 3 months of follow-up.

Key words: Optic neuritis; Retinal nerve fiber layer; Ganglion cell layer; Visual evoked potential; Multiple sclerosis; Central nervous system; Optic nerve head

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INTRODUCTION

Optic neuritis (ON) is defined as inflammation of the optic nerve, and in most cases, the cause is idiopathic.[1] ON is a common cause of visual impairment in young individuals having an incidence of 1–5 in 100,000 people/year.[2] It usually affects patients aged 15–49 years with female preponderance.[3] It may be an isolated episode or the first presentation of a central nervous system (CNS) demyelinating event. Visual function usually improves spontaneously over weeks, and 95% of patients return to visual acuity of at least 20/40 within 12 months.[4] A high dose of steroids hastens the rate, not the vision outcome.[5] Prognostic factors of visual recovery in ON are visual acuity at presentation, race, ethnicity, comorbidities, ocular signs such as retro bulbar pain, optic disc edema, and retinal thinning in optical coherence tomography (OCT).[6]

OCT is a rapid, non-invasive, non-contrast, transpupillary, and reproducible imaging technique using low coherence interferometry to produce cross-sectional images of the retina and optic nerve head (ONH) with high temporal accuracy.[7] Its capacity to depict the ONH, peripapillary retinal nerve fiber layer (RNFL), and cellular layers of the macula enables quantitative and qualitative assessment of optic nerve diseases.[7] In ON, the RNFL thickens acutely from axoplasmic flow stasis caused by inflammatory demyelination. In the post-acute phase, RNFL and ganglion cell layer (GCL) thinned out due to retrograde axonal and axonal degeneration, respectively.[8-10] Several studies have shown peripapillary RNFL, and macular thickness analysis may be used to detect axonal loss in ON and multiple sclerosis.[11,12]

Visual evoked potentials (VEPs) are generated in the visual cortex as a response to a visual stimulus. Pattern reversal visual evoked response (PSVER) is the preferred study in most adults, and it elicits a robust and reproducible response. The latency of the VEP accurately reflects the amount of demyelination in the visual pathway, whereas the amplitude correlates with the axonal damage. An increase in VEP latency is not a specific sign of retinal diseases affecting different parts of the sensory retina and this is increased latency with reduced VEP amplitude is seen in macular disease.[13,14] The rapid changes in RNFL and VEP study after ON make it logical to think of testing neuroprotective strategies for a short-term frame.

Vitamin D acts as an anti-inflammatory and neuroprotective agent, affects immune function, CNS development, and possesses immune-regulatory capacity.[15-18] Some *in vitro* and animal studies showed that Vitamin D supplementation reduced inflammatory cell infiltration in the CNS by suppressing antigen-presenting cells' function.[19] Vitamin D inhibits brain gamma-glutamyl transpeptidase, protects neurons against superoxide and hydrogen peroxide, and reduces nitric oxide levels by inhibiting nitric oxide synthetase in CNS. Besides this, Vitamin D reduces calcium-mediated excitotoxicity and stimulates neurotrophin release, and maintains homeostasis.[20] Few studies have shown the potential benefits of Vitamin D in the recovery of ON.[21,22]

We hypothesized that ON may be associated with low serum Vitamin D levels.

MATERIALS AND METHODS

This observational analytical longitudinal study was conducted in indoor as well as outdoor in the Department of Neurology of NRSMCH, Kolkata from May 2019 to November 2020 among 30 patients of ON and 30 age and sex-matched controls.

Inclusion criteria

Unilateral ON characterized by acute or sub-acute onset(within 2 weeks), partial or complete loss of vision in one eye, retrobulbar pain with or without disc swelling, impaired color vision, and relative afferent pupillary defect were included in the study.

Exclusion criteria

Patients were unable to provide informed consent, presentation inconsistent with primary demyelinating ON, previous history of ON in either eye, on Vitamin D supplementation, medications that influence prognosis of ON like beta-interferon, use of systemic steroids in the past 3 months, significant cataract and or ocular disease such as pathological myopia and glaucoma, pregnancy, renal insufficiency, hypercalcemia, nephrolithiasis, systemic disease, and coexisting other neurological disorders and obese individuals with ON were excluded from the study.

