DOI:10.14744/bej.2018.41736
Beyoglu Eye J 2018;3(2): 52-57

Original Article

Distinguishing Non-Arteritic Ischemic Optic Neuropathy from optic Neuritis with Serum Vitamin B12, Ferritin and Folic Acid Level

Hande Guclu,[1] Zeynep Banu Doganlar[2]

[1]Department of Ophthalmology, Trakya University, Edirne, Turkey
[2]Department of Medical Biology, Trakya University, Edirne, Turkey

Abstract

Objectives: The aim of this study was to compare the levels of vitamin B12 (Vit B12), folic acid, serum ferritin, serum iron, and total iron binding capacity (TIBC) in patients with optic neuritis (ON) and non-arteritic ischemic optic neuropathy (NAION). It was hoped to determine whether these simple and inexpensive laboratory measurements are indicative for making a distinction between ON and NAION.

Methods: In this retrospective study, the data of patients who were diagnosed with ON and NAION between September 2005 and December 2016 were reviewed. In all, 42 patients with NAION, 70 patients with ON, and 76 members of a control group, a total of 188, were enrolled in the study. All of the participants underwent a full ophthalmological examination and complete physical examination, including a detailed medical history and blood count and evaluation of the biochemical parameters, serum ferritin, Vit B12, folic acid, serum iron, and TIBC.

Results: The mean serum ferritin level was 236±458.4 ng/mL for the NAION patients, 32.8±34.6 ng/mL for the ON patients, and 76.1±84.6 ng/mL for the control group. The mean serum Vit B12 level was 478±306.3 pg/mL for the NAION patients, 291.7±136.9 pg/mL for the ON patients, and 417.1±163.4 pg/mL for the control group. The mean serum folic acid level was 11.4±6.3 ng/mL for the NAION patients, 6.6±2.7 ng/mL for the ON patients, and 14.5±5.2 ng/mL for the control group.

Conclusion: A higher serum ferritin level was significantly associated with NAION, and lower Vit B12 and folic acid was associated with ON patients. Serum ferritin, Vit B12, and folic acid measurements could be a useful method for distinguishing between NAION and ON before using complicated and invasive methods.

Keywords: Folic acid, non-arteritic ischemic optic neuropathy, optic neuritis, serum ferritin, vitamin B12.

Introduction

Optic neuritis (ON) and non-arteritic ischemic optic neuropathy (NAION) are the most common acute optic neuropathies of adults, and they can be difficult to differentiate (1). Distinguishing between these 2 diseases is usually possible using multiple findings, such as pain and typical visual field deficit, as well as with some characteristic clues related to the nature of the diseases, such as age of onset, degree of visual healing, and association with systemic diseases (diabetes, hypertension, multiple sclerosis, neuromyelitis optica) (1-3).

Vitamin B12 (Vit B12) is very important in terms of peripheral and central nervous system functions. A deficiency of Vit B12 has been found to be related to multiple neuro-ophthalmological conditions, including optic neuropathy, bilateral abducens palsy, internuclear ophthalmolplegia, and nystagmus (4-5). Folic acid is required for the formation of tetrahydrofolate, which plays a key role in the detoxification of formate (6, 7). In the case of a folic acid deficiency, formate can block mitochondrial oxidative phosphorylation by inhibiting cytochrome oxidase (6, 8). A folic acid deficiency has been demonstrated to be associated with optic neu-

Address for correspondence: Hande Guclu, MD. Department of Ophthalmology, Trakya University, Edirne, Turkey
Phone: +90 284 235 76 41 **E-mail:** hande83_toprakci@hotmail.com
Submitted Date: March 31, 2018 **Accepted Date:** May 09, 2018 **Available Online Date:** June 10, 2018

ropathy in multiple studies (7-9). Serum ferritin is an inflammatory marker, which has been reported to be related to the pathogenesis of some diseases, such as diabetes, cancer, heart diseases, neurodegenerative diseases, and age-related macular degeneration (10, 11). Iron plays an important role in cell functions, such as oxygen transport, cell division, mitochondrial adenosine triphosphate formation, and myelin production (12, 13).

