



Is the clinical course of non-arteritic ischemic optic neuropathy associated with oxidative damage and the dynamics of the antioxidant response?

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Abstract

Purpose Oxidative stress is known to be a decisive factor in the wide etiopathogenesis of optic neuropathy. This study aimed to comprehensively evaluate the interaction of optic neuropathy's clinical course with systemic oxidative damage and antioxidant response dynamics in a large series.

Methods This case-controlled clinical study included 33 non-arteritic anterior ischemic optic neuropathy (NAION) patients and 32 healthy individuals. Extensive systemic oxidation profiles were statistically compared between the two groups, and correlations between the clinical and biochemical data in the study group were analyzed.

Results Vitamin E and malondialdehyde (MDA) levels were significantly higher in the study group. Significant correlations were observed in the analyses between clinical findings and oxidative stress parameters. Correlations between vitamin E and intraocular pressure (IOP), between B₁₂ and cup-to-disk ratio (c/d), between antioxidant glutathione and superoxide dismutase (SOD) enzyme systems, and between uric acid (UA) and age were found to be very significant. As significant correlations were found in either clinical and biochemical data or in oxidative stress parameters, correlations between vitamin E and cholesterol, MDA were found to be very significant.

Conclusions This study not only supplies significant information regarding oxidative damage and antioxidant response in NAION, but also points out the specific interactions of neuromodulators, like vitamin E, in intracellular signaling pathways and regulation

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mechanisms. A better reading of these connections may help improve diagnosis, follow-ups and treatment criteria and strategies.

Keywords 8-hydroxy-2'-deoxyguanosine · Non-arteritic anterior ischemic optic neuropathy · Stress · Oxidative · Vitamin B₁₂ · Vitamin E

Introduction

Non-arteritic anterior ischemic optic neuropathy (NAION) is one of the major causes of vision loss in the elderly, and oxidative stress and apoptosis of the retinal ganglion cells play a role in the pathophysiology of the neurological damage [1]. The entire nerve system is rich in lipids and extremely sensitive to oxidative damage [2]. Oxidation degradation products are the sign of oxidative damage in serum and tissue; in lipids, the product is MDA [3]. Molecules, such as UA, and vitamins A, C, E remain stable after binding with ionized oxygen, which enables them to exert antioxidant action. Vitamins A and E are liposoluble vitamins. Such compounds, once formed, block the chain reaction by transferring their ions to the systems in which SOD and glutathione peroxidase are rate-limiting enzymes [4]. The DNA damage due to oxidative stress can be determined specifically from urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels [4, 5]. Elevated serum MDA levels were reported in cataract patients which implies that an ocular pathology may cause signs of oxidative stress in serum [6]. In an observation study, no antioxidant but folate was found to be deficient in prisoners with optic neuropathy [7]. Vitamin B₁₂, on the other hand, is another compound which is of vital importance for neurological damage and required for methylmalonic acid (MMA) metabolism [8]. The aim of this study was to comprehensively evaluate the interactions between clinical optic neuropathy findings, systemic oxidative damage, and antioxidant response dynamics as an exploratory analysis to assess for potential interactions between clinical and biochemical variables.

Methods

Thirty-three patients diagnosed and followed-up in Izmir Dokuz Eylül University Neuroophthalmology

Clinic as NAION, and 32 healthy individuals were included in this study. Patients with no known ocular or systemic concomitant disorders, neurological diseases, recent surgeries, or antioxidant and/or corticosteroid usage were selected. Biochemical and clinic findings were noted. For oxidative stress analysis, plasma MDA as a lipid oxidation product and urine 8-OHdG as an oxidatively induced DNA damage product were assessed. Along with antioxidants ceruloplasmin (Cp), UA, and vitamins A, B₁₂, C, E and folate, antioxidant systems SOD, total (tGSH) and reduced glutathione (GSH) were also evaluated. Care was taken to obtain both the samples and clinical data at the same day, and few out-of-range values in certain parameters—due to errors occurring during and before measurements, were excluded. As extensive systemic oxidation profiles statistically compared the two groups, correlations between clinical and biochemical data in the study group were also statistically analyzed.