Objectives

The objectives of the study are as follows:

1. To find any correlation between Vitamin D levels and ON severity in acute and chronic ON
2. To find any correlation between Vitamin D levels and RNFL in acute and chronic ON
3. To find any correlation between Vitamin D levels and GCL thickness in OCT
4. To find any correlation between Vitamin D levels and the latency and amplitude of VEP P100 potential.

Considering the reduced footfall of non-COVID cases due to the pandemic situation and lockdown regulations, a complete enumeration method was used and all patients

fulfilling the inclusion and exclusion criteria were selected consecutively. This study was approved by the Institutional Ethics Committee of NRSMCH and prior informed written consent was taken from all patients.

All participants underwent general examination including body mass index (BMI) determination as well as detailed nervous system examinations and findings were recorded in a structured questionnaire. They have undergone through complete ophthalmological examination including best-corrected monocular visual acuity assessment, standard automated perimetry, and dilated fundoscopy using an indirect ophthalmoscope. Routine investigations including complete blood count, renal and liver function test, vasculitis profile, serum angiotensin-converting enzyme, viral serology, and serum Vitamin B12 level were carried out in all subjects. Serum anti-aquaporin four antibody and anti-myelin oligodendrocyte glycoprotein (MOG) antibody were also done. CSF analysis was done for microscopic examination, biochemistry, oligoclonal band, immunoglobulin G index, and pan-neurotropic virus panel.

Serum Vitamin D was measured from venous blood. Three blood samples were obtained at 5-day interval during the acute phases of ON within 14 days of the attack and were kept at −80°C at the central laboratory of said medical college and then the mean Vitamin D value was obtained. Serum 25 (OH) Vitamin D ng/mL was measured by a commercially available radio-immune assay kit. Neuroimaging using magnetic resonance imaging (MRI) of the brain and bilateral orbits along with spine including a short tau inversion recovery sequence by 1.5 T MRI scanner was done.

PSVER was recorded from both eyes by using Neuron-Spectrum-4/EPM neurosoft machine. The VEP parameters recorded were latencies to N70, P100, and N155 waves and peak-to-peak amplitude of the P100 wave. Spectral domain OCT (SD-OCT) device was used to measure RNFL thickness which produces 3D data volumes. The peripapillary region was scanned on all eyes using SD-OCT. All scans were performed through dilated pupil RT vue –100 SD-OCT (Optovue Inc., software version 6.9.0.27) was used to measure the average peripapillary RNFL thickness. For our measurement, the ONH scan protocol was used which consists of 12 radial scans of 3.4 mm in length and six concentric ring scans ranging from 2.5–4 mm in diameter centered on the optic disc.

Macular scan protocol using optovue SD-OCT was followed. 7 mm square area consisting of a 7 mm horizontal and 15 mm vertical line spaced at 0.5 mm intervals and centered 0.75–1 mm temporal to fovea.

All the data collected were compiled in MS Excel 2013 and checked for consistency and completeness. These were analyzed using SPSS Version 16. Descriptive statistics were described in numbers and percentages for categorical variables as well as mean (standard deviation [SD]), median (interquartile range [IQR]) and range in cases of continuous variables.

Nonparametric measures like Mann–Whitney U-test, Wilcoxon signed-rank test Kruskal–Wallis test and Spearman Rho correlation were applied as continuous variables were not normally distributed as evident from statistically significant P value in both Kolmogorov–Smirnov test as well as Shapiro–Wilk test. $P<0.05$ will be considered statistically significant.

RESULTS

Around half (43.3%) of study participants belonged to the age group 20–29 years with the mean (SD) age of 23.3 (8.4) years ranged from 7 years to 37 years. Majority of them were females, (53.3%), Hindu (66.7%), and having idiopathic etiology (43.3%) (Table 1). One-fifth (20%) had Vitamin D deficiency and two-fifth (40%) had Vitamin D insufficiency. Mean (SD) Vitamin D was 27.71 (8.58) ng/mL ranged from 9 ng/mL to 42 ng/mL with median of 27.8 ng/mL. (Table 2).

The mean value of VEP P100 latency in the control population was 108±2.2 ms and 108.2±2.4 ms in the left and right eye, respectively, in Vitamin D sufficient patients and 104±1.9 ms and 105±1.7 ms in the left and right eye, respectively, in Vitamin D insufficient control population.