Multifocal visual evoked potential, ultrasonography, diffusion-weighted magnetic resonance imaging, visual field tests, fluorescein angiography, optical coherence tomography, and laser speckle flowgraphy have been reported to be helpful in distinguishing ON from NAION in multiple studies. (1, 3, 14-20). The aim of this study was to demonstrate and compare the levels of Vit B12, folic acid, serum ferritin, serum iron, and total iron binding capacity (TIBC) in patients with ON and NAION. The objective was to determine whether these simple and inexpensive laboratory methods could be used to differentiate between ON and NAION.

Methods

In this retrospective study, the data of patients who were diagnosed with NAION and ON between September 2005 and December 2016 were reviewed. A total of 188 participants: 42 patients with NAION, 70 patients with ON, and 76 individuals in a control group, were enrolled in the study. All of the patients underwent a complete neuro-ophthalmological examination, including a detailed medical and ocular history, best corrected visual acuity assessment, pupillary examination, color vision evaluation (Ishihara color vision test), intraocular pressure measurement, automated visual field examination with a Humphrey field analyzer (HFA II 750; Zeiss Medical Technology/Carl Zeiss Meditec AG, Jena, Germany), and slit lamp and fundus examination. In addition, cranial magnetic resonance imaging and visual evoked potential measurements were also performed. The criteria used for NAION were: unilateral disc swelling accompanied by a clinical characterization of NAION, a lack of pain, no improvement in visual acuity in the first month of follow-up, and altitudinal visual field deficit, The criteria used to define ON were: unilateral decreased visual acuity accompanied by or without unilateral disc swelling, pain with ocular movement, and improvement of visual acuity in the first month of follow-up. Exclusion criteria were a positive temporal artery biopsy; a history of any ocular pathology or ocular surgery; the presence of decreased visual acuity of more than 15 days; the presence of inflammatory, rheumatological, or infectious disease; a high sedimentation rate; malignancy; liver disease; kidney disease; hematological disease; coronary artery disease; use of any medications that could cause hematological effects, such as antiaggregants, oral contraceptives, steroids; or the use of any medications that could have an anti-inflammatory effect, such as angiotensin-converting enzyme inhibitors, statins, and beta blockers. Venous blood samples were taken at the time of diagnosis of the NAION and ON patients.

The blood count parameters were measured using a Sysmex XE-2100 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Serum Vit B12 and folic acid levels were measured with a Centaur XP immunoassay analyzer using original kits (Siemens Healthcare Diagnostics, Erlangen, Germany). Serum iron and unsaturated iron binding capacity (UIBC) levels were measured using an Architect C16000 clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA) using commercial kits (Archem Diagnostic Ind. Ltd., Istanbul, Turkey) and the TIBC level of patients was calculated according to the following formula: TIBC= Iron + UIBC.

Statistical Analysis

The results were shown as mean±SD, and categorical variable results were presented as a number (percentage). The Kruskal-Wallis test was used for a comparison of age, ferritin, VitB12, folic acid, TIBC, serum iron, hemoglobin (Hb), and hematocrit (Htc) between groups, and then the Mann-Whitney U test with the Bonferroni adjustment was used for multiple comparisons when a significant difference was found. Receiver operating characteristic curve (ROC) analysis was used to assess the ION, ON, and control groups. Area under the curve (AUC) values and cut-off points were calculated, and then sensitivity and specificity values at these points were calculated. Moreover, discriminant analysis was used to assess the groups based on the ferritin, Vit B12, and folic acid levels. A p value <0.05 was considered statistically significant. IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis.

Results

In this retrospective study, there were 42 ION patients, 70 ON patients, and 76 healthy controls. The mean age was 60.6±16.5 years for the ION patients, 37.3±8.5 years for the ON patients, and 49.1±16.6 years in the control group. There were statistically significant differences between the ages of the groups (p<0.001).