Clinical examinations

The patients underwent a detailed ophthalmic examination, including mean logarithm of the minimum angle of resolution-best corrected visual acuity (LogMAR-BCVA), color vision (Ishihara test) and IOP with PASCAL Dynamic Contour Tonometry (Nidek inc., Fremont, CA, USA) measurements. Patients whose IOPs were higher than 21 mm/Hg were excluded. Posterior segment analyses with optical coherence tomography (OCT) were taken with a spectral domain OCT (Spectralis; Heidelberg Engineering Ltd, Heidelberg, Germany). c/d, retinal nerve fiber layer thickness (RNFL) and macular thickness (MT) were also recorded. 30-2 visual field analysis via standard achromatic perimetry (SAP) was conducted with a Humphrey Field Analyzer Model 740i (Carl Zeiss Inc. Dublin, CA, USA), and mean (MD) and pattern standard (PSD) deviations were obtained. Each parameter with basic clinical findings (visual acuity, color vision, age, and gender) was recorded for each patient.

Biochemical analyses

Serum and fist morning urine samples were collected from patients and healthy subjects. MDA measurement was performed according to the study of Hong

et al. [9]. The method is based on the condensation of MDA with thiobarbituric acid (TBA) in the presence of reducing substances. Samples were analyzed using Prominence LC 20 (Shimadzu, Kyoto, Japan) HPLC set equipped with a Sil-20AC autosampler, a LC-20AD pump, and a RF-10Ax1 fluorescence detector. MDA was detected by fluorescence (excitation 515 nm; emission 553 nm). The results were expressed in $\mu\text{mol/L}$.

GSH and tGSH activities in serum were determined by HPLC (Shimadzu, Toyo, Japan). This method was based on derivative of GSH with o-Phthalaldehyde (OPA) [10]. The analysis was performed using Prominence LC 20 (Shimadzu, Kyoto, Japan) HPLC set, equipped with a Sil-20AC autosampler, a LC-20AD pump, and a RF-10Ax1 fluorescence detector. GSH and tGSH were detected by fluorescence (excitation 340 nm, emission 420 nm). Results were expressed as $\mu\text{mol/L}$.

The SOD activity was determined using a RAN-SOD kit (Randox Labs, Crumlin, UK). The absorbance was measured at 505 nm on a microplate reader (Bio-Tek Instruments, Inc., VT, USA). Results were expressed as $\mu\text{mol/mg}$ protein. Vitamin C levels were colorimetrically determined at the Dokuz Eylul University Hospital, via ARLab using the BioTek Synergy HT ELISA Reader (Winooski, USA) with the Ascorbic Acid Assay Kit (Sigma-Aldrich MAK074, Taufkirchen, Germany). The results were expressed in $\mu\text{g/ml}$.

LC–MS/MS analysis

- a. **Plasma MMA analysis:** Plasma samples were collected in tubes containing EDTA anticoagulant and were centrifuged immediately at 4 °C and 2000xg for ten minutes. The concentration of MMA was measured by LC-MS/MS (Shimadzu Prominence UFLC, Japan and 4000 QTRAP, Applied Biosystems, Germany, respectively), as described previously [11]. Intra- and inter-day MMA precisions were <3.8% and <5.7%, respectively. Results were given in $\mu\text{mol/L}$. The cut-off value for plasma MMA levels was 0.271 $\mu\text{mol/L}$, which has been frequently used in the previous literature [12].
- b. **8-OHdG assessment in urine:** To evaluate oxidative DNA damage, first morning urine samples were spiked with aliquot of 8-OH-dG- $^{15}\text{N}_5$

as internal standard and LC-MS/MS analyses were performed using a an HPLC system (Shimadzu, Kyoto, Japan) coupled to a triple quadrupole ion-trap mass spectrometer (4000 QTRAP Applied Biosystems, CA, USA) equipped with a TurboIonSpray™ source, as described previously [13, 14]. The Analyst Software Version 1.5 (Applied Biosystems) was used for data analyses. Urinary creatinine concentrations were used for normalization of the results. The results were expressed as nmol/mmol creatinine.