Table 1: Background characteristics of study participants (n=30)

Attributes	Number (%)
Age	
<10	1 (3.3)
10–19	8 (26.7)
20–29	13 (43.3)
≥30	8 (26.7)
Gender	
Male	14 (46.7)
Female	16 (53.3)
Religion	
Hindu	20 (66.7)
Muslim	10 (33.3)
Etiology	
Idiopathic	13 (43.3)
MOG	8 (26.7)
MS	4 (13.3)
NMO	1 (3.4)
NMOSD	4 (13.3)

MOG: Myelin oligodendrocyte glycoprotein, MS: Multiple sclerosis, NMO: Neuromyelitis optica, NMOSD: Neuromyelitis optica spectrum disorder

The mean value of VEP P100 amplitude in the right and left eye in the control population was 4.1±0.8 μV and 4.3±0.8 μV (vitamin D sufficient) and, 3.7±1.1 μV and 3.9±1.9 μV (Vitamin D insufficient) respectively. The range of RNFL thickness and GCL volume in control subjects was 94.65±9.44 μm and 73.6±4.7 μm, respectively, in Vitamin D sufficient group and 93.54±8.2 μm and 72.7±3.3 μm in Vitamin D insufficient group, respectively.

In the affected eye, the mean (SD) VEP P 100 was 126.27 (8.12) ranged from 113 to 142 with a median of 127.5 whereas mean (SD) VEP amplitude was 6.13(3.16) ranged from 0.26 to 11.2 with a median of 6.7. In the unaffected eye, the mean (SD) VEP P 100 was 99.80 (10.38) ranged from 81 to 116 with a median of 102 whereas mean (SD) VEP amplitude was 8.60 (2.55) ranged from 2.9 to 11.8 with a median of 9.2. Affected eye showed a significantly higher baseline VEP P 100 and significantly lower baseline VEP Amplitude (Figure 1).

In affected eye, mean (SD) retinal nerve fiber layer (RFNL) in baseline was 126.56 (12.55) ranged from 98.8 to 145.1 with a median of 126.2 which was significantly reduced during follow-up to get mean (SD) RFNL during follow-up was 77.88 (4.25) ranged from 69.90 to 85.44 with a median of 77.7. In the unaffected eye, the mean (SD) RFNL in baseline was 88.30 (5.73) ranged from 79.2 to 98.4 with a median of 87.9 which was significantly reduced during follow-up to get the mean (SD) RFNL during follow-up was 83.82 (8.4) ranged from 73.4 to 96.33 with a median of 80.3. However, the reduction of RFNL value from baseline to follow-up is significant higher in affected eye compared to the unaffected eye (Figure 2).

In the affected eye, the mean (SD) GCL in baseline was 75.32 (3.15) and ranged from 69.74 to 81.67 with a median of 75.35 which was significantly reduced during follow-up to get the mean (SD) GCL during follow-up was 66.48 (2.92) ranged from 61.33 to 72.53 with a median of 66.07. In the unaffected eye, the mean (SD) GCL in baseline was 76.38 (3.83) ranged from 70.43 to 84.30 with a median of 74.9 which was increased during follow-up to get the mean (SD) GCL during follow-up was 77.21 (4.15) ranged from 70.69 to 84 with a median of 76.56. However, the reduction of GCL value from baseline to follow-up is significant higher in affected eye compared to unaffected eye (Figure 3).

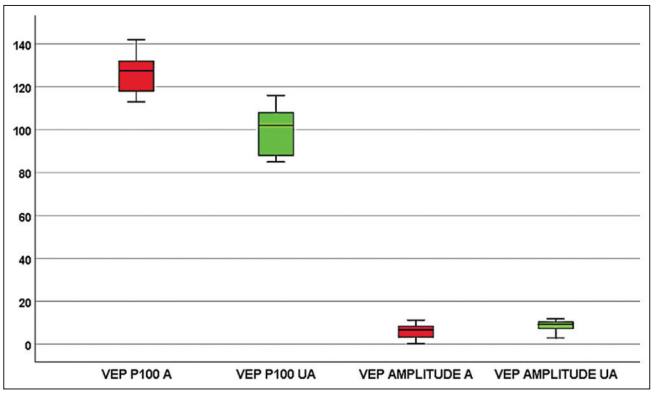

Figure 1: Baseline visual evoked potential among eyes of study subjects (n=30)

