CASE REPORT

Peripapillary Retinal Nerve Fibre Layer Thinning Secondary to Nutritional Folic Acid Deficiency

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ABSTRACT

Folic acid has a fundamental role in central nervous system (CNS) function at all ages, especially the methionine synthase-mediated conversion of homocysteine to methionine, which is essential for nucleotide synthesis and genomic and non-genomic methylation. Folic acid and vitamin B12 may have roles in the prevention of disorders of CNS development, mood disorders, and dementias, including Alzheimer disease and vascular dementia in elderly people. The authors examined the peripapillary retinal nerve fibre layer thickness (RNFLT) in patients with nutritional folic acid deficiency using optical coherence tomography (OCT). Patients were divided into two groups according to blood folic acid levels: blood folic acid <7 nmol/L as Group 1 and >7 nmol/L as Group 2. Peripapillary RNFL measurements were performed. There were significant positive correlations between serum folate levels and RNFLT in all quadrants ($p < 0.05$), except for the temporal quadrant ($p = 0.41$).

Keywords: Folic acid deficiency, optical coherence tomography, peripapillary retinal nerve fibre layer thickness

INTRODUCTION

A water-soluble vitamin, folic acid (pteroyl monoglutamate) is a combination of pteric acid (combination of *para*-aminobenzoic acid and pteridine) and L-glutamic acid. The active form of folic acid results from the reduction of pteroyl monoglutamate by dihydrofolate reductase and becomes tetrahydrofolic acid. It has a part in the important metabolic actions of purine, thymidylate, and methionine synthesis, serine-glycine conversion, and histidine catabolism, and it carries single-carbon units.^{1–3}

Although mammals can synthesise all components of this vitamin, they cannot form the bond between pterin and amino benzoic acid.³ The polyglutamate form of folate in nutrients is transformed into monoglutamate before absorption. This catabolism

is done by pteroylpolyglutamate hydrolase (glutamate carboxypeptidase). The enzyme group of glutamate carboxypeptidase is active in a soluble form, and binds the membranes at the brush border of the human jejunal mucosa.^{4,5}

Causes of folic acid deficit include physiological conditions, nutritional deficiency, malabsorptions (celiac disease, tropical sprue, ulcerative colitis), haemolytic anaemia, smoking, alcohol, and drugs.^{3,6} A decrease in serum folate level in old age is related to disruptions of diet and intestinal folate absorption. Researchers have found that intestinal hydrolysis of folic polyglutamate is disrupted with aging, which causes a decrease in absorption.^{7,8} Neuropsychological studies have found general and specific impairments in cognitive functions, such as attention, episodic and visuospatial memory, abstract

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reasoning, and peripheral neuropathy, that were attributed to folate deficiency.^{9–11}

In the literature, there are no published studies on the relationship between folic acid deficiency and peripapillary retinal nerve fibre layer thickness (RNFLT). Therefore, in this study, we examined RNFLT in patients with folic acid deficiency, using spectral-domain optical coherence tomography (OCT).

MATERIALS AND METHODS

The study adhered to the tenets of the Declaration of Helsinki. It was approved by the local ethics committee (meeting: May 2011, document no.: 27) and written informed consents were obtained from all patients before they were recruited into the study.

In the Erzurum Training and Research Hospital and Kafkas University ophthalmology departments, 69 patients of similar ages were divided into two groups: those who had an insufficient serum folic acid level (Group 1) and a normal group (Group 2). Patients with isolated folic acid deficiency were referred to the eye clinic by the internal medicine clinic. All participants underwent a standard ophthalmologic examination, which included oto-refractometry, visual acuity, Goldmann applanation tonometry, measuring of intraocular pressure, frontal and +90 D non-contact lens, and posterior segment examination by biomicroscopy. A spectral-domain OCT device was used for RNFLT (RTVue version 4.0; Optovue, Optovue, Fremont, CA).