Statistical analyses

Statistical analyses were conducted using the IBM Statistical Package for Social Sciences (SPSS) software, version 21.0. The normality of the data was evaluated via the Shapiro–Wilk test. In comparisons between two groups, *t*-tests and Mann–Whitney U tests were applied for variables showing parametric and nonparametric distributions, respectively. Chi-Square test was used for categorical variables. Correlation coefficients were determined with Pearson's and Spearman's correlation tests for variables showing parametric and nonparametric distributions, respectively. For absolute values of correlation coefficients, 0–0.19, 0.2–0.39, 0.40–0.59, 0.6–0.79 and 0.8–1 were regarded as very weak, weak, moderate, strong and very strong correlations, respectively. *p* values less than 0.05 and 0.01 were considered statistically significant and very significant, respectively.

Results

No significant differences were found in gender distributions of the patient and control groups. Ages, however, in the control group were significantly higher than the study group (Table 1). Regarding oxidative stress markers, however, significant differences were observed between two groups (Fig. 1). Compared to the controls, the levels of GSH; vitamins A, B₁₂ and E; folic acid; MMA; MDA; and 8-OHdG were higher, while tGSH; SOD; vitamin C; Cp; and UA were slightly lower, in the study group. Statistically, increments in MDA ($t=2.3$ $p=0.025$) and Vitamin E ($z=3.648$ $p<0.001$) were found to be significant and very significant in the study group, respectively

Table 1 Comparison of demographic data between control and study groups

		Control	Patient	<i>p</i> value
Gender ^a	Female(n)/Male(n)	23/9	17/16	0.092
Age ^b	Mean ± Std. Dev. (<i>n</i>)	64.09 ± 13.24 (32)	57.42 ± 10.51(33)	0.028*

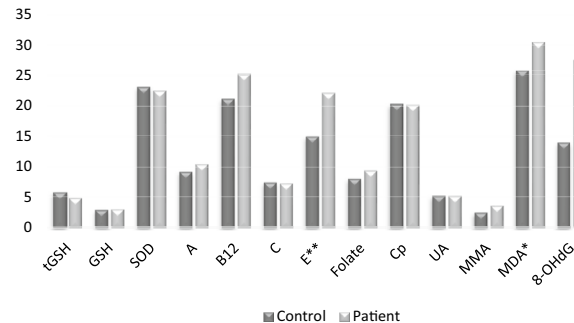
^aChi-Square Test^b*t*-test**p* < 0.05

Fig. 1 Comparison of blood biochemistry and oxidative stress parameters between patient and control groups. tGSH: Total glutathione (mmol/L × 100), GSH: Reduced glutathione, (mmol/L × 100), SOD: Superoxide dismutase (μmol/mg), A: Vitamin A (μg/L /100), B12: Vitamin B₁₂ (pg/mL /10), C: Vitamin C (ng/μL × 100), E: Vitamin E (μg/L), Folate (ng/mL),

Cp: Ceruloplasmin (mg/dL), UA: Uric acid, (μg/L), MMA: Methylmalonic acid (μmol/L × 10), MDA: Malonyl dialdehyde (μmol/L × 10), 8-OHdG: 8-hydroxy-2'-deoxyguanosine/Creatinine (nmol/mmol), *: *p* < 0.05 significant, **: *p* < 0.001 very significant

(Table 2). Comparison of oxidative stress parameters according to gender in study group is given in Online Resource 1 (ESM_1).

In the study group, significant and very significant correlations were observed in statistical analyses performed between clinical findings and oxidative stress parameters, along with correlations between clinical and biochemical data. In the correlation analysis between clinical and biochemical data in the study group, negative correlations between color vision with age, as well as between RNFL and vision, were statistically very significant. Positive correlation between age and vision and negative correlation between color vision and RNFL were also significant (Table 3). Correlation analysis between clinical and biochemical data showing parametric (ESM_2) and nonparametric (ESM_3) distributions in the study group is given in Online Resources 2 and 3, respectively.

