Received:
16 January 2018

Revised:
07 June 2018

Accepted:
04 July 2018

<https://doi.org/10.1259/bjr.20180086>

Cite this article as:

Kalita J, Soni N, Dubey D, Kumar S, Misra UK. Evaluation of optic nerve functions in subacute combined degeneration using visual evoked potential and diffusion tensor imaging—a pilot study. *Br J Radiol* 2018; **91**: 20180086.

FULL PAPER

Evaluation of optic nerve functions in subacute combined degeneration using visual evoked potential and diffusion tensor imaging—a pilot study

¹JAYANTEE KALITA, DM, ²NEETU SONI, DM, ¹DEEPANSHU DUBEY, DM, ²SUNIL KUMAR, DM and ¹USHA KANT MISRA, DM

¹Department of Neurology, Sanjay Gandhi Post Graduate medical Sciences, Lucknow, India

²Department of Radiodiagnosis, Sanjay Gandhi Post Graduate medical Sciences, Lucknow, India

Address correspondence to: Professor Usha Kant Misra
E-mail: drukmisra@rediffmail.com

Objective: To evaluate optic nerve involvement in subacute combined degeneration (SACD) using diffusion tensor imaging (DTI) and visual evoked potential (VEP) studies, and their changes following cobalamine treatment.

Methods: Six patients with SACD and six healthy matched controls were included. Visual acuity, field of vision, and color vision were tested. Pattern shift VEP was done, and P100 latency and amplitude were measured. Optic nerve MRI, and DTI of optic nerve to muscle ratio were measured, and fractional anisotropy ratio (FAR), axial diffusivity ratio (ADR), radial diffusivity ratio (RDR), and mean diffusivity ratio (MDR) were calculated. The patients received hydroxyl cobalamine 1000 µg intramuscularly and their clinical examination, VEP and DTI studies were repeated at 3 months.

Results: The age of the patients ranged between 16 and 60 years and two were females. Their visual acuity, field of vision, and color vision were normal. P100 latency

was prolonged in five patients (10 eyes) and amplitude was reduced in one (1 eye). The SACD patients had reduced FAR (1.94 ± 0.55 vs 2.81 ± 0.42 ; $p = 0.01$) and increased MDR (1.00 ± 0.04 vs 0.95 ± 0.01 ; $p = 0.01$) and RDR (0.96 ± 0.03 vs 0.89 ± 0.01 ; $p = 0.002$) compared to the controls. The FAR value correlated with P100 latency ($r = -0.88$). At 3 months, FAR value increased which was associated with improvement in P100 latency.

Conclusion: In SACD patients, optic nerve FAR is reduced and correlates with P100 latency. Both these parameters improve on cobalamine treatment.

Advances in knowledge: Subclinical VEP abnormalities are common in SACD but conventional MRI sequence of optic nerve is normal. DTI of optic nerve reveals reduced fractional anisotropy (FA) values which improve after cobalamine treatment. FA values correlate with prolongation of P100 latency. DTI and VEP abnormalities suggest subclinical optic nerve myelin dysfunction.

INTRODUCTION

Vitamin B12 deficiency results in hematological, neurological, and behavioral abnormalities. The neurological manifestation of vitamin B12 deficiency is subacute combined degeneration (SACD), which is characterized by sensory ataxia, spasticity, and peripheral neuropathy. Cognitive impairment has been reported in 18–50% of patients with vitamin B12 deficiency neurological syndrome (VBDNS) and majority of them are associated with myelopathy.^{1–3} In earlier studies, nutritional amblyopia was attributed to vitamin B12 deficiency with tobacco consumption. In recent studies on VBDNS, vision is seldom affected which may be attributed to early diagnosis and treatment.^{4–6} In our earlier studies, we have found only one patient with visual loss out of 57 patients with VBDNS.⁶ Optic nerve involvement in vitamin B12 deficiency has been reported