OCT was performed through undilated pupils using the RTVue version 4.0 (Optovue) by the same investigators, E. Ceylan and M. Ekinici. Only scans that reached signal strength of at least ≥ 6 , which indicates a high-quality scan, were accepted for the analyses. The RNFL scan pattern completes four circular scans in 0.15 s at a diameter of 3.45 mm, targeted around the optic nerve head. Peripapillary RNFLT was measured in each eye as superior and inferior hemispheres, and average values were considered for evaluation. The average of the two measurements was taken; the differences between readings of the masked physicians were found to be within 10% of the mean.

Serum vitamin B12 and folic acid levels were evaluated using proper kits within an Elecsys ZOTO 2010 device (Roche Diagnostics, Indianapolis, IN). Vitamin B12 levels were measured as pg/mL, then convert into pmol/L by multiplying by 0.738, whereas results for folic acid were measured as nmol/L. The healthy control group (Group 2) consisted of persons whose serum folic acid level >7 nmol/L, whereas Group 1 consisted of patients whose serum folic acid level <7 nmol/L.¹²

Inclusion criteria for this study were (1) the absence of any systemic or ocular disease; (2) visual acuity of

20/20 (absolute) on the Snellen chart, whether uncorrected or best corrected; (3) refraction defect ≤ 3 around dioptre borders and anisometropia >1 for dioptre; (4) normal vitamin B12 levels; (5) no neurodegenerative disease such as Alzheimer, Parkinson, and multiple sclerosis; (6) non-smoker; and (7) should not use phenytoin, barbiturates, or alcohol.

Statistical Analysis

The findings were analysed using SPSS (SPSS, Chicago, IL, USA). Data were checked for normal distribution using the Kolmogorov-Smirnov test and for equality of variances using Levene's test. When comparing groups, Student's *t*-test was used for normally distributed data and the Mann-Whitney *U* test for data that were not normally distributed. In addition, a chi-square test was used to compare descriptive variables with frequency distributions and qualitative data. The results were evaluated with a 95% confidence interval, and $p < 0.05$.

RESULTS

The mean age was 71.71 ± 4.83 (65–82 years) and there were 37 (53.6%) males and 32 (46.4%) females in the study group. There were 34 patients (49.3%) in Group 1 and 35 (50.7) in Group 2. There were no significant differences between two groups with respect to median age ($t = -1.417$, $p = 0.1619$) and gender distribution ($\chi^2 = 0.013$, $p = 0.911$) (Table 1).

Serum folate levels in Group 1 and Group 2, respectively, were 2.62 ± 1.68 and 8.06 ± 1.08 nmol/L. Statistically significant differences were found between the serum folate levels of the two groups ($t = -19.08$, $p < 0.001$). Vitamin B12 levels in Group 1 and Group 2, respectively, were 357.15 ± 7.44 and 355.26 ± 7.26 pmol/L. There were no statistically significant differences between vitamin B12 levels of the two groups ($t = 1.068$, $p = 0.290$). Blood count and biochemical parameters were within normal limits in both groups (Table 1).

Average RNFLT were found to be 93.41 ± 2.47 μm (90–97) in Group 1 and 110.54 ± 3.76 μm (105–117) in Group 2; this difference is statistically significant ($t = -22.790$, $p < 0.001$). In both groups, whereas RNFLT were minimal in the temporal quadrant, they were maximal in the inferior quadrant (Table 2). There were significant correlations between serum folate levels and mean ($r = 0.76$, $p < 0.001$), upper quadrant ($r = 0.48$, $p < 0.01$), inferior quadrant ($r = 0.36$, $p < 0.05$), and nasal quadrant ($r = 0.36$, $p < 0.05$) RNFLT; only the temporal quadrant correlation was non-significant ($r = -0.14$, $p = 0.41$).

TABLE 1 Descriptive features of the groups.

Feature	Group 1	Group 2	<i>t</i> or χ^2	<i>p</i>
Number of cases	34	35		
Age (mean $\pm$ SD)	70.74 $\pm$ 4.07	72.31 $\pm$ 5.10	<i>t</i> = -1.417	<i>p</i> = 0.161
Female/Male	16/18	16/19	χ^2 = 0.013	<i>p</i> = 0.911
Vitamin B12 (pmol/L)	357.15 $\pm$ 7.44	355.26 $\pm$ 7.26	<i>t</i> = 1.068	<i>p</i> = 0.290
Folic acid (nmol/L)	2.62 $\pm$ 1.68	8.06 $\pm$ 1.08	<i>t</i> = -19.080	<i>p</i> < 0.001*

*Statistically significant difference.