VEP	Unaffected eye (UA)	Affected eye (A)	P-value¥
P100			
Mean (SD)	99.80 (10.38)	126.27 (8.12)	0.001*
Median (IQR)	102 (88, 108)	127.5 (118, 132.5)	
Range	31,116	113,142	
Amplitude			
Mean (SD)	8.60 (2.55)	6.13 (3.16)	0.001*
Median (IQR)	9.2 (7.28, 10.45)	6.7 (3.08, 8.55)	
Range	2.9,11.8	0.26, 11.2	

¥Mann–Whitney U-test, *Statistically significant, IQR: Interquartile range, SD: Standard deviation, VEP: Visual evoked potential

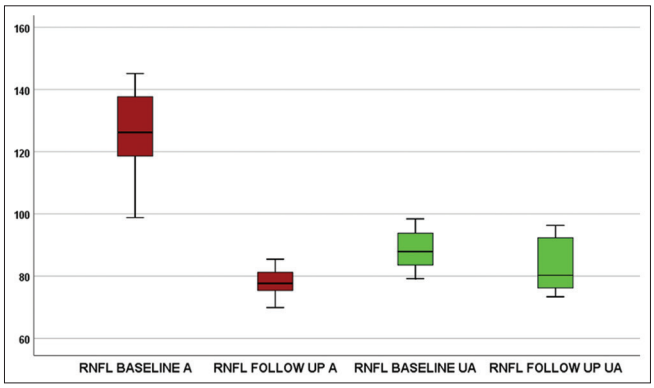

Figure 2: Retinal nerve fiber layer in baseline and after 3-month follow-up among eyes of study subjects (n=30)

RFNL	Unaffected eye (UA)	Affected eye (A)	P-value¥
Baseline			
Mean (SD)	88.30 (5.73)	126.56 (12.55)	<0.001*
Median (IQR)	87.9 (83.6, 93.8)	126.2 (118.3, 137.7)	
Range	79.2, 98.4	98.8, 145.1	
Follow-up			
Mean (SD)	83.82 (8.4)	77.88 (4.25)	0.019*
Median (IQR)	80.3 (76.1, 92.5)	77.7 (75.2, 81.3)	
Range	73.4, 96.33	69.90, 85.44	
Change (baseline-follow-up)			
Mean (SD)	4.48 (5.65)	48.68 (10.54)	<0.001*
Median (IQR)	4.45 (1.41, 6.84)	49.8 (42.9, 56.9)	
Range	−6.09, 23.91	23.4, 69.4	
P-value§	<0.001*	<0.001*	

¥Mann–Whitney U-test, §Wilcoxon Signed Rank Test, *Statistically significant, IQR: Interquartile range, SD: Standard deviation, VEP: Visual evoked potential, RFNL: Retinal nerve fiber layer

In comparison to the unaffected eye, the affected eye showed significantly more thinning of RFNL and GCL values in all levels of Vitamin D. Significantly higher thinning of RFNL and GCL in the affected eye was noticed in Vitamin D deficient group followed by people with Vitamin D insufficiency and normal Vitamin D. However, in case of unaffected eye, thinning in RFNL is significantly higher in Vitamin D deficient and insufficient group compared to Vitamin D sufficient group. Moreover, no significant difference of improvement of GCL was seen in unaffected eye (Figures 4 and 5).

Vitamin D showed moderate to high and statistically significant negative correlation with VEP P 100 in both affected and unaffected eyes. Which denoted with decrease of Vitamin D level, there was an increase in VEP P100 in both affected and unaffected eyes or subclinically affected eye. Vitamin D also showed a moderate and statistically significant positive correlation with VEP amplitude in both affected and unaffected eyes which denoted with decrease of Vitamin D level there was a significantly decrease in VEP amplitude in both affected and unaffected eyes. Thus, Vitamin D might have a pathophysiological role in the severity of ON which is more marked in the affected eye (symptomatic) compared to the unaffected eye (asymptomatic). Vitamin D showed moderate to high and statistically significant negative correlation with RFNL and GCL in baseline as well as follow-up in both affected and unaffected eyes which denoted with decrease of Vitamin D level, there was increase in RFNL and GCL in both affected and unaffected eyes (subclinical eye). Significantly higher decreasing value of RFNL, that is, severe thinning in affected eye is seen with increasing Vitamin D level in both affected eye and unaffected eye. In the case of GCL, significantly higher decreasing value of GCL, that is, thinning in affected eye is noticed with decreasing Vitamin D level in the affected eye however no significant correlation of improvement of GCL and Vitamin D in unaffected eye was noticed (Table 3). The multivariate analysis has been shown in Table 4.