Regarding oxidative stress parameters, UA showed very significant and significant positive correlations

with age and IOP, respectively. Significant positive and negative correlations were observed between cholesterol and MDA, and between glucose and Vitamin A, 8-OHdG, respectively. Whereas a strong and very significant negative correlation was observed between Vitamin B₁₂ and c/d ratio, Vitamin E was very significantly and significantly correlated with cholesterol and IOP, respectively. MD showed very significant and significant negative correlations with MMA and Cp, respectively (Table 4). Correlation analysis of oxidative stress parameters with clinical and biochemical data showing parametric (ESM_4) and nonparametric (ESM_5) distributions in the study group is given in Online Resources 4 and 5, respectively.

In the correlation analysis between oxidative stress parameters in the study group, antioxidant glutathione and SOD enzyme systems were found to correlate very significantly. tGSH showed negative and positive significant correlations with both Vitamin B₁₂ and MMA, respectively. Significant correlations between MMA and Cp, SOD and

Table 2 Comparison of blood biochemistry and oxidative stress parameters between control and study groups

		Control	Patient	<i>p</i>
Cholesterol ^a	Mean ± Std. Dev. (<i>n</i>)	209.81 ± 42.80(31)	206.28 ± 46.00(32)	0.754
Glucose ^b	Mean ± Std. Dev. (<i>n</i>)	91.10 ± 30.77(31)	96.77 ± 35.69(30)	0.263
	Median (25th–75th Perc.)	85(74–94)	89.5(77–106)	
tGSH ^b	Mean ± Std. Dev. (<i>n</i>)	0.06 ± 0.04(32)	0.05 ± 0.017(33)	0.386
	Median (25th–75th Perc.)	0.051(0.041–0.063)	0.047(0.039–0.060)	
GSH ^b	Mean ± Std. Dev. (<i>n</i>)	0.031 ± 0.015(32)	0.032 ± 0.013(33)	0.679
	Median (25th–75th Perc.)	0.03(0.02–0.037)	0.029(0.023–0.04)	
SOD ^b	Mean ± Std. Dev. (<i>n</i>)	23.01 ± 15.70(32)	22.39 ± 10.06(33)	0.486
	Median (25th–75th Perc.)	20.97(11.46–30.49)	24(13.51–30.27)	
A ^b	Mean ± Std. Dev. (<i>n</i>)	929.85 ± 303.73(31)	1051.18 ± 259.52(33)	0.065
	Median (25th–75th Perc.)	882.3(676.4–1174.6)	1072.8(925.8–1211.7)	
B12 ^b	Mean ± Std. Dev. (<i>n</i>)	251.16 ± 169.74(31)	210.93 ± 85.98(30)	0.644
	Median (25th–75th Perc.)	215(144–275)	193(159–265)	
C ^b	Mean ± Std. Dev. (<i>n</i>)	0.075 ± 0.032(32)	0.074 ± 0.037(33)	0.454
	Median (25th–75th Perc.)	0.074(0.057–0.095)	0.067(0.056–0.086)	
E ^b	Mean ± Std. Dev. (<i>n</i>)	15.03 ± 8.07(32)	22.06 ± 8.54(33)	< 0.001**
	Median (25th–75th Perc.)	13.22(11–17.3)	21.6(18.3–28.5)	
Folate ^b	Mean ± Std. Dev. (<i>n</i>)	8.17 ± 3.27(31)	9.46 ± 5.04(32)	0.372
	Median (25th–75th Perc.)	7.64(5.5–10.7)	8.85(5.9–10.9)	
Cp ^b	Mean ± Std. Dev. (<i>n</i>)	20.27 ± 3.31(31)	20.13 ± 4.04(31)	0.899
	Median (25th–75th Perc.)	19.8(17.5–21.8)	20.2(17–22.4)	
MMA ^b	Mean ± Std. Dev. (<i>n</i>)	0.379 ± 0.496(32)	0.269 ± 0.223(24)	0.345
	Median (25th–75th Perc.)	0.202(0.148–0.345)	0.153(0.126–0.408)	
Uric acid ^a	Mean ± Std. Dev. (<i>n</i>)	5.38 ± 1.58(31)	5.34 ± 1.17(32)	0.899
MDA ^a	Mean ± Std. Dev. (<i>n</i>)	2.57 ± 0.72(31)	3.04 ± 0.89(32)	0.025*
8-OHdG ^b	Mean ± Std. Dev. (<i>n</i>)	12.41 ± 9.09(27)	14.93 ± 10.36(28)	0.523
	Median (25th–75th Perc.)	11.17(5.52–16.35)	11.85(5.56–19.72)	