The serum ferritin level was greater in the ION patients than in the ON patients or the control group. There were statistically significant differences between the groups (p=0.03). Vitamin B12 and folic acid levels were lower in the ON group than in the other groups and the difference was statistically significant. (p=0.001, p<0.001). The serum iron level was lower in the ION patients, but Hb and Htc levels were lower in the ON patients and the differences were

statistically significant (p<0.001, p=0.009, p=0.017) (Table 1). Significant cut-off values were determined to discriminate NAION from ON: >26.3 for ferritin (sensitivity 65.8%, specificity 74.3%), >306 for VitB12 (sensitivity 57.1%, specificity 74.3%), and >11.2 for folic acid (sensitivity 47.6%, specificity 99.0%). Similarly, significant cut-off values were determined to classify the ON group and the control group: ≤26.3 for ferritin (sensitivity 74.3%, specificity 68.4%), ≤306 for VitB12 (sensitivity 74.3%, specificity 78.9%), and ≤11.3 for folic acid (sensitivity 100.0%, specificity 65.8%). The greatest AUC value was determined for folic acid (AUC=0.910; p<0.001) in the analysis of the ON group and the control group. However, cut-off values were not sufficient to distinguish the NAION group from the controls (Table 2, Fig. 1). The classification function coefficients of Fisher's linear discriminant functions are provided in Table 3 and the results are shown in Table 4. According to the discriminant analysis, overall, 66.0% of the cases were correctly classified in their original group: the individual results were 28.6% in the NAION group, 85.7% in the ON group, and 68.4% in the control group.

Table 1. Laboratory results of non-arteritic ischemic optic neuropathy patients, optic neuritis patients, and controls

	NAION (n=42)	ON (n=70)	Control (n=76)	p
Ferritin	236±458.4	32.8±34.6	76.1±84.6	0.030
Vit B12	478±306.3	291.7±136.9	417.1±163.4	0.001
Folic Acid	11.4±6.3	6.6±2.7	14.5±5.2	<0.001
TIBC	352.1±69.7	342.7±68.4	324.3±67.7	0.257
Serum iron	57.9±41.4	65.9±34.1	88.8±33.1	<0.001
Hb	13.8±5.1	12.6±1.6	13.6±1.3	0.009
Htc	46.8±34.8	38.1±4.6	41.1±3.6	0.017

Hb: hemoglobin; Htc: hematocrit; NAION: non-arteritic ischemic optic neuropathy; ON: optic neuritis; TIBC: total iron binding capacity; Vit B12: vitamin B12.

Discussion

The results of this study demonstrated that there was a higher serum ferritin level in the ION group compared with the ON group and the controls. None of the biomarkers of Vit B12, folic acid, serum iron, TIB, Hb, or Htc was related to a diagnosis of ION. The serum folic acid and Vit B12 levels were found to be significantly lower in the ON group compared with the ION group and the control group.

NAION is an acute ischemic event resulting from interruption of the vascular supply to the optic nerve (1, 21, 22). Diabetes, hypertension, and atherosclerosis are known to increase the development of this ischemic disease (21). Inflammation has been demonstrated to be related to NAION

Table 3. Classification function coefficients of Fisher's linear discriminant analysis

	Groups		
	NAION	ON	Control
Ferritin	0.00016	-0.00236	-0.00443
Vit B12	0.01012	0.00657	0.00822
Folic acid	0.40168	0.26600	0.62811
(Constant)	-5.827	-2.899	-7.188

NAION: non-arteritic ischemic optic neuropathy; ON: optic neuritis; Vit B12: Vitamin B12.

Table 2. Diagnostic values of laboratory results of non-arteritic ischemic optic neuropathy patients, optic neuritis patients, and controls

	Cut-off	AUC	P	Sensitivity	Specificity	LR+	LR-
NAION vs ON							
Ferritin	>26.3	0.665	0.050	65.8	74.3	2.59	0.45
Vit B12	>306	0.668	0.030	57.1	74.3	2.22	0.58
Folic Acid	>11.2	0.732	0.002	47.6	99.0		
NAION vs Control							
Ferritin	>155.9	0.543	0.614	33.3	89.5	3.17	0.75
Vit B12	≤308	0.524	0.790	47.6	78.9	2.26	0.66
Folic Acid	≤9.39	0.654	0.054	47.6	84.2	3.02	0.62
ON vs Control							
Ferritin	≤26.3	0.662	0.014	74.3	68.4	2.35	0.38
Vit B12	≤306	0.774	<0.001	74.3	78.9	3.53	0.33
Folic Acid	≤11.3	0.910	<0.001	100.0	65.8	2.92	0.00

AUC: Area under the curve; LR +: likelihood ratio positive; LR-: likelihood ratio negative; NAION: non-arteritic ischemic optic neuropathy; ON: optic neuritis; Vit B12: vitamin B12.

