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Effect of nutritional-deficiency anemia on peripapillary retinal nerve fiber layer: A North Indian study

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Abstract:

PURPOSE: The purpose of this study was to evaluate the effect of nutritional-deficiency anemia (NDA) on peripapillary retinal nerve fiber layer thickness (PPRNFLT) using spectral-domain optical coherence tomography and to determine any correlation arising thereof. This was a single-center, cross-sectional, observational study.

MATERIALS AND METHODS: A total 115 eyes of 115 NDA patients (50 of each with iron-deficiency anemia [IDA] and Vitamin B12-deficiency anemia [BDA], and 15 with folic acid-deficiency anemia [FDA]) aged 18–65 years were compared with a total 100 eyes of 50 age- and sex-matched healthy controls. All subjects underwent comprehensive clinical, ophthalmic, and hematological evaluation, followed by PPRNFLT assessment for the mean total, superior, inferior, nasal, and temporal quadrants.

RESULTS: PPRNFLT for the mean total and all four quadrants in IDA patients, for the mean total, inferior, nasal, and temporal quadrants in BDA patients, and for the mean total, inferior, and nasal quadrants, in FDA patients, was significantly lower as compared to the controls ($P < 0.05$). The mean total PPRNFLT of all NDA patients correlated significantly ($P < 0.05$) with their relevant hematological parameters with Pearson's coefficient (r) value of 0.613, 0.610, 0.336, 0.295, 0.337, 0.374, and – 0.509, respectively, for serum haemoglobin (Hb), iron, ferritin, mean corpuscular volume (MCV), mean cell hemoglobin, mean corpuscular hemoglobin concentration, and total iron binding capacity in IDA; 0.310, 0.435, and – 0.386, respectively, for serum Hb%, Vitamin B12, and MCV in BDA; and 0.557, 0.358, and – 0.294 for Hb%, folate, and MCV, respectively, in FDA cases. Mean total retinal nerve fiber layer thinning of all NDA patients showed progression with the increasing severity grades of anemia, except in very severe BDA where an inverse relationship was documented.

CONCLUSION: Our study revealed that PPRNFLT is significantly thinner in all NDA patients (total and all four quadrants in IDA; total, inferior, nasal, and temporal in BDA; and total, inferior, and nasal in FDA) correlating well with their relevant hematological parameters. Early detection of this may be crucial in preventing potential blinding sequelae and differentiating glaucomatous and other neuro-ophthalmic disorders.

Keywords:

Folic acid-deficiency anemia, iron-deficiency anemia, nutritional-deficiency anemia, retinal nerve fiber layer thickness, spectral-domain optical coherence tomography, Vitamin B12 deficiency anemia

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Introduction

Nutritional-deficiency anemia (NDA) can be caused due to deficiencies of micronutrients such as iron (iron-deficiency anemia [IDA]), Vitamin B12 (Vitamin B12-deficiency anemia [BDA]), and folic acid (folic acid-deficiency anemia [FDA]), with iron deficiency being the most common cause of anemia.

In various studies, it has been reported that the body iron store (ferritin) was low

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in almost 60%–70% of the Indian population.[1,2] Retinal neuronal cells can be damaged due to the reduction of normal perfusion and oxygen saturation (hypoxia), as well as due to the inhibition of normal functioning of oligodendrocytes and myelination process.[3]

Vitamin B12 deficiency (BD) is recently reported to be endemic in India, and its prevalence may be as high as 70%–100%.[4] This may also be because nearly 30% of the Indian population is vegetarian.[5] Although the prevalence of Folic Acid Deficiency (FD) is not as high as BD, the causes are almost similar in both (nutritional, intestinal malabsorption, increased physiological demand, and drugs)[6-8] Both also have a similar intricate relationship in their metabolic function ad hematological and clinical manifestations. Other than anemia, chronic BD or FD (not merely subnormal serum level) may lead to various neurological or neuropsychiatric manifestations or even life-threatening atherosclerosis because of increased serum homocysteine level. A chronic BD definitively proved to cause optic neuropathy and loss of vision, possibly due to derangement of superoxide scavenging mechanism as well as myelin synthesis.[9,10] Whereas, a chronic FD results in optic neuropathy secondary to the axonal transport impairment resulting from decrease in oxidative phosphorylation, and demyelination resulting from accumulation of neurotoxic formate level.[11]