in 1 out of 153 patients highlighting the rarity of visual impairment in VBDNS.⁷ Subclinical optic nerve involvement as assessed by visual evoked potential (VEP) has been reported in 58.8% of patients.⁵ Modern neuroimaging studies have provided valuable information on the mechanism of underlying pathophysiology. Diffusion tensor imaging (DTI) provides tissue microstructural information by measuring the average and directional water diffusivity for a given voxel in terms of mean diffusivity (MD) and fractional anisotropy (FA). FA is sensitive to microstructural integrity, MD to cellularity, edema, and necrosis, while axial diffusivity (AD) decreases in axonal injury and radial diffusivity (RD) increases in white matter demyelination. In multiple sclerosis, DTI abnormality is detected not only in the patients with symptomatic optic neuritis but also in those without any visual impairment.⁸ DTI study of optic nerve may also provide valuable information about

the pathophysiology of subclinical VEP abnormality in SACD. Toxic neuropathies usually result in axonal involvement, but in vitamin B12 deficiency, there is characteristic myelin dysfunction which responds to cobalamine treatment.^{2,3,9} There is no study correlating DTI of optic nerve with VEP changes in the patients with SACD. In this communication, we report the DTI findings of optic nerve in the patients with SACD and correlate these with VEP changes as well as their response to cobalamine treatment.

METHODS AND MATERIALS

The patients with SACD diagnosed on the basis of characteristic clinical features and low serum vitamin B12 level ($<211 \text{ pg ml}^{-1}$) and/or megaloblastic bone marrow were included. Patients with history of stroke, structural brain lesion, hepatic or renal failure, seizures or toxic exposure were excluded. The project was approved by the Institute Ethics Committee, and the patients or their representative provided written consent (IEC code; 2014–49-IP-75).

Dietary habit, malabsorption, gastrointestinal surgery, and smoking habits were noted. Duration of illness, presenting symptoms including forgetfulness, walking difficulty, paresis, numbness, and visual impairment were enquired. Cognitive functions were evaluated by Mini Mental State Examination (MMSE) scale. Field of vision was tested by computerized test, color vision by Ishihara chart, and visual acuity by Snellen's chart. Fundus examination was done and presence of optic atrophy and macular or retinal changes were noted. Muscle power was assessed by Medical Research Council scale. Tone and tendon reflexes were categorized as normal, hypo, and hypertonia. Sensations of pinprick, joint position, and vibration were noted.

Investigations

Hemoglobin, blood counts, red blood cell indices, and reticulocyte counts were measured. Fasting and post-prandial blood sugar, serum creatinine, transaminases, bilirubin and lactate dehydrogenase, HIV serology, and thyroid stimulating hormone were measured. Antiparietal cell antibody was assayed by enzyme-linked immuno-sorbent assay. Fasting serum vitamin B12 level was measured using vitamin B12 assay kit (Siemens Healthcare Diagnostics Technical Services). In selected patients, bone marrow was examined.

Visual evoked potential

Pattern shift visual evoked potential (PSVEP) was done using Key point (Key point, Dentec, Netherlands). The patient was explained about the test and was allowed to sit comfortably on a chair with neck supported. The recording electrode was placed at Oz referred to Fpz as per 10–20 system of electrode placement. The ground electrode was placed at Cz. The electrode impedance was kept below 5 k Ω . The filters were set at 1–300 Hz and sweep time 250–500 ms. The distance between eye to center of the screen was 100 cm and fixation point for full field size was 8°. The check size was 56' arc and luminance of central field was 50 cd m⁻² and a contrast level of 50–80%. Stimulation rate was 1 Hz producing reversal pattern every 500 ms. 100 epochs were twice averaged for reproducibility. The latency and amplitude of P100

waveform were measured and compared with our laboratory normal values. The values were considered abnormal if exceeded more than 2.5 standard deviation of normal. The upper limit of normal P100 latency is 106 (96.9 $\pm$ 3.6) ms and lower limit of amplitude 3.0 (7.8 $\pm$ 1.9) μ v.^{10,11}