Table 4. Results of discriminant analysis

		Predicted group membership			Total
		NAION	ON	Control	
Original group membership					
n					
	NAION	12	16	14	42
	ON	6	60	4	70
	Control	12	12	52	76
%					
	NAION	28.6	38.1	33.3	100.0
	ON	8.6	85.7	5.7	100.0
	Control	15.8	15.8	68.4	100.0

NAION: non arteritic ischemic optic neuropathy, ON: optic neuritis.

in multiple studies (23, 24). Bernstein et al. (24) reported findings of early inflammatory alterations and stressed that inflammatory-based treatments could be useful in the early phase of the disease (24). Salgado et al. (23) suggested that NAION-related optic nerve damage is associated with thrombosis or hypoperfusion. These 2 conditions result in tissue edema in the sheath of optic nerve, which causes a compartment syndrome. The damage mechanism is similar to central nervous system white matter stroke. Bernstein et al. (25) claimed that NAION is a stroke of the optic nerve. Middle cerebral artery occlusion leads to cortical cellular inflammation; therefore, NAION could result in inflammation similar to central nervous system strokes (23, 26). Serum ferritin is a well-known marker of iron body stores, iron-related oxidative stress, and inflammation (10, 27, 28). Gye et al. (10) observed higher serum ferritin levels in male glaucoma patients; however, other iron-related markers, such as iron, transferrin, and TIBC were not associated with glaucoma. Similarly, in this study, we detected a higher serum ferritin level in the NAION patients, but we found no relationship between the other biomarkers of serum iron, TIBC, Vit B12, and folic acid level with NAION. We theorized that NAION's inflammatory character could be a result of this higher ferritin level and that this laboratory test could be useful to distinguish between NAION and ON in older age patients. In addition, the increased incidence of atherosclerosis and diabetes that are accompanied by inflammation in NAION patients may have contributed to the detection of high ferritin levels in the NAION patients.

ON is also an inflammatory demyelinating disease of the myelin sheath surrounding the retinal ganglion cell axons of the optic nerve (29). ON may be immune-mediated with known antibodies, such as neuromyelitis optica, or associat-