It is prudent to measure the retinal nerve fiber layer thickness (RNFLT) by utilizing optical coherence tomography (OCT) in NDA patients, considering that retinal nerve fiber layer thinning (RNFL-t) might precede the development of frank optic neuropathy, as is the rule in cases with glaucomatous optic neuropathy. Typical pattern of RNFL-t has also been reported in various ischemic retinal diseases, myopia, chronic obstructive pulmonary disease (COPD), and neurological (e.g. multiple sclerosis, Parkinson's disease, and migraine) and neuropsychiatric illnesses (Alzheimer's disease).[12-19]

Spectral-domain OCT (SD-OCT) is especially known for its capability of enhancing the image resolution, improved quality of measurements, reduced scan time, and reproducibility.[20]

Currently, retinal nerve fiber layer (RNFL) study on NDA is scarce as per our knowledge. Therefore, the aim of the study was to analyze the effect of NDA on peripapillary RNFLT in adult patients using SD-OCT and to determine any correlation arising thereof.

Materials and Methods

Study population and design

This cross-sectional observational study was performed for a period of 1.5 years (September 2017 to February 2019) at our sub-Himalayan tertiary care multispecialty referral center in North India that caters to a large population of this state and neighboring states as well. This study adhered to the tenets of the Declaration of Helsinki. Necessary approval was taken from the Institutional Ethics Committee (Approval number: SGRR/IEC/43/18). Patients' informed consent was obtained at the beginning of this study.

Inclusion and exclusion criteria

In this study, 115 eyes of 115 patients (aged 18–65 years) with clinically diagnosed anemia (IDA group: 50 patients, BDA group: 50 patients, and FDA group: 15 patients), who were recruited from the Department of Medicine in- and outpatient clinic, were included. A total 50 age- and sex-matched healthy nonanemic subjects were also included as a control group. Patients who were nonglaucomatous having normal intraocular pressure of <21 mmHg, open anterior chamber angle, having no cup–disc ratio (C: D ratio) abnormalities, or documented glaucomatous visual field defects, in either or both eyes, were included in this study.

Patients with a history of external trauma, ocular surgery or laser/injection procedures, best-corrected visual acuity <6/6 (BCVA; Snellen), myopia or hypermetropia of >6 diopters, uveitis, pseudoexfoliation syndrome, squint, amblyopia, alcoholism, smoking, systemic diseases other than NDA (known case of diabetes, hypertension, COPD, thyroid disorders, Alzheimer's disease, Parkinson's disease, and multiple sclerosis), structural or organic ocular disease (acquired or congenital), pregnancy, significant ocular media opacity, and signal strength of <6 in SD-OCT were excluded.

Description of parameters

Hematological parameters adopted for the diagnosis of IDA were serum hemoglobin (Hb%) <11 g/dL, iron <50 μg/dL, ferritin <15 μg/dl, and total iron binding capacity (TIBC) >300 μg/dL; for BDA were serum Hb% <11 g/dL and B12 <200 pg/mL; and for FDA were serum Hb% <11 g/dL and folic acid (FA) <3.00 ng/mL. Blood biochemistry measurements were performed using VITROS 5600. Anemia was graded according to the Hb% level as mild: 11–9 g/dL, moderate: 9–7 g/dL, severe: 5–7 g/dL, and very severe: <5 g/dL.