Cholesterol (mg/dL), Glucose (mg/dL), tGSH: Total glutathione (mmol/L), GSH: Reduced glutathione, (mmol/L), SOD: Superoxide dismutase (µmol/mg), A: Vitamin A (µg/L), B12: Vitamin B₁₂ (pg/mL), C: Vitamin C (ng/µL), E: Vitamin E (µg/L), Folate (ng/mL), Cp: Ceruloplasmin (mg/dL), UA: Uric acid, (µg/L), MMA: Methylmalonic acid (µmol/L), MDA: Malonyl dialdehyde (µmol/L), 8-OHdG: 8-hydroxy-2'-deoxyguanosine/Creatinine (nmol/mmol), *p*: *p* value; *n*: Number of samples

^a*t*-test

^bMann–Whitney U Test

p* < 0.05 *p* < 0.001

Table 3 Significant and highly significant correlations between clinical and biochemical data in the study group

			<i>r</i>	<i>p</i>	<i>n</i>	
Age	–	Vision	0.405	0.19*	32	M
Age	–	C Vs	–0.582	< 0.001**	32	S
Vision	–	C Vs	–0.633	< 0.001**	32	S
Vision	–	RNFL	–0.601	< 0.001**	32	S
C Vs	–	RNFL	0.354	0.047*	32	W

C Vs: Color vision; RNFL: Retinal nerve fiber layer analysis; *r*: Pearson correlation coefficients; *p*: *p* value; *n*: Number of samples

p* < 0.05 *p* < 0.001

Table 4 Significant and highly significant correlations of oxidative stress parameters with clinical and biochemical data in the study group

			<i>r</i>	<i>p</i>	<i>n</i>	
Age	–	UA	0.551	0.001**	31	M
MD	–	Cp	–0.412	0.015*	30	M
MD	–	MMA	–0.569 ^a	0.004**	24	M
Ch	–	MDA	0.431	0.014*	32	M
Ch	–	E	0.52	0.002**	32	M
IOP	–	E	0.459 ^a	0.007**	32	M
IOP	–	UA	0.387 ^a	0.029*	32	M
c/d	–	B12	–0.700 ^a	<0.001***	29	S
Glu	–	A	–0.399 ^a	0.029*	30	W
Glu	–	8-OHdG	–0.467 ^a	0.019*	25	M

c/d: Cup disk ratio; Ch: Cholesterol (mg/dL); Glu: Glucose (mg/dL); tGSH: Total glutathione (mmol/L); GSH: Reduced glutathione (mmol/L); SOD: Superoxide dismutase (μmol/mg); A: Vitamin A (μg/L); B12: Vitamin B₁₂ (pg/mL); C: Vitamin C (ng/μL); E: Vitamin E (μg/L); Folate (ng/mL); Cp: Ceruloplasmin (mg/dL); IOP: Intraocular pressure; UA: Uric acid, (μg/L); MMA: Methylmalonic acid (μmol/L); MD: Mean deviation; MDA: Malonyl dialdehyde (μmol/L); 8-OHdG: 8-hydroxy-2'-deoxyguanosine/Creatinine (nmol/mmol); *r*: Pearson correlation coefficients; *p*: *p* value, *n*: Number of samples