MRI study

Data acquisition

Cranial MRI was performed on a 3T MR GE scanner (Signa HDxt; General Electric, Milwaukee, WI), using 16-channel head, neck, and spine coil (with inbuilt 12-channel head coil). In addition to routine imaging sequences of brain [axial T_1 , T_2 , fluid-attenuated inversion-recovery (FLAIR), and diffusion-weighted imaging] and orbit (coronal T_1 , T_2 , and FLAIR), DTI of both the optic nerves was done with the following parameters: a single shot axial echo planar imaging sequence using b value 1000 s mm⁻², slice thickness 1.6 mm, interslice gap 0, field of view 24 mm, voxel size of 1.09 $\times$ 1.09 $\times$ 1.6 mm, matrix 128 $\times$ 128, and 15 diffusion directions. Using the same sequence parameters, DTI was done in six age and gender matched healthy controls whose vitamin B12 levels were normal. After 3 months of treatment, the patients underwent follow-up MRI study using the same protocol. Controls were scanned once and used for initial and follow-up comparison with SACD patients. During the DTI sequence acquisition, patient was instructed to keep the eyes close and avoid moving the eyes.

Data fusion and region of interest analysis

The DTI data of individual patient was analyzed independently using FuncTool software – ADW4.4 GE workstation. The radiologist was unaware of the clinical details. After using a motion correction algorithm for the head motion and image distortion due to eddy currents FA, apparent diffusion co-efficient (ADC) and structural diffusion tensor maps were generated. For quantitative analysis, an oval region of interest (ROI) measuring 10–14 mm² was placed manually in the mid-length of the intra-orbital part of the visualized optic nerve on the color-coded tensor axial images (10 mm directly behind the globe of the eye) on both the optic nerves. FA, RD, AD, and MD were measured in ROI. For normalization, FA, MD, RD, and AD were calculated from the temporalis muscle in the same slice as selected for assessing optic nerve DTI parameters. ROIs were in the similar range for both optic nerve and muscle. The ROIs were placed on the most medial part of the temporalis muscle just outside the lateral orbital wall. The optic nerve to muscle ratio of the DTI parameters was calculated. The optic nerve diameter was also measured at the same level.

Treatment and follow up

Patients were treated with parenteral cobalamine 1000 μ g daily for 10 days followed by weekly for a month and thereafter monthly. Patients were followed up at 3 months and their clinical, VEP, and DTI studies were repeated.

Statistical analysis

Fractional anisotropy ratio (FAR), axial diffusivity ratio (ADR), radial diffusivity ratio (RDR), and mean diffusivity ratio (MDR) of optic nerve were compared between patients and

controls using Mann-Whitney U test. The initial and 3-month follow-up DTI and VEP parameters (P100 latency and amplitude) were compared using paired *t* test. The baseline FAR was correlated with age, gender, duration of illness, vitamin B12, mean corpuscular volume, hemoglobin, and latency and amplitude of P100 using Spearman or Karl-Pearson correlation test. Statistical analysis was done using SPSS v. 16 software and the variable having a two-tailed *p* value < 0.05 was considered significant.

RESULTS

Nine patients with SACD underwent DTI study of optic nerve. In three patients, the quality of images was suboptimal; therefore the results are based on six patients. The age of the patients ranged between 16 and 60 years and two were females. The duration of illness ranged between 1.5 and 36 months. All the patients had sensory ataxia and spasticity, four patients had associated neuropathy and one had forgetfulness (MMSE 21). All but one patient had anemia and mean corpuscular volume was increased in four patients. Serum homocysteine was increased in all and ranged between 16.6 and 62.3 $\mu\text{g dl}^{-1}$. Serum bilirubin was elevated (1.8 mg dl^{-1}) in one and lactate dehydrogenase (>450 U l^{-1}) in two patients. Four patients were vegetarian and none had any gastrointestinal disease or surgery. Antiparietal cell antibody was present in two and one patient had hypothyroidism.