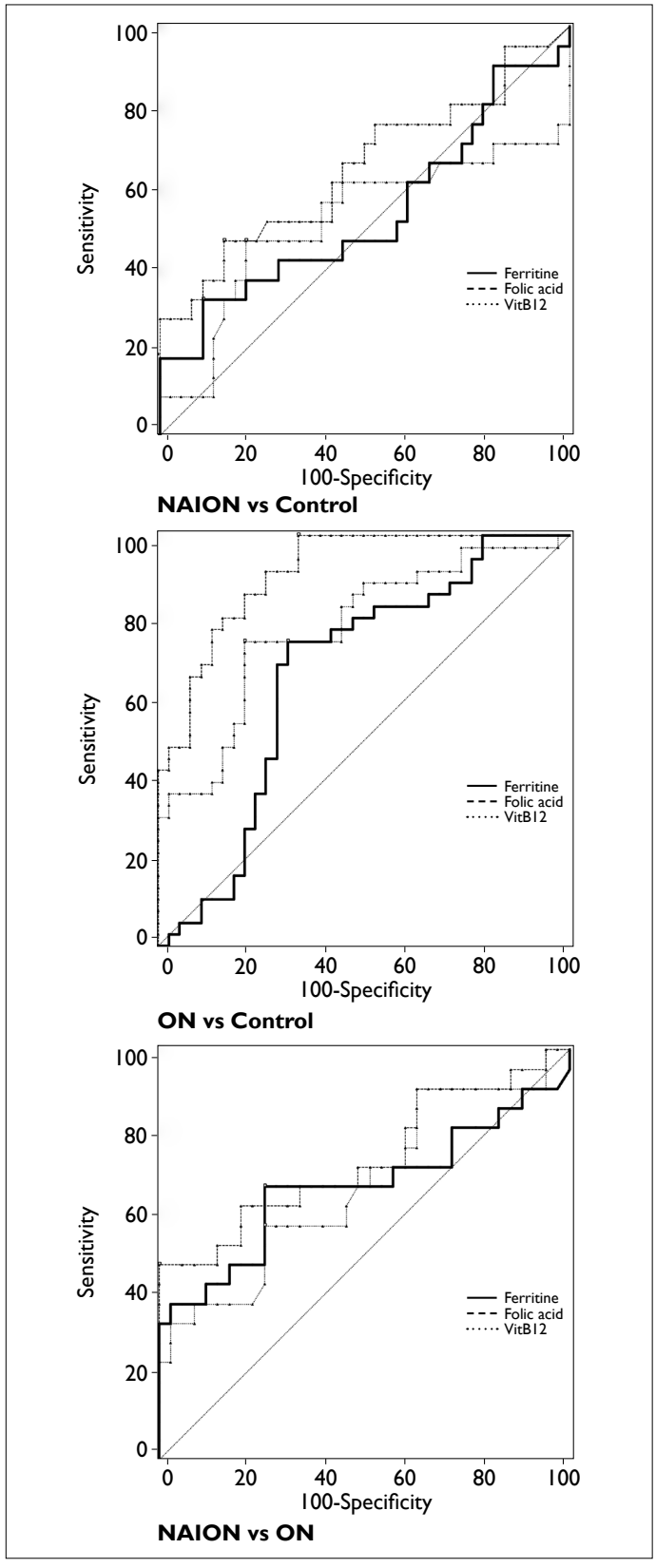

Figure 3. ROC analysis for discriminating the NAION, ON, and control groups.

NAION: non-arteritic ischemic optic neuropathy; ON: optic neuritis; Vit B12: Vitamin B12.

ed with other systemic diseases, like multiple sclerosis (29). Vit B12 is a coenzyme in multiple cell reactions, including DNA synthesis and folate metabolism (30). Vit B12 deficiency is a well-known cause of optic neuropathy that occurs due to insufficient myelin production, resulting in axonal degeneration and demyelination (31). In the present study, we found significantly lower Vit B12 levels in ON patients; however, the mean Vit B12 level was within normal limits. In the literature, Vit B12 concentrations have been reported as normal at ≥200 pmol/L, borderline when 150–200 pmol/L, and low when the level is <150 pmol/L (32). It was also demonstrated in a previous study that 42.6% of aquaporin-4 antibody-positive neuromyelitis optica patients had a Vit B12 level of <300 pmol/L.

Folic acid concentration is important for myelin basic protein methylation, which is required for nerve myelination and nerve function (8, 33). Multiple studies have reported that folic acid deficiency could lead to nutritional optic neuropathy (6-8). We found significantly lower folic acid levels in the ON patients; however, similar to the VitB12 level, the folic acid level was within normal limits and was not at the level of deficiency. In a previous study, the folic acid level was classified as normal at ≥7 nmol/L, borderline when 5-7 nmol/L, and low <5 nmol/L (32).

Although older patients have an increased risk of Vit B12 and folic acid deficiency (32), interestingly, in the present study, the ON patients (who were younger than the NAION patients and the controls) had lower Vit B12 and folic acid levels. Also, in our study, significant cut-off values were determined to discriminate ON from the controls: ≤306 for Vit B12 and ≤11.3 for folic acid. We suggest that lower (but not of deficiency level) folic acid and Vit B12 levels could be related to ON. The higher serum ferritin levels observed in NAION patients could be associated with that additional risks for inflammation, such diabetes and atherosclerosis in comparison with the younger ON patients. Yet it is noteworthy that more aggressive local inflammation of the optic nerve would typically be seen in NAION patients than in cases of ON aside from the relationship to other systemic diseases.