Clinical examination

Each patient underwent comprehensive ocular examination for both the eyes, and a randomly selected eye was included in the study. Examination included assessment of BCVA, refraction by auto-refracto-keratometer (ARK-1, Nidek Co. Ltd., Japan), slit-lamp biomicroscopy (Haag-Straight®, Koenz, Switzerland), tonometry (Goldmann applanation tonometry), perimetry (Humphrey Visual Field Analyzer;

Carl Zeiss® Meditec Inc. Dublin, CA, USA.), fundoscopy with 90 D and 20 D lens, and RNFLT assessment by SD-OCT (RS-3000 Nidek Gamagori, Aichi 443-0038, Japan). Peripapillary RNFLT scans were obtained by a single-blinded second operator for total, inferior, superior, nasal, and temporal retinal quadrants using optic disc 200 × 200 pixels on 6 mm × 6 mm 2 cube scan protocol within a 3.45 mm diameter centered on the ONH. During the scan, the best possible fixation was ensured in each case after full dilation of the pupil by a combination of 0.8% tropicamide and 5% phenylephrine eye drops.

Statistical analysis

All data were analyzed by the second observer (co-author) using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA) and recorded on Microsoft Excel Windows 10. The quantitative data were expressed as mean with standard deviation. Categorical and nominal data were expressed in percentage. Paired *t*-test was used for analyzing quantitative data (intragroup analysis), Mann–Whitney *U*-test was used to analyze nonparametric data, and Chi-square test was used to analyze categorical data. Pearson's correlation coefficient analysis was used to correlate intragroup quantitative variables. Statistical significance was considered as $P < 0.05$.

Results

The clinical and hematological parameters of NDA are presented in Tables 1-3. In the present study, mean age, gender differences, C/D ratio, rim area, and disc area between the IDA, BDA, and FDA group cases were similar ($P > 0.05$). Similarly, except a significant difference ($P < 0.05$) of the relevant blood parameters for the individual anemic group (serum Hb%, mean corpuscular volume [MCV], mean cell hemoglobin [MCH], mean corpuscular hemoglobin concentration (MCHC), iron, ferritin, and TIBC for IDA cases; and serum Hb%, MCV, and B12 for BDA cases; and serum Hb%, MCV, and FA level for FDA cases), all other blood parameters were found similar ($P > 0.05$) to that with their corresponding control cases.

For IDA cases, there was a significant RNFL-t of mean total, superior, inferior, nasal, and temporal quadrants of retina as compared to the control (92.62 ± 8.22 μm vs. 97.28 ± 5.06 μm, $P < 0.001$; 117.82 ± 13.87 μm vs. 123.23 ± 8.13 μm, $P < 0.001$; 119.18 ± 11.90 μm vs. 126.03 ± 7.88 μm, $P < 0.001$; 68.53 ± 5.79 μm vs. 74.82 ± 6.02 μm, $P < 0.001$; and 62.91 ± 8.06 μm vs. 66.20 ± 6.10 μm, $P < 0.0014$, respectively) [Table 1]. A significant Pearson's correlation of mean total RNFLT was established positively with serum Hb%, iron, ferritin, MCV, MCH, and MCHC ($r = 0.613$, $P < 0.001$; $r = 0.610$, $P < 0.001$; $r = 0.336$, $P = 0.001$; $r = 0.295$, $P = 0.003$; $r = 0.337$, $P = 0.001$; and $r = 0.374$, $P \leq 0.001$, respectively) and negatively with serum TIBC ($r = 0.509$, $P < 0.001$) [Table 4 and Figure 1].

For BDA cases, there was significant RNFL-t of mean total, inferior, nasal, and temporal quadrants, as compared to the control (92.26 ± 8.0 μm vs. 97.28 ± 5.06 μm, $P < 0.001$; 119.11 ± 11.8 μm vs. 126.03 ± 7.88 μm, $P < 0.001$; 69.74 ± 8.30 μm vs.