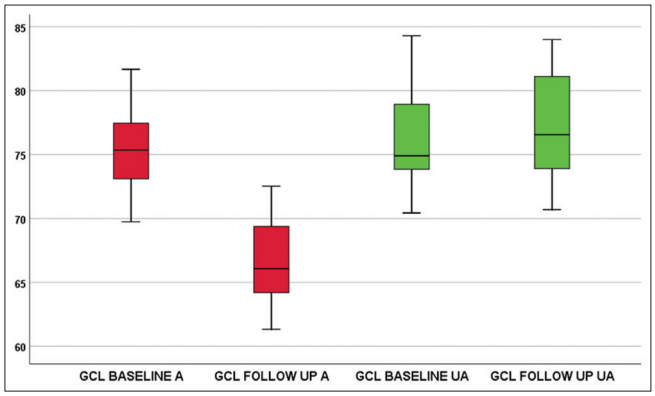

Figure 3: Ganglion cell layer in baseline and after 3-month follow-up among eyes of study subjects (n=30)

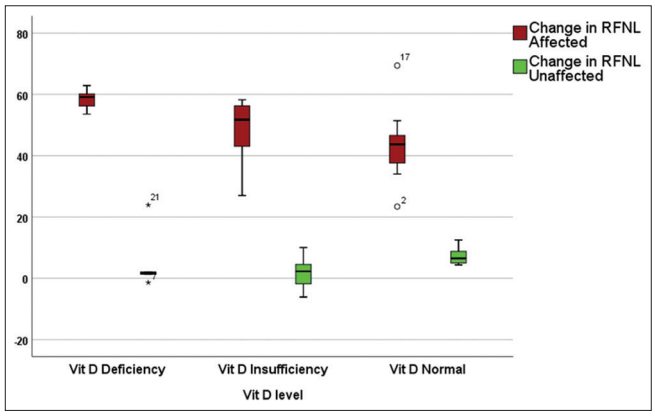

Figure 4: Change in retinal nerve fiber layer among eyes of study subjects with various degrees of Vitamin D deficiency (n=30)

GCL	Unaffected eye (UA)	Affected eye (A)	P-value¥
Baseline			
Mean (SD)	76.38 (3.83)	75.32 (3.15)	0.433
Median (IQR)	74.9 (73.83, 79.15)	75.35 (73, 77.13)	
Range	70.43, 84.30	69.74, 81.67	
Follow-up			
Mean (SD)	77.21 (4.15)	66.48 (2.92)	<0.001*
Median (IQR)	76.56 (73.87, 81.14)	66.07 (64.11, 69.38)	
Range	70.69, 84.00	61.33, 72.53	
Change (Baseline-Follow-up)			
Mean (SD)	−0.84 (3.87)	8.84 (5.32)	<0.001*
Median (IQR)	−0.33 (−2.25, 1.33)	9.62 (2.83, 11.6)	
Range	−9.1, 7.2	0.39, 8.57	
P-value§	<0.001*	<0.001*	

¥Mann–Whitney U-test, §Wilcoxon Signed Rank Test, *Statistically significant, IQR: Interquartile range, SD: Standard deviation, VEP: Visual evoked potential, GCL: Ganglion cell layer

Change in RFNL	Unaffected eye (UA)	Affected eye (A)	P-value¥
Vitamin D deficiency (<20)			
Mean (SD)	4.85 (9.42)	58.52 (3.24)	<0.001*
Median (IQR)	1.6 (1.3, 2.1)	59.14 (56.25, 60.15)	
Range	−1.4, 23.91	53.56, 62.87	
Vitamin D insufficiency (20–29)			
Mean (SD)	1.71 (4.57)	49.16 (9.02)	<0.001*
Median (IQR)	2.26 (−1.81, 4.55)	51.75 (43.04, 56.27)	
Range	−6.09, 10.01	27.01, 58.22	
Vitamin D normal (≥30)			
Mean (SD)	7.08 (2.66)	43.26 (11.04)	<0.001*
Median (IQR)	6.48 (5.01, 8.77)	43.72 (37.59, 46.63)	
Range	4.37, 12.50	23.4, 69.4	
P-value§	0.008*	0.003*	