The greatest limitation of the present study is its retrospective nature, which meant that we could not measure methylmalonic acid and homocysteine levels, 2 markers that are important for Vit B12 and folic acid deficiency. However, to the best of our knowledge, this is the first study to analyze the use of serum ferritin, Vit B12, and folic acid levels to distinguish between 2 diseases that can be difficult to differentiate: NAION and ON.

In conclusion, instead of using complicated, invasive, and expensive methods, measurement of serum ferritin, Vit B12, and folic acid levels are useful, inexpensive, noninvasive, and effective laboratory methods for distinguishing NAION from ON. Further studies will reveal additional relationships between the levels of serum ferritin, Vit B12, and folic acid and NAION, ON, and treatment.

Disclosures

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (HG, ZBD); preparation and review of the study (HG, ZBD); data collection (HG); and statistical analysis (ZBD).

References

1. Erlich-Malona N, Mendoza-Santiesteban CE, Hedges TR 3rd, Patel N, Monaco C, Cole E. Distinguishing ischaemic optic neuropathy from optic neuritis by ganglion cell analysis.Acta Ophthalmol 2016;94:721–6. [CrossRef]
2. Rizzo JF, Lessell S. Optic neuritis and ischaemic optic neuropathy. Overlapping clinical profiles. Arch Ophthalmol 1991;109:1668–72.
3. Kim MK, Kim US. Analysis of fundus photography and fluorescein angiography in nonarteritic anterior ıschemic optic neuropathy and optic neuritis. Korean J Ophthalmol 2016;30:289–94.
4. Pineles SL, Avery RA, Liu GT. Vitamin B12 optic neuropathy in autism. Pediatrics 2010;126:e967–70. [CrossRef]
5. Akdal G, Yener GG, Ada E, Halmagyi GM. Eye movement disorders in vitamin B12 deficiency: two new cases and a review of the literature. Eur J Neurol 2007;14:1170–2. [CrossRef]
6. Hsu CT, Miller NR, Wray ML. Optic neuropathy from folic acid deficiency without alcohol abuse. Ophthalmologica 2002;216:65–7. [CrossRef]
7. Golnik KC, Schaible ER. Folate-responsive optic neuropathy. J Neuroophthalmol 1994;14:163–9. [CrossRef]
8. Hodson KE, Bowman RJ, Mafwiri M, Wood M, Mhoro V, Cox SE. Low folate status and indoor pollution are risk factors for endemic optic neuropathy in Tanzania. Br J Ophthalmol 2011;95:1361–4. [CrossRef]
9. De Silva P, Jayamanne G, Bolton R. Folic acid deficiency optic neuropathy: a case report. J Med Case Rep 2008;2:299.
10. Gye HJ, Kim JM, Yoo C, Shim SH, Sung KC, Lee MY, et al. Relationship between high serum ferritin level and glaucoma in a South Korean population: the Kangbuk Samsung health study. Br J Ophthalmol 2016;100:1703–7. [CrossRef]
11. Hahn P, Milam AH, Dunaief JL. Maculas affected by age-related macular degeneration contain increased chelatable iron in the retinal pigment epithelium and Bruch's membrane. Arch Ophthalmol 2003;121:1099–105. [CrossRef]
12. Acir NO, Dadaci Z,Cetiner F, Yildiz M, Alptekin H, Borazan M. Evaluation of the peripapillary retinal nerve fiber layer and ganglion cell-inner plexiform layer measurements in patients with iron deficiency anemia with optical coherence tomography. Cutan Ocul Toxicol 2016;35:131–6. [CrossRef]
13. Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. Glia 2009;57:467–78. [CrossRef]