Table 1: The demographic and clinical characteristics of the iron-deficiency anemia and control groups

IDA	Variables	Mean±SD		*P*
		Anemic group, (*n*=50)	Control group, (*n*=50)	
	Age (years)	38.08±11.83	42.40±12.90	0.084
	Sex (female: male) (%)	31:19 (62/38)	28:22 (56/44)	0.061
Blood parameters	Hb% (g/dL)	7.39±1.99	13.58±1.43	<0.001
	MCV (fL)	67.04±10.67	89.58±2.25	<0.001
	MCH (pg)	20.42±5.62	30.41±1.42	<0.001
	MCHC (g/dL)	29.63±3.06	31.69±0.99	<0.01
	Serum iron (μg/dL)	23.29±12.28	87.07±2.45	<0.001
	Serum ferritin (μg/dL)	10.44±6.71	38.50±10.57	<0.001
	Serum TIBC (μg/dL)	347.98±39.92	292.81±16.10	<0.001
	Serum Vitamin B12 (pg/mL)	820.48±181.29	830.86±157.96	0.758
	Serum folic acid (ng/mL)	10.9±4.63	11.08±4.60	0.845
Optic disc parameters	C/D ratio	0.44±0.12	0.45±0.11	0.576
	Rim area (mm^2)	1.40±0.43	1.43±0.37	0.087
	Disc area (mm^2)	2.27±0.49	2.31±0.45	0.475
Peripapillary RNFLT	Superior (μm)	117.82±13.57	123.23±8.13	<0.001
	Inferior (μm)	119.18±11.90	126.03±7.88	<0.001
	Nasal (μm)	68.53±5.79	74.82±6.02	<0.001
	Temporal (μm)	62.91±8.06	66.20±6.10	0.0014
	Total (μm)	92.62±8.22	97.28±5.06	<0.001

IDA=Iron-deficiency anemia, Hb=Hemoglobin, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, TIBC=Total iron binding capacity, RNFLT=Retinal nerve fiber layer thickness, C/D=Cup: disc, SD=Standard deviation

Table 2: The demographic and clinical characteristics of the Vitamin B12-deficiency anemia and control groups

BDA	Variables	Mean±SD		*P*
		Anemic group, (*n*=50)	Control group, (*n*=50)	
	Age (years)	43.58±13.40	42.40±12.90	0.650
	Sex (%)	27:23 (54/46)	28:22 (56/44)	0.214
Blood parameters	Hb% (g/dL)	7.62±1.86	13.58±1.43	<0.01
	MCV (fL)	110.23±7.63	89.58±2.25	<0.01
	Serum Vitamin B12 (pg/mL)	175.3±22.71	830.86±157.96	<0.001
	Serum folic acid (ng/mL)	10.8±4.26	11.08±4.60	0.752
	Serum iron (µg/dL)	82.19±18.74	87.07±2.45	0.070
	Serum. ferritin (µg/dL)	41.91±7.23	38.50±10.57	0.062
Optic disc parameters	C/D ratio	0.43±0.12	0.45±0.11	0.221
	Rim area (mm^2)	1.47±0.33	1.43±0.37	0.422
	Disc area (mm^2)	2.39±0.47	2.31±0.45	0.221
Peripapillary RNFLT	Superior (µm)	121.62±10.03	123.23±8.13	0.214
	Inferior (µm)	119.11±11.8	126.03±7.88	<0.001
	Nasal (µm)	69.74±8.30	74.82±6.02	<0.001
	Temporal (µm)	63.28±7.87	66.20±6.10	0.003
	Total (µm)	92.26±8.0	97.28±5.06	<0.001

BDA=Vitamin B12 deficiency anemia, Hb=Hemoglobin, MCV=Mean corpuscular volume, RNFLT=Retinal nerve fiber layer thickness, C/D=Cup: disc, SD=Standard deviation

Table 3: The demographic and clinical characteristics of the Folic acid-deficiency anemia and control groups