^aSpearman's rho correlation coefficients

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

glutathiones were positive and negative, respectively. Whereas positive correlation of MDA and cholesterol (Table 4) was significant, very significant positive correlations between Vitamin E with vitamin A and MDA (Table 5) were the other prominent findings regarding antioxidant response dynamics. Despite statistically significant *p* values

in the correlations between GSH and SOD, glucose and vitamin A, correlation coefficients were below 0.500 which implies a low correlation (Table 5). Correlation analysis between oxidative stress parameters and biochemical data showing parametric (ESM_6) and nonparametric (ESM_7) distributions in the study group is given in Online Resources 6 and 7, respectively.

Table 5 Significant and highly significant correlations between oxidative stress parameters and biochemical data in the study group

			<i>r</i>	<i>p</i>	N	
tGSH	–	B12	–0.402 ^a	0.028*	30	M
tGSH	–	MMA	0.417 ^a	0.043*	24	M
tGSH	–	GSH	0.447	0.009**	33	M
tGSH	–	SOD	–0.471	0.006**	33	M
Cp	–	MMA	0.417 ^a	0.043*	24	M
GSH	–	SOD	–0.388	0.026*	33	W
MDA	–	E	0.489	0.005**	32	M
A	–	E	0.567	0.001**	33	M

Cp: Ceruloplasmin; MMA: Methylmalonic acid; MDA: Malonyl dialdehyde; tGSH: Total glutathione (mmol/L); GSH: Reduced glutathione (mmol/L); SOD: Superoxide dismutase (μmol/mg); A: Vitamin A (μg/L); B12: Vitamin B₁₂ (pg/mL); E: Vitamin E (μg/L); Cp: Ceruloplasmin (mg/dL); MMA: Methylmalonic acid (μmol/L); MDA: Malonyl dialdehyde (μmol/L); *r*: Pearson correlation coefficients

^aSpearman's rho correlation coefficients

* $p < 0.05$ ** $p < 0.01$

Discussion

The optic neuropathies are various groups of disorders characterized by visual loss resulting from optic nerve dysfunction. According to the World Health Organization, a form of optic neuropathy is responsible for approximately 15% of the global burden of visual impairment [15]. However, clinical studies on the connection between optic neuropathy and oxidative damage mostly focus on glaucoma, a specific form of optic neuropathy. As it is a valid working hypothesis that a special form of ischemia is the reason for ganglion cell death in glaucoma [16], the mechanisms responsible for ischemic optic neuropathy are known to include oxidative stress, excitotoxicity, angiogenesis, neuroinflammation, and apoptosis following retinal ischemia [15] as well.

A random-effects meta-analysis of Parkinson's disease, conducted by Wei et al., has shown that patients with Parkinson's have significantly higher levels of blood oxidative stress markers—ferritin, 8-OHdG, nitrite, and MDA—compared with healthy subjects. In contrast, concentrations of UA, catalase, tGSH, and total cholesterol are significantly lower in Parkinson's patients compared with healthy subjects. Interestingly, blood levels of Mn, Cu, Zn, Fe, SOD, albumin, GSH, vitamin E, Cp, triglycerides, LDL-cholesterol, lactoferrin, transferrin, and HDL-cholesterol show no significant differences between Parkinson's patients and healthy subjects. In peripheral blood, significant heterogeneity is reported for 21 of the 22 oxidative stress markers, except for lactoferrin [17]. In comparing the blood biochemistry and oxidative stress parameters between the patient and control groups in the present study, only vitamin E and MDA showed statistically significant increments. This finding is highly concordant with both the meta-analysis of Wei et al. and a similar study carried out by the first author and another research group in 2010. Thirty-one healthy individuals and 160 glaucoma patients were included in the latter multicenter case–control study. SOD and GSH were found to be decreased, and MDA and vitamins A and E were found to be increased, in the study group. For vitamin E and MDA, this increase was determined to be very significant [18]. Serum MDA levels are increased, not only in the cataract patients [6], but also in primary angle closure glaucoma (PACG) patients [19]. These reports are significant regarding nerve tissue damage,

for MDA is an oxidation degradation product and is the most important marker of lipid oxidative damage [1, 19]. The increased vitamin E levels reported in patients with optic neuropathy can be explained with the existence of sensitive mechanisms for regulating tissue levels vitamin E. In addition to being an important liposoluble antioxidant, however, molecules of the vitamin E family exert neuroprotective and anti-inflammatory activities [20].