14. Chen JJ, AbouChehade JE, Lezzi R Jr, Leavitt JA, Kardon RH. Optical coherence angiographic demonstration of retinal changes from chronic optic neuropathies. Neuroophthalmology 2017;41:76–83 [CrossRef]
15. Giambene B, Virgili G, Menchini U. Retinal nerve fiber layer thickness by Stratus and Cirrus OCT in retrobulbar optic neuritis and nonarteritic ischemic optic neuropathy. Eur J Ophthalmol 2017;27:80–5. [CrossRef]
16. Jayaraman M, Gandhi Ra, Ravi P, Sen P. Multifocal visual evoked potential in optic neuritis, ischemic optic neuropathy and compressive optic neuropathy. Indian J OPhthalmol 2014;62:299–304.
17. Maekubo T, Chuman H, Nao-I N. Laser speckle flowgraphy for differentiating between nonarteritic ischemic optic neuropathy and anterior optic neuritis. Jpn J Ophthalmol 2013;57:385–90.
18. Dehghani A, Giti M, Akhlaghi MR, KArami M, Salehi F. Ultrasonography in distinguishing optic neuritis from nonarteritic anterior ischemic optic neuropathy. Adv Biomed Res 2012;1:3.
19. He M, Cestari D, Cunnane MB, Rizzo JF 3rd. The use of diffusion MRI in ischemic optic neuropathy and optic neuritis. Semin Ophthalmol 2010;25:225–32. [CrossRef]
20. Traversi C, Bainciardi G, Tasciotti A, Berni E, Nuti E, Luzi P, et al. Fractal analysis of fluoroangiographic patterns in anterior ischaemic optic neuropathy and optic neuritis: a pilot study. Clin Exp Ophthalmol 2008;36:323–8. [CrossRef]
21. Kesler A, Irge D, Rogowski D, Bornstein N, Berliner S, Shapira I, et al. High sensitivity C recative protein measurements in patients with non-arteritic anterior ischaemic optic neuropathy: a clue to the presence of a microinflammatory response. Acta Ophthalmol 2009;87:216–21. [CrossRef]
22. Hayreh SS Anterior ischemic optic neuropathy. Arch Neurol 1981; 38:675-78 [CrossRef]
23. Salgado C, Vilson F, Miller NR, Bernstein SL. Cellular inflammation in nonarteritic anterior ischemic optic neuropathy and its primate model. Arch Ophthalmol 2011;129:1583–91. [CrossRef]
24. Bernstein SL, Johnson MA, Miller NR. Nonarteritic anterior ischemic optic neuropathy (NAION) and its experimental models. Prog Retina Eye Res 2011;30:167–87. [CrossRef]
25. Bernstein SL, Guo Y, Kelman SE, Flower RW, Johnson MA. Functional and cellular responses in a novel rodent model of anterior ischemic optic neuropathy. Invest Ophthalmol Vis Sci 2003;44:4153–62. [CrossRef]
26. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, et al. Temporal and spatial Dynamics of cerebral immune cell accumulation in stroke. Stroke 2009;40:1849–57.
27. Yeap BB, Divitini ML, Gunton JE, Olynyk JK, Beilby JP, McQuillan B, et al. Higher ferritin levels, but not serum iron or transferrin saturation, are associated with Type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis. Clin Endocrinol 2015;82:525–32. [CrossRef]
28. Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics 2014;6:748–73. [CrossRef]
29. Boudreault K, Durand ML, Rizzo JF3rd. Investigation-directed approach to inflammatory optic neuropathies. Semin Ophthalmol 2016;31:117–30. [CrossRef]
30. Pan Y, Liu Y, Guo H, Jabir MS, Liu X, Cui W, et al. Associations between Folate and Vitamin B12 levels and inflammatory bowel disease: a meta-analysis. Nutrients 2017;9.pii:E382. [CrossRef]
31. Gökçe Çokal B, Güneş HN, Güler SK, Yoldaş TK. Visual and somotosensory evoked potentials in asymptomatic patients with vitamin B12 deficiency. Eur Rev Med Pharmacol Sci 2016;20:4525–9.
32. Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, et al. Screening for vitamin B-12 and folate deficiency in older persons. Am J Clin Nutr 2003;77:1241–7. [CrossRef]
33. Scott JM. Folate and vitamin B12. Proc Nutr Soc 1999;58:441–8. [CrossRef]