¥Mann–Whitney U-test, §Kruskal–Wallis Test, *Statistically significant, IQR: Interquartile range, SD: Standard deviation, VEP: Visual evoked potential, RFNL: Retinal nerve fiber layer

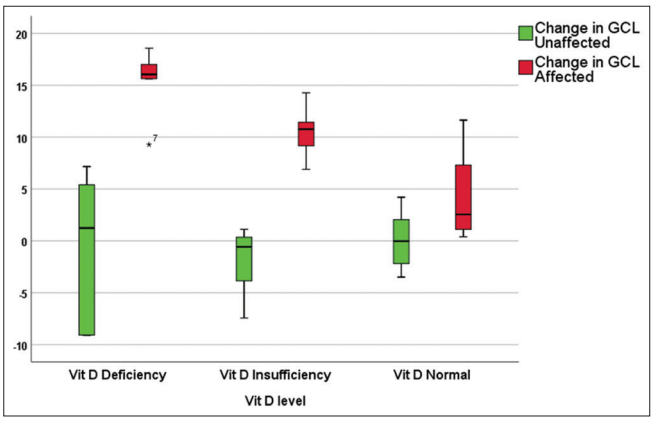

Figure 5: Change in Ganglion cell layer among eyes of study subjects with various degrees of Vitamin D deficiency (n=30)

Change in GCL	Unaffected eye (UA)	Affected eye (A)	P-value¥
Vitamin D deficiency (<20)			
Mean (SD)	−0.52 (7.05)	15.43 (3.20)	<0.001*
Median (IQR)	1.24 (−9.07, 5.41)	16.05 (15.63, 17)	
Range	−9.10, 7.16	9.28, 18.57	
Vitamin D insufficiency (20–29)			
Mean (SD)	−1.84 (3.07)	10.35 (2.04)	<0.001*
Median (IQR)	−0.57 (−3.85, 0.36)	10.77 (9.16, 11.43)	
Range	−7.44, 1.11	6.90, 14.27	
Vitamin D normal (≥30)			
Mean (SD)	0.01 (2.35)	4.04 (3.83)	<0.001*
Median (IQR)	−0.02 (−2.18, 2.05)	2.56 (1.11, 7.31)	
Range	−3.49, 4.21	0.39, 11.64	
P-value§	0.473	<0.001*	

¥Mann–Whitney U-test, §Kruskal–Wallis Test, *Statistically significant, IQR: Interquartile range, SD: Standard deviation, VEP: Visual evoked potential, GCL: Ganglion cell layer

Table 2: Distribution of serum Vitamin D level among study subjects (n=30)

Vitamin D level (ng/mL)	Number (%)	Descriptive statistics of Vitamin D level (ng/mL)
Deficiency (<20)	6 (20.0)	Mean (SD): 27.71 (8.58)
Insufficiency (20–29)	12 (40.0)	Median (IQR): 27.80 (22.35, 35.25)
Normal (≥30)	12 (40.0)	Range: (Minimum, Maximum): 9, 42
Total	30 (100.0)	

IQR: Interquartile range, SD: Standard deviation

Table 3: Correlation of Vitamin D, VEP and RFNL, and GCL in study subjects (n=30)

Parameters	Vitamin D			
	Affected eye		Unaffected eye	
	Coefficient	P-value	Coefficient	P-value
VEP P100	−0.733	<0.001*	−0.468	0.009
VEP Amplitude	0.511	0.004*	0.500	0.005
Baseline RFNL	−0.779	<0.001*	−0.871	<0.001*
Follow-up RFNL	−0.687	<0.001*	−0.767	<0.001*
Change RFNL	−0.667	<0.001*	0.567	0.001*
Baseline GCL	−0.708	<0.001*	−0.720	<0.001*
Follow-up GCL	0.687	<0.001*	0.788	<0.001*
Change GCL	−0.753	<0.001*	0.005	0.980

*Spearman Rho Correlation, *Statistically significant, VEP: Visual evoked potential, RFNL: Retinal nerve fiber layer, GCL: Ganglion cell layer