FDA	Variables	Mean±SD		*P*
		Anemic group, (*n*=15)	Control group, *n*=50)	
	Age (years)	42.40±17.30	42.40±12.90	1.000
	Sex (%)	30:20 (60/40)	28:22 (56/44)	0.150
Blood parameters	Hb% (g/dL)	9.7±1.75	13.58±1.43	<0.001
	MCV (fL)	108±11.39	89.58±2.25	<0.001
	Serum Vitamin B12 (pg/mL)	736.86±228.35	830.86±157.96	0.074
	Serum folic acid (ng/mL)	2.27±0.32	11.08±4.60	<0.001
	Serum iron (µg/dL)	85.14±6.57	87.07±2.45	0.08
	Serum ferritin (µg/dL)	45.2±20.6	38.50±10.57	0.06
Optic disc parameters	C/D ratio	0.43±0.12	0.45±0.11	0.394
	Rim area (mm^2)	1.37±0.29	1.43±0.37	0.416
	Disc area (mm^2)	2.25±0.50	2.31±0.45	0.533
Peripapillary RNFLT	Superior (µm)	124.9±5.4	123.23±8.13	0.293
	Inferior (µm)	122.8±6.76	126.03±7.88	0.04
	Nasal (µm)	68.43±4.04	74.82±6.02	<0.001
	Temporal (µm)	64.23±3.60	66.20±6.10	0.09
	Total (µm)	95.07±3.45	97.28±5.06	<0.001

FDA=Folic acid-deficiency anemia, Hb=Hemoglobin, MCV=Mean corpuscular volume, RNFLT=Retinal nerve fiber layer thickness, C/D=Cup: disc, SD=Standard deviation

Table 4: Correlations between retinal nerve fiber layer thickness and hematological parameters within the iron-deficiency anemia study group

IDA	Correlation	Serum Hb%	Serum iron	Serum ferritin	Serum TIBC	MCV	MCH	MCHC
Mean total RNFLT (92.62±8.22 µ)	Pearson's correlation (*r*)	0.613	0.610	0.336	–0.509	0.295	0.337	0.374
	P	0.000	0.000	0.001	0.000	0.003	0.001	0.000
	Eyes (*n*)	98	98	98	98	98	98	98

IDA=Iron-deficiency anemia, RNFLT=Retinal nerve fiber layer thickness, Hb=Hemoglobin, TIBC=Total iron binding capacity, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration

74.82 ± 6.02 µm, $P < 0.001$; and 63.28 ± 7.87 µm vs. 66.20 ± 6.10 µm, $P < 0.003$, respectively) but not superior quadrant (121.62 ± 10.03 µm vs. 123.23 ± 8.13 µm, $P = 0.214$) [Table 2]. A significant Pearson's correlation of mean total RNFLT was established positively with serum Hb% and B12 ($r = 0.310$, $P = 0.002$, and $r = 0.435$, $P < 0.001$, respectively) but negatively with serum MCV ($r = –0.386$, $P < 0.001$) [Table 5 and Figure 2]. Only eight (0.16%) BDA patients had associated nonspecific neurological features that were mostly limited to paresthesia of extremities,