All those interactions also imply that the significant and very significant interactions between vitamin E, MDA and cholesterol observed in the present study are noteworthy. In a systematic review of 14 observational and seven randomized controlled clinical trials, vitamin E was found to correlate significantly with MDA and SOD [21]. In a clinical study carried out in Africa, patients demonstrated higher plasma atherogenic indices and higher levels of total cholesterol, along with at least 1,334-fold of MDA concentration above normal levels. Also, in the same patients, the higher atherogenic plasmatic index significantly ($p < 0.05$) increased with MDA concentration [22]. Sole supplementation of vitamin E, on the other hand, may act as a stimulant to HMG-CoA activity and, therefore, demonstrate hypercholesterolemic activity [23]. The hypocholesterolemic effects of vitamin E, however, are also well known via several membrane proteins [24].

In correlation analysis between the clinical and biochemical data, significant correlations between color vision and age, as well as between RNFL and vision, are consistent with current clinical knowledge and the extant literature [25]. Prominent results related with vitamins E and B₁₂ were observed in the correlation analysis of oxidative stress parameters with clinical and biochemical data, as well as other oxidative stress parameters in the study group. Vitamin E was found to significantly correlate with IOP in NAION patients. Engin et al. have reported that α -tocopherol exert vasoregulatory effects on the retina, which is important for avoiding ischemia [26]. Another parameter significantly correlated with IOP—UA—is also known to exert protective effects against oxidative damage in the central nervous system. Negative association between UA levels and disease severity has been shown in PACG [27] patients. Significantly higher UA levels, however, have been reported in NTG patients [28]. A recent large-scale meta-analysis results, however, revealed

no significant associations between NAION and glaucoma or IOP in 1095 and 46 cases, respectively [29].

Along with MDA and cholesterol, Vitamin A was the third molecule to show very significant correlation with vitamin E in this study. Vitamins A and E are fat soluble vitamins absorbed in the small intestine through different, but interrelated, mechanisms. Similar to vitamin E, vitamin A possesses antioxidative properties and the ability to regulate gene expression by interacting with the retinoic acid receptors and the retinoid X receptors, acting as transcription factors [30]. Furthermore, the antioxidant properties of vitamin E are shown to help to prevent vitamin A oxidation and, therefore, serve to enhance vitamin A absorption when there is concomitant consumption of vitamin A and E [31]. Negative correlation of vitamin A with glucose was found to be weak but significant. It is evident that hepatic glucose and lipid metabolism are regulated by vitamin A metabolites at many different levels [32].

A significant negative correlation between tGSH and SOD has been observed in this study. After screening 12 articles with 646 long-lived participants and 1052 controls, Belenguer-Varea et al. [33] have presented significantly lower SOD and higher glutathione reductase activities in elderly individuals, concordant with the present results. Low intake of folate and vitamin B₁₂ has also been found to be associated with reduced levels of tGSH in patients with type 2 diabetes mellitus [34]. In this study, tGSH showed negative and positive significant correlations with both vitamin B₁₂ and MMA, respectively. MMA was also significantly correlated with Cp. Cp is the carrier of the Cu⁺⁺ ion, which is a cofactor of oxidation enzymes, and its deficiency affects optic nerve function, and the nervous system in general [35]. Vitamin B₁₂ is another antioxidant whose deficiency causes antioxidant response disorders related to the glutathione system. However, vitamin B₁₂ deficiency can be difficult to diagnose. Due to the lack of a clear association between serum vitamin B₁₂ and its deficiency, it has been proposed that functional vitamin B₁₂ deficiency can occur at any serum level. Moreover, it was stated that normal or high serum vitamin B₁₂ levels can sometimes be seen in a vitamin B₁₂ deficient state, and high levels of MMA have been identified as a better indicator of B₁₂ deficiency than the actual serum B₁₂ level itself [36]. On the other hand, vitamin B₁₂ deficiency is reported to be related