Visual testing

Visual acuity, field of vision, and color vision were normal in all the patients. P100 latency of PSVEP was prolonged (range

106.8–126.9 ms) in five patients (10 eyes) bilaterally. The amplitude of P100 was reduced (<3 μV) in one patient (1 eye) only.

MRI study

Cranial MRI on T_1 , T_2 , FLAIR, and DWI did not reveal any signal changes in the optic nerve and brain parenchyma. The optic nerve diameter was not significantly different between patients and controls (3.62 ± 0.12 vs 3.61 ± 0.09 mm; $p = 0.83$). On DTI study, the mean FAR of the optic nerve was significantly reduced (mean + standard deviation, 1.94 ± 0.55 vs 2.81 ± 0.42 ; $p = 0.01$), and MDR (1.00 ± 0.04 vs 0.95 ± 0.01 ; $p = 0.01$) and RDR (0.96 ± 0.03 vs 0.89 ± 0.01 ; $p = 0.002$) were increased in the patients compared to the control. The FA value ranged between 0.37 and 0.54 in the patients; whereas in the controls it ranged between 0.55 and 0.70. The mean RD in the patients ranged between 4.1 and 4.3; whereas in the controls it ranged between 3.03 and 4.11. The AD and ADR values were not different between the patients and the controls (Figure 1).

Correlation

The baseline FAR correlated with P100 latency ($r = -0.88$; $p = 0.02$), but not with amplitude ($r = -0.28$; $p = 0.58$). The FAR also did not correlate with serum vitamin B12 level ($r = 0.23$; $p = 0.66$), age ($r = -0.27$; $p = 0.60$), duration of illness ($r = -0.13$; $p = 0.81$), hemoglobin level ($r = -0.44$; $p = 0.38$), mean corpuscular volume ($r = 0.32$; $p = 0.53$), and serum homocysteine level ($r = -0.01$; $p = 0.99$). The details of clinical, VEP, and DTI findings are presented in Table 1 and Figure 2.

Figure 1. Error bar diagram shows DTI findings in the patients with subacute combined degeneration and controls. AD, axial diffusivity; DTI, diffusion tensor imaging; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

Table 1. Clinical, hematological, biochemical, VEP, and DTI of optic nerves in the patients with subacute combined degeneration

Sl No	Age/sex	Duration (months)	B ₁₂	Syndrome	Hb		MCV		Hcy	VEP L/A		FA value	
					Before	After	Before	After		Right	Left	Right	Left
1	27/M	1.50	<150	Myeloneuropathy with cognitive	9.20	11.00	105.30	86.00	54.10	126.90/7.35	121.80/7.06	0.537	0.545
2	16/F	1.50	<150	Myelopathy	12.0	12.60	100.60	88.20	39.00	106.80/5.14	110.00/7.95	0.496	0.477
3	45/F	2.00	291	Myelopathy	11.6	12.10	86.00	84.00	45.43	96.50/14.60	97.00/14.50	0.528	0.519
4	42/M	1.50	111	Myeloneuropathy	10.7	11.80	112.00	75.90	21.23	112.00/6.16	112.00/6.21	0.563	0.576
5	60/M	36.00	>1200	Myeloneuropathy	13.6	13.10	96.00	88.50	16.63	106.80/4.1	112.00/3.9	0.405	0.327
6	40/M	2.00	234	Myelopathy	9.00	11.10	124.20	104.50	62.30	113.30/3.76	111.50/3.21	0.478	0.528

A, amplitude in μV ; B₁₂, serum vitamin B₁₂; DTI, diffusion tensor imaging; FA, fractional anisotropy; Hb, hemoglobin; Hcy, homocysteine; L, latency in ms; MCV, mean corpuscular volume; VEP, visual evoked potential.