DISCUSSION

Our study shows the age of affection of ON is more common in the third decade of life (43.3%) (mean age-23.3 years) with a female preponderance (53.3%). This is consistent with the demographic findings of a previous study.[23] Obesity is associated with diminished thickness in several SD-OCT measurements through various direct and indirect mechanisms.[24] BMI is associated with acute ON severity in males but not in female patients. The BMI range of our patients was 19–24 kg/m^2. Age, gender, and axial length should be considered when measuring the GCL thickness and volume with OCT. RNFL was significantly thicker in males compared to females. In a previous study, all global and sectorial RNFL thickness parameters significantly decreased with increasing age.[25] Existing literature also showed a definite gender difference in VEP parameters, with females having shorter P100 latencies and higher amplitude.[26] We have taken the mean value of RNFL thickness, GCL thickness, and VEP latencies from age and gender-matched control subjects to reduce the confounding influence. About 60% of our study population has Vitamin D insufficiency (30 ng/mL, i.e., 75nmol/L) in the background of 19 out of 30 (63.3%) age and sex-matched control populations are Vitamin D insufficient. About 37% of the general population being Vitamin D insufficient (30–50 nmol/L) in previous studies.[27,28] There is a widespread prevalence of Vitamin D deficiency in 70–100% of the general population in the Indian subcontinent, as reported by Ritu and Gupta.[29]

The median VEP P100 latency in the affected eye was significantly higher in the Vitamin D insufficient group. The VEP P100 latency value of the affected eye had a good negative correlation with serum Vitamin D level, which was statistically significant. This finding suggests that decreased serum 25(OH) Vitamin D level is somehow associated with increased severity of ON as evidenced by increased VEP P100 latency, although the same cannot be proven in the control group with Vitamin D sufficiency and deficiency. To the best of our literature survey, we have not found similar observations in any previous study. This may be a novel finding and demands further analysis to support our statement.

Table 4: Stratified analysis showing comparison of different variables in subjects with Vitamin D deficiency and subjects with adequate Vitamin D level (n=30)

Variables	Vitamin D level (ng/mL)		P-value¥
	Deficient (<30)	Adequate (≥30)	
VEP P 100 affected			
Mean (SD)	129.78 (7.97)	121.0 (4.99)	
Median (IQR)	132 (124.75, 136)	120 (118, 124.80)	0.006*
Range	116, 142	113, 130	
VEP P 100 difference			
Mean (SD)	−27.06 (10.22)	−25.6 (9.9)	
Median (IQR)	−23.5, (−33.5, −21.5)	−27.5, (−32, −18.5)	0.99
Range	−50, −11	−43, −9	
VEP amplitude affected			
Mean (SD)	5.5 (3.13)	7.08 (3.01)	
Median (IQR)	6.35 (2.66, 7.9)	7.35 (5.8, 9.6)	0.200
Range	0.26, 10.40	1.4, 11.2	
VEP amplitude difference			
Mean (SD)	2.58 (1.75)	2.3 (1.44)	
Median (IQR)	2.42 (1.28, 3.11)	2.3 (1.33, 3.1)	0.884
Range	0.5, 6.8	0.2, 5.6	
RNFL affected baseline			
Mean (SD)	132.21 (10.69)	118.01 (10.4)	
Median (IQR)	135.09 (122.9, 139.5)	118 (113.5, 122.2)	0.001*
Range	104.9, 145.1	98.8, 139.3	
GCL affected baseline			
Mean (SD)	76.82 (2.04)	73.06 (3.2)	
Median (IQR)	76.6 (75.28, 78.68)	72.2 (71, 73.7)	0.001*
Range	73.17, 79.90	69.74, 81.67	
RNFL affected follow up			
Mean (SD)	79.93 (3.42)	74.8 (3.5)	
Median (IQR)	78.96 (77.26, 83.74)	74.6 (72.04, 78)	0.001*
Range	74.6, 85.44	69.9, 81.2	
GCL affected follow-up			
Mean (SD)	64.78 (1.9)	69.02 (2.22)	
Median (IQR)	64.79 (63.13, 66.28)	69.4 (67.9, 70.3)	0.001*
Range	61.33, 69.22	64.76, 72.53	
RNFL difference (UA–A) baseline			
Mean (SD)	−40.39 (7.88)	−35.06 (10.25)	
Median (IQR)	−42.39 (−46.15, −35.28)	−35.6 (−41.4, −25.7)	0.079
Range	−49.9, −16.3	−54.5, −17.7	
RNFL difference (UA–A) follow-up			
Mean (SD)	9.14 (6.91)	1.13 (3.5)	
Median (IQR)	10.33 (6.8, 14.5)	1.64 (−2.4, 3.9)	0.001*
Range	−11.27, 16.29	−4.56, 6.57	
GCL difference (UA–A) baseline			
Mean (SD)	1.68 (3.3)	01.23 (4.11)	
Median (IQR)	2.6 (−0.75, 4.32)	1.4 (−1.35, 3.07)	0.267
Range	−5, 5.7	−9.89, 5.16	
GCL difference (UA–A) follow-up			
Mean (SD)	15.13 (4.16)	4.2 (3.004)	
Median (IQR)	15.39 (12.24, 18.66)	3.06 (1.75, 6.32)	0.001*
Range	7.12, 22.67	1.12, 9.65	