to both symptomatic and asymptomatic small fiber loss and retinal nerve fiber thickness in glaucomatous other non-glaucomatous optic neuropathies [37], which highlights the very significant negative correlation of vitamin B₁₂ with the c/d ratio. In this study, MD showed significant negative correlations with both MMA and Cp. It has been reported on the association of copper deficiency with the development of concomitant neurologic deficits manifested as peripheral neuropathies and myeloneuropathy indistinguishable from the clinical and T2-weighted MRI findings seen in vitamin B12 deficiency [38].

MDA is an important end product for lipid peroxidation, and 8-OHdG is a critical marker for DNA damage, and increases in both MDA and 8-OHdG, implying oxidative damage in lipid tissue, have been reported in PACG [19] patients. Although MDA and 8-OHdG are products of lipid oxidation, damage can occur at various organelles and levels, depending on the composition of the tissue or organ from which the oxidation originates. On the other hand, these two compounds are elements of a complex mechanism and interact with each other. MDA is a known mutagenic and carcinogenic [39] and reacts physiologically with forming deoxyguanosines, including 8-OHdG [3]. Despite MDA being significantly increased among the NAION patients in the present study, neither increase nor correlations of 8-OHdG were statistically significant, except for a negative correlation with glucose. Although 8-OHdG is identified as important biomarkers in the pathogenesis process of type 2 diabetes mellitus, understandably enhances in blood level as the disease progress [40]. Because reviewing clinical studies on 8-OHdG currently generates interest concerning whether it is an actual marker of DNA damage in lipid tissue due to oxidative stress, variable reports are encountered. 8-OHdG has been reported to be increased in the plasma of Leber's hereditary optic neuropathy [41] and in both the aqueous humor and plasma of POAG patients [42]. Yuki et al., however, studied urine samples in NTG cases and reported decreased urine 8-OHdG/creatinine ratio, which is concordant with the present research [5].

In a recent study among 18 newly diagnosed NAION patients and 17 healthy subjects, along with serum total oxidant and antioxidant status, solely advanced oxidation protein products were studied, and no significant differences were reported between

the patients and the control group [43]. Although having limitations in age mismatch between control and study groups and statistical risk of type I error due to numerous pairwise comparisons, the present study is much more comprehensive, with a larger NAION series. Furthermore, the researchers have specifically focused on the products and defense dynamics of lipid oxidation using more sophisticated assay techniques. This study not only obtained clear information regarding oxidative damage and antioxidant response in a frequent form of optic neuropathy, but it also pointed out specific interactions between neuromodulators, like vitamin E, and intracellular signaling and regulation mechanisms. Significant elevations in both vitamin E and MDA levels in the study group and their significant interactions with vitamin A, cholesterol—and with certain clinical parameters as well, observed in the present study are noteworthy. Meaningfully, significant correlations between Glutathione/Vitamin B₁₂ and SOD enzyme systems were also revealed. Better reading of these connections and focusing on oxidative nerve tissue damage may aid the development of more suitable diagnosis, follow-up and treatment criteria and strategies, alongside a fuller understanding of NAION.

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Author contributions EKN constructed the hypothesis and wrote the project, contributed to material preparation, data collection, and wrote the manuscript. The project was produced and conducted by EKN, as S-BM, GG, İH, YA contributed to development of the ideas, design and supervision of the course of the project. D-EC and H-KD collected data, and all the authors except Y-ÖS contributed to material preparation and data analysis. Y-ÖS performed the statistical analysis. All authors have read and approved the final version of the manuscript.

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Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent/licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval The research adhered to the tenets of the Declaration of Helsinki. Approval from the Ethics Committee of Dokuz Eylul University, as well as informed consent from the subjects, was obtained.

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