Follow up

Clinical

At 3 months follow up, all the patients improved and were independent for activities of daily living. The patients with low MMSE also scored to 30. The sense of joint position though improved but remained impaired in all the patients. Loss of ankle reflex also persisted in all.

VEP and DTI

At 3 months follow up, P100 latency improved from baseline in all SADC patients although it remained prolonged in 5 eyes (111.76 ± 9.01 vs 105.34 ± 6.63 ms; $p = 0.02$; Figure 3). P100 amplitude however did not show significant change (6.84 ± 4.02 vs 6.44 ± 3.20 μV ; $p = 0.55$). The improvement in VEP paralleled with improvement in FAR (1.94 ± 0.55 vs 2.60 ± 0.57 ; $p = 0.04$) at 3 months compared to baseline. The details are presented in Table 2. The follow-up FAR in the patients were comparable to the baseline values in the controls (Figure 3).

DISCUSSION

In the present study, subclinical optic nerve dysfunction in the patients with SADC was evident on DTI and VEP studies. DTI of optic nerve revealed reduced FAR and increased MDR and RDR compared to the controls. FAR correlated with P100 latency but not with amplitude of VEP. Cobalamine treatment for 3 months resulted in normalization of FAR and improvement in P100 latency. The DTI findings of optic nerve and prolongation of P100 latency are consistent with myelin dysfunction. The prototype of optic nerve demyelination is observed in multiple sclerosis (MS) which is reported in 75–97% with definite MS.¹¹ Optic nerve demyelinating plaque in MS however is hyperintense on T₂ and FLAIR, and enhanced on gadolinium in about 64% patients with clinical optic neuritis.¹² In a study on 104 patients with MS, 38 had history of optic neuritis. Optic nerve DTI revealed reduced FA, which was most apparent in slowly progressive MS, and FA value dropped by 19% in the patients who had optic neuritis. The DTI abnormality in optic nerve correlated with thickness of retinal nerve fiber layer, loss of visual acuity, and history of optic neuritis. Optic nerve DTI though correlated with VEP in MS, but in the patients without optic neuritis did not reveal any change in DTI compared to the controls.⁸ In another study on 37 patients with unilateral optic neuritis, DTI was performed at 1, 3, and 6 months, and it correlated with outcome. AD was reduced at baseline, 1 and 3 months, and correlated with retinal nerve fiber layer thickness at 6 and 12 months. Reduction in AD in acute MS and at 1 month suggests axonal loss and poor visual outcome.¹³

Optic nerve demyelinating changes in MS are different from SADC, because SADC patients are usually asymptomatic, conventional MRI sequences do not reveal any signal abnormality, although half the patients have prolongation of P100 latency in VEP which normalizes following cobalamine treatment.^{1,5,7} These changes are consistent with myelin dysfunction of optic nerve which is further supported by reduced FAR and increased RDR and MDR on DTI with prolonged P100 latency on PSVEP in the present study. Moreover, optic nerve diameter in SADC patients was comparable to controls.

Figure 2. (A) FA, (B) ADC, and (C) structural diffusion tensor index maps obtained in a 27-year-old male with subacute combined degeneration. Regions of interest are placed in the middle part of both the optic nerves. (D) VEP of the same patient revealed prolonged P100 latency on both right and left eye (126.9 ms/121.8 ms) which normalized (102.5 ms/101.5 ms) at 3 months after treatment. ADC, apparent diffusion co-efficient; FA, fractional anisotropy; VEP, visual evoked potential.