TABLE 2 Peripapillary OCT findings for the two groups.

Finding	Group 1	Group 2	<i>t</i>	<i>p</i>
ARNFLT	93.41 $\pm$ 2.47	110.54 $\pm$ 3.76	<i>t</i> = -22.790	<i>p</i> < 0.001*
SRNFLT	108.18 $\pm$ 1.73	127.31 $\pm$ 2.23	<i>t</i> = -39.703	<i>p</i> < 0.001*
NRNFLT	85.12 $\pm$ 2.19	103.69 $\pm$ 3.37	<i>t</i> = -27.146	<i>p</i> < 0.001*
IRNFLT	114.94 $\pm$ 1.92	129.60 $\pm$ 2.15	<i>t</i> = -29.764	<i>p</i> < 0.001*
TRNFLT	81.06 $\pm$ 2.14	100.20 $\pm$ 3.62	<i>t</i> = -26.764	<i>p</i> < 0.001*

ARNFLT = average RNFLT; SRNFLT = superior RNFLT; NRNFLT = nasal RNFLT; IRNFLT = inferior RNFLT; TRNFLT = temporal RNFLT.

*Statistically significant difference.

DISCUSSION

We examined RNFLT in patients with folic acid deficiency, using spectral-domain OCT. There was significant peripapillary RNFL thinning in patients with nutritional folic acid deficiency. To the best of our knowledge, in the medical literature, this is the first study to investigate the relationship between folic acid deficiency and RNFL thinning.

Folic acid is located in the cytosol and mitochondria and it participates as a single-carbon unit carrier in metabolic reactions such as histidine catabolism, and methionine synthesis from purine, thymidylate, and homocysteine, with the help of methionine synthase.¹⁻³ Especially for rapidly dividing tissues, folate balance is very important.

Causes of folic acid deficit include physiological conditions, nutritional deficiency, malabsorption (celiac disease, tropical sprue, ulcerative colitis), haemolytic anaemia, smoking, alcohol, and drugs.^{3,6} Researchers have found that intestinal hydrolysis of folic polyglutamate is disrupted with aging, which causes a decrease in absorption.^{7,8} Chronic alcohol intake and glutamate carboxypeptidase inhibitor intake with meals reduces the activity of glutamate carboxypeptidase and impairs folate absorption.³ Patients with haemolytic anaemia are at risk for folate deficiency due to peripheral destruction of red blood cells and, in response, increased erythropoiesis.¹³ Smoking is reported to be one of the factors that negatively influences the balance of folic acid, and there are studies reporting that in smokers, due to loss of folic acid, cellular and subcellular changes such as chromosomal abnormalities and increased bronchial metaplasia can be seen.^{6,14} The use of drugs,

especially phenytoin or barbiturates, can cause lack of folic acid.⁹

In the literature, together with megaloblastic anaemia, which is secondary to folic acid deficiency, spinal cord, peripheral nerve, and mental disorders are described.^{15,16} Again in the literature, it is emphasised that inadequate maternal intake of folate increases the risk of neural tube defects, especially anencephaly or spina bifida.^{17,18} Reynolds⁹ identified changes in 34 patients who had isolated folic acid deficiency: 27% had cognitive changes, 56% had affective disorder, and 18% had peripheral neuropathy, with no identifiable subacute combined degeneration of the cord (SCD). In his study, Pincus identified SCD in 25 of his patients who had isolated folic acid deficiency.¹⁹ Reynolds⁹ pointed out that folic acid deficiency induced by phenytoin or barbiturates is commonly associated with mental changes, especially depression, apathy, psychomotor retardation, and cognitive decline. When Reynolds added 5 mg folic acid daily for 1-3 years in the treatment of 26 epilepsy patients who had folic acid deficiency, he observed improved driving, initiative, alertness, concentration, mood, and sociability in most, and an increase in seizure frequency in some of his patients.²⁰

Impairment of oxidative phosphorylation in mitochondria has an important role in the pathophysiology of optic neuropathy. Oxidative phosphorylation in the mitochondria provides the production of ATP (adenosine triphosphate) at one end, and the transfer of electrons to oxygen at the other end. Proteins containing sulphur amino acids and vitamins such as folic acid and B12 are especially important for oxydative phosphorilation. Their deficiency results in a reduction in the production of ATP.