*Significant in 95% confidence interval, ¥Mann–Whitney U-test, VEP: Visual evoked potential, RFNL: Retinal nerve fiber layer, GCL: Ganglion cell layer, IQR: Interquartile range, SD: Standard deviation

Significantly higher RNFL thickness value in the affected eyes was noted at baseline among the baseline Vitamin D insufficient compared to the Vitamin D sufficient group. This higher RNFL thickness value in the Vitamin D insufficient group is possibly caused by greater axoplasmic flow stasis following inflammatory demyelination. This finding may be related to vitamin D insufficiency. The change in RNFL thickness in the affected eye and the unaffected eye follow-up at the end of 3 months was also significantly more in the Vitamin D insufficient group than in the sufficient group. There was greater RNFL thinning in the affected eyes of subjects with ON who had Vitamin D insufficiency due to greater retrograde axonal degeneration. Similarly, the significant change in follow-up of RNFL thickness in the

unaffected eyes may be related to subclinical affection of unaffected eyes based on different etiologies of ON, that is, MOG and neuromyelitis optica (NMO) related demyelinating ON. Vitamin D insufficiency may be involved in greater RNFL thinning. There was a statistically negative correlation between RNFL thickness and serum 25(OH) Vitamin D level at baseline. The change in RNFL thickness of subjects with ON was statistically more significant in the Vitamin D insufficient group than in the other group. This observation in our study corroborates with the previous studies.[21,30,31]

Significantly higher GCL volume in the affected eye was noted at baseline in the Vitamin D insufficient group than sufficient group (median 76.6 vs. 72.7 μm and P=0.001). At 3-month follow-ups, the change and the thinning in GCL volume were significantly greater among subjects with Vitamin D insufficiency. Kupersmith et al., also observed GCL and inner plexiform layer thinning within 1–2 months of onset, and this may be an early biomarker of structural injury.[32]

We have also noted that seven individuals out of thirty patients have subclinical affection in the asymptomatic eye, corroborating with increased VEP latency and RNFL and GCL thickness during the acute presentation. Out of seven subjects with subclinical affection, six patients had Vitamin D insufficiency and are diagnosed MOG (five subjects) and NMO (two subjects) related ON. These findings are consistent with the previous reports of ongoing subclinical structural damage with axonal loss observed in patients with MOG and NMO but without clinical ON.[33]

Limitations of the study

This study has certain limitations. The sample size was small due to the COVID-19 pandemic. Genetic variants of the DVR gene, known to influence the serum 25(OH) Vitamin D, and the impact of seasonal changes on serum Vitamin D levels were not determined. The impact of different etiologies of ON on RNFL and GCL has not been addressed because of the limited sample size.

CONCLUSION

- Vitamin D insufficiency was found to be present in 60% of individuals with ON in our case series.
- There is an increased prevalence of ON in female subjects, affecting individuals mostly in the third decade of life.
- Subjects with lower Vitamin D levels correlated with increased severity of affection of optic nerve in the acute inflammatory state as demonstrated by significantly increased baseline thickness of RNFL and GCL and significantly greater RNFL and GCL thinning at the end of 3-month follow up.
- The severity of ON has been found in the Vitamin D insufficient group, as evidenced by significantly prolonged VEP P100 latency.
- Our findings support the hypothesis that low serum Vitamin D levels are related to unfavorable ON disease outcomes. Further studies are required to validate our observations and also whether Vitamin D prophylaxis minimizes the disease severity.

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