DTI has also been studied in acute ischemic optic neuropathy. In a study on 26 patients with AION, DTI of optic nerve revealed increased ADC and orthogonal eigenvalue $\lambda_{\perp}$, and reduced FA value in the affected optic nerve compared to the unaffected nerve and controls. The VEP amplitude correlated with ADC and orthogonal eigenvalue $\lambda_{\perp}$ but not with FA. These findings are consistent with axonal and demyelinating changes in optic nerve.¹⁴

The studies in early 20th century, visual loss due to vitamin B12 deficiency has been described when cobalamine therapy was

not available.¹⁵ In the recent years, symptomatic visual dysfunction has not been reported which may be due to early detection and treatment.^{2,5-7} In a study on 10 patients with SACD, VEP revealed mild prolongation of P100 latency in 7 patients, suggesting axonal loss with secondary demyelination.⁷ Subclinical nature of VEP abnormalities and normalization following treatment suggests myelin dysfunction rather than axonal loss especially in the early stage. In our earlier study on 17 patients with VBDNS, VEP was abnormal in 10 and had moderate to severe prolongation in 8 patients. None of these patients had

Figure 3. The error bar diagram shows FA ratio at baseline and at 3 months following treatment in the patients with SACD in comparison to controls. FA, fractional anisotropy; SACD, subacute combined degeneration.

Table 2. Initial and 3-month follow-up study of VEP and DTI of optic nerve to muscle ratio in the patients of subacute combined degeneration

Mean values	Pre-treatment (N 12 eyes)	Post-treatment (N 12 eyes)	p value
VEP P100 latency ms	111.76 ± 9.01	105.34 ± 6.63	0.018
VEP P100 amplitude μ V	6.84 ± 4.02	6.44 ± 3.20	0.55
FA ratio	1.94 ± 0.55	2.60 ± 0.57	0.04
MD ratio	1.00 ± 0.04	0.99 ± 0.05	0.82
RD ratio	0.96 ± 0.03	0.93 ± 0.04	0.10
AD ratio	1.07 ± 0.06	0.98 ± 0.22	0.34
ADC ratio	1.01 ± 0.16	0.98 ± 0.22	0.79

AD, axial diffusivity; ADC, apparent diffusion co-efficient; FA, fractional anisotropy; DTI, diffusion tensor imaging; MD, mean diffusivity; RD, radial diffusivity; VEP, visual evoked potential.

clinical visual abnormality and VEP improved following cobalamin treatment. Amplitude of P100 waveform in these patients was not significantly reduced.⁵ Similar demyelinating changes in VEP in SACD have also been reported by others.¹⁶ Autopsy study revealed axonal degeneration with patchy demyelination of optic nerve and optic tract.¹⁷ Since autopsy studies are based on terminal patients with pernicious anemia, hence may not be consistent with the VEP and DTI results in our patients.

This study is limited by small sample size. Performing DTI study of optic nerve is technically challenging because of small size of optic nerve, mobility, high signal intensity from surrounding CSF and orbital fat, close proximity to the paranasal sinuses, and surrounding arterial and venous pulsations. We have not used

the CSF and fat suppression technique. We had to exclude three patients because of technical reason.

CONCLUSION

DTI and VEP studies suggest subclinical myelin dysfunction of optic nerve in the patients with SACD which improve following cobalamin treatment.

ACKNOWLEDGEMENTS

We thank Mr Shakti Kumar for secretarial help.

ETHICS APPROVAL

This study was approved by Institute Ethics Committee, SGP GIMS, Lucknow INDIA.