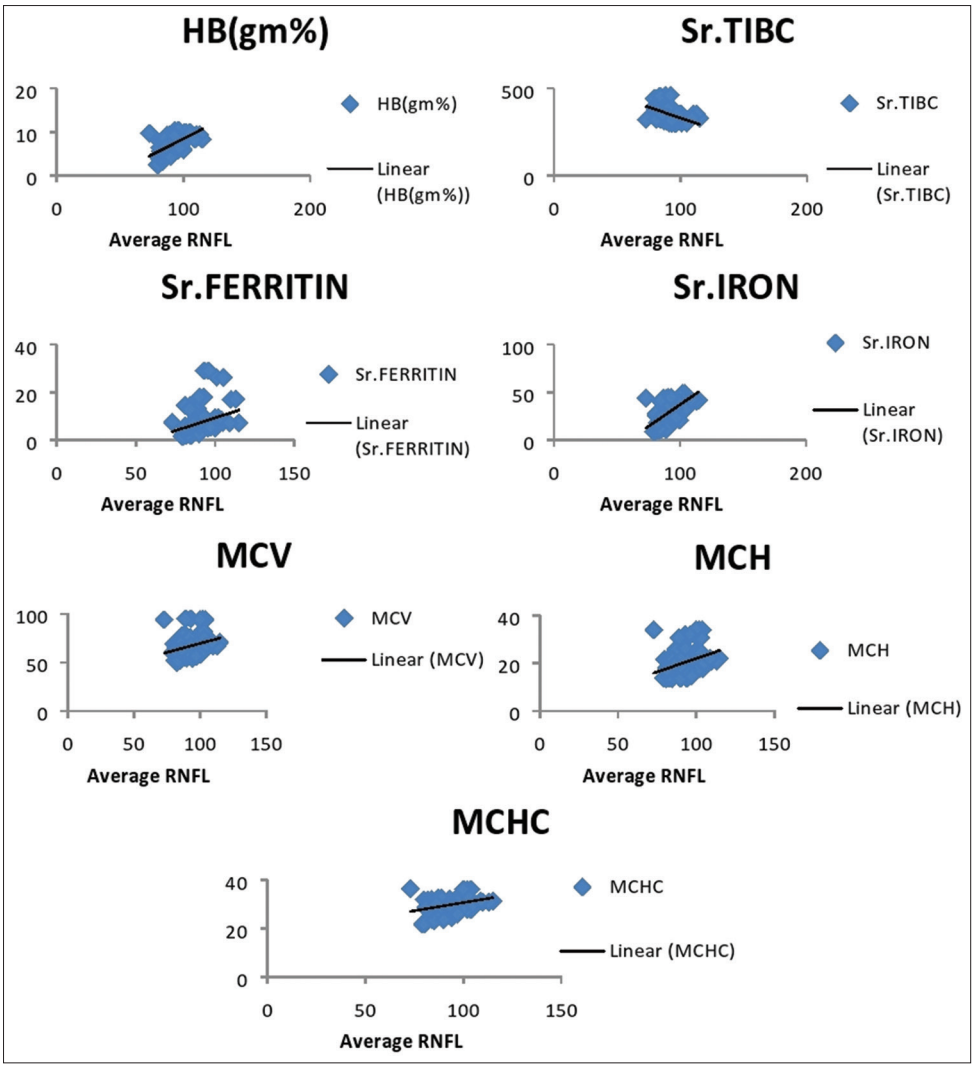

Figure 1: Correlation analysis of mean total RNFLT and different laboratory parameters in the IDA group. Average RNFL: Mean total retinal nerve fiber layer, Hb: Hemoglobin, TIBC: Total iron binding capacity, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, IDA: Iron-deficiency anemia

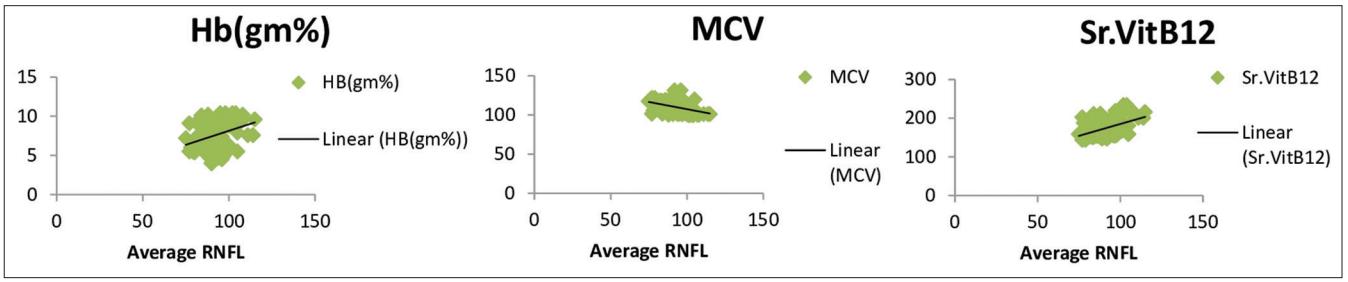

Figure 2: Correlation analysis of mean total RNFLT and different laboratory parameters in the BDA group. Average RNFL: Mean total retinal nerve fiber layer, Hb: Hemoglobin. MCV: Mean corpuscular volume, RNFLT: Retinal nerve fiber layer thickness, BDA: B12-deficiency anemia

movement disorders, auditory symptoms, and mental depression.