Nutrient deficiency and exposure to toxins reduce the production of ATP in all cells' mitochondria. In addition, in neurons that have demyelinated, long axons are more attenuated. This shows that the optic nerve is more susceptible to a decrease in ATP production.^{21,22} Folic acid is also needed for the production of tetrahydrofolate, which participates in formate detoxification. Formate accumulation blocks mitochondrial oxidative phosphorylation by inhibiting cytochrome oxidase. Martin-Amat *et al.* showed that toxic levels of formate injection caused optic nerve damage characterised by intra-axonal swelling and mitochondrial damage.²³ Sadun *et al.* showed that an increased formate level due to methanol injections caused mitochondrial damage and reduced ATP production in rats.²⁴ It has been thought that degradation in axonal transport primarily effects the less myelinated optic nerves and retinal nerve fibres. Also, the same study showed that rats that lack folic acid were more adversely affected than normal ones. Golnik and Schaible have suggested that formic acidemia occurs as a result of the inability to detoxify endogenous formate in people who lack folic acid. In the same study, in the patients diagnosed with tobacco-alcohol amblyopia and low folic acid-normal serum vitamin B12 levels, after folic acid supplementation therapy, vision and colour vision recovery were demonstrated.²⁵ de Silva *et al.*²⁶ reported on a 44-year-old woman who consulted their clinic with painless bilateral vision loss. They assessed a bilateral vision level 6/60, inability to see any plates in the Ishihara tests, and a history of 14 U of alcohol (1.5 bottles, i.e., 1125 mL of 12.5% wine) per week and 20 cigarettes per day. She also had a serum folic acid level of 1.5 ng/mL (2.5–18 ng/mL), red cell folate 208 nmol/L (220–620 nmol/L), and haemoglobin 14 g/dL (11.5–16 g/dL), with a mean corpuscular volume (MCV) of 122.8 fL (78–100 fL). They reported her bilateral vision level increased to 6/36 after 4 weeks of 5 mg/day oral folate supplementation.

In our study, we showed that folic acid deficiency causes thinning in all quadrants of the retinal nerve fibre layer, and that thinning in the superior, inferior, and nasal quadrants were correlated with serum folic acid levels. It is thought that thinning of the retinal nerve fibre layer in patients with folic acid deficiency occurs because of axonic transport impairment due to a decrease in oxidative phosphorylation and demyelination. In our study, there was not significant correlation between serum folate levels and the temporal quadrant RNFLT. It is reported that macular fibres occupy the central part at the proximal end of the optic nerve and keeps this position due to the anterior part of the chiasma.²⁷ We think that the protection of the macular fibres from demyelination can be occur because of the path of macular fibres in the optic nerve.

Folate deficiency has been consistently associated with evidence of depression, and cognitive decline, and specific impairments of intellectual function, including attention, episodic and visuospatial memory, and abstract reasoning.⁹ In our study group, the most physiological pathology observed was depression ($n = 10$; 29.4%).

This study on RNFLT has clinical significance in the monitoring of neurodegenerative diseases such as Parkinson disease, Alzheimer disease, and multiple sclerosis.^{28–30} While following up these diseases, which mostly affect elderly patients, it should be considered that folic acid can affect RNLF values. A second important consideration is that the mechanism of homocysteine needs B12 and folic acid, and blood levels of homocysteine increase in the absence of these vitamins.³¹ It is well known that an increased homocysteine level increases age-related macular degeneration and risk of stroke; that is why these risks should be considered when evaluating patients who lack folic acid.^{32,33}

One weakness of our report is that we did not measure the red blood cell folate level, which is evaluated as a better measure of chronic folate deficiency than serum folate level.

We suggest that further prospective longitudinal studies are needed to determine whether the RNFLT changes in folic acid-deficient patients can be treated over time.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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