REFERENCES

1. Kalita J, Misra UK. Benefit of vitamin B-12 supplementation in asymptomatic elderly: a matter of endpoints. *Am J Clin Nutr* 2015; **102**: 529–30. doi: <https://doi.org/10.3945/ajcn.115.118380>
2. Kalita J, Misra UK. Vitamin B12 deficiency neurological syndromes: correlation of clinical, MRI and cognitive evoked potential. *J Neurol* 2008; **255**: 353–9. doi: <https://doi.org/10.1007/s00415-008-0660-x>
3. Misra UK, Kalita J, Das A. Vitamin B12 deficiency neurological syndromes: a clinical, MRI and electrodiagnostic study. *Electromyogr Clin Neurophysiol* 2003; **43**: 57–64.
4. Heaton EB, Savage DG, Brust JC, Garrett TJ, Lindenbaum J. Neurologic aspects of cobalamin deficiency. *Medicine* 1991; **70**: 229–45. doi: <https://doi.org/10.1097/00005792-199107000-00001>
5. Pandey S, Kalita J, Misra UK. A sequential study of visual evoked potential in patients with vitamin B12 deficiency neurological syndrome. *Clin Neurophysiol* 2004; **115**: 914–8. doi: <https://doi.org/10.1016/j.clinph.2003.11.013>
6. Misra UK, Kalita J. Comparison of clinical and electrodiagnostic features in B12 deficiency neurological syndromes with and without antiparietal cell antibodies. *Postgrad Med J* 2007; **83**: 124–7. doi: <https://doi.org/10.1136/pgmj.2006.048132>
7. Fine EJ, Soria E, Paroski MW, Petryk D, Thomasula L. The neurophysiological profile of vitamin B12 deficiency. *Muscle Nerve* 1990; **13**: 158–64. doi: <https://doi.org/10.1002/mus.880130213>
8. Smith SA, Williams ZR, Ratchford JN, Newsome SD, Farrell SK, Farrell JA, et al. Diffusion tensor imaging of the optic nerve in multiple sclerosis: association with retinal damage and visual disability. *AJNR Am J Neuroradiol* 2011; **32**: 1662–8. doi: <https://doi.org/10.3174/ajnr.A2574>
9. Hemmer B, Glocker FX, Schumacher M, Deuschl G, Lücking CH. Subacute combined degeneration: clinical, electrophysiological, and magnetic resonance imaging findings. *J Neurol Neurosurg Psychiatry* 1998; **65**: 822–7. doi: <https://doi.org/10.1136/jnnp.65.6.822>
10. Celesia GG, Bodis-Wollner I, Chatrian GE, Harding GF, Sokol S, Spekrijse H. Recommended standards for electroretinograms and visual evoked potentials. Report of an IFCN committee. *Electroenceph Clin Neurophysiol* 1993; **87**: 421–36. doi: [https://doi.org/10.1016/0013-4694\(93\)90157-Q](https://doi.org/10.1016/0013-4694(93)90157-Q)
11. Misra UK, Kalita J. Visual evoked potential. In: Misra U. K, Kalita J, eds. *Clinical Neurophysiology*. Elsevier, a division of Reed Elsevier India Pvt Ltd., New Delhi, India; 2014. pp. 283–9.
12. Merandi SF, Kudryk BT, Murtagh FR, Arrington JA. Contrast-enhanced MR imaging of optic nerve lesions in patients with acute optic neuritis. *AJNR Am J Neuroradiol* 1991; **12**: 923–6.
13. van der Walt A, Kolbe SC, Wang YE, Klistorner A, Shuey N, Ahmadi G, et al. Optic nerve diffusion tensor imaging after acute optic neuritis predicts axonal and visual outcomes. *PLoS One* 2013; **8**: e83825. doi: <https://doi.org/10.1371/journal.pone.0083825>
14. Wang MY, Qi PH, Shi DP. Diffusion tensor imaging of the optic nerve in subacute anterior ischemic optic neuropathy at 3T. *AJNR Am J Neuroradiol* 2011; **32**: 1188–94. doi: <https://doi.org/10.3174/ajnr.A2487>
15. Cohan H. Optic atrophy as the presenting sign in pernicious anemia. *Lancet* 2: 1202–3.
16. Troncoso J, Mancall EL, Schatz NJ. Visual evoked responses in pernicious anemia. *Arch Neurol* 1979; **36**: 168–9. doi: <https://doi.org/10.1001/archneur.1979.00500390086010>
17. Agamanolis DP, Chester EM, Victor M, Kark JA, Hines JD, Harris JW. Neuropathology of experimental vitamin B12 deficiency in monkeys. *Neurology* 1976; **26**: 905–14. doi: <https://doi.org/10.1212/WNL.26.10.905>