For FAD cases, significant RNFL-t was observed in mean total, nasal, and inferior quadrants of retina ($P < 0.05$) but not in superior and temporal quadrants (124.9 ± 5.4 μm vs. 123.23 ± 8.13 μm, $P = 0.293$, and 64.23 ± 3.60 μm vs. 66.20 ± 6.10 μm, $P = 0.09$, respectively) [Table 3]. Further, mean total RNFLT had a significant positive correlation with serum Hb% and FA ($r = 0.557$, $P = 0.001$, and $r = 0.358$, $P < 0.0001$, respectively) but a significant negative correlation with serum MCV ($r = -0.294$, $P = 0.002$) [Table 6 and Figure 3].

Mean total RNFL-t in all NDA patients showed progression with the increasing severity of anemia,

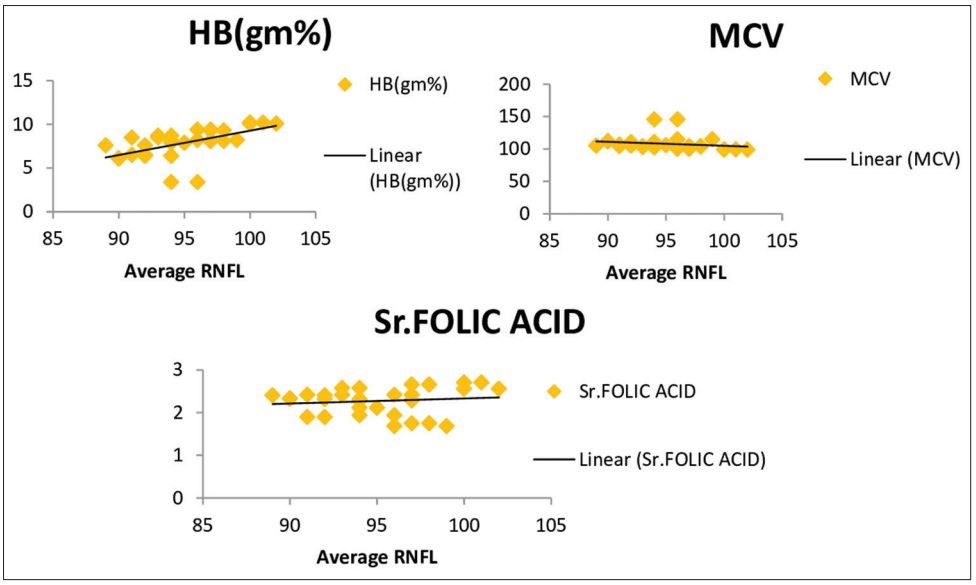

Figure 3: Correlation analysis of mean total RNFLT and different laboratory parameters in the FDA group. Average RNFL: Mean total retinal nerve fiber layer, Hb: Hemoglobin, MCV: Mean corpuscular volume, RNFLT: Retinal nerve fiber layer thickness, FDA: Folic acid-deficiency anemia

Table 5: Correlations between retinal nerve fiber layer thickness and hematological parameters within the Vitamin B12 deficiency anemia study group

BDA	Correlation	Serum Hb%	MCV	Serum B12
Mean total RNFLT (92.26±8.0 μ)	Pearson's correlation (*r*)	0.310	−0.386	0.435
	P	0.002	0.000	0.000
	Eyes (*n*)	99	99	99

BDA=Vitamin B12 deficiency anemia, Hb=Hemoglobin, MCV=Mean corpuscular volume, RNFLT=Retinal nerve fiber layer thickness

Table 6: Correlations between retinal nerve fiber layer thickness and hematological parameters within the folic acid-deficiency anemia study group

FDA	Correlation	Serum Hb%	MCV	Serum folic acid
Mean total RNFLT (95.07±3.45 μ)	Pearson correlation	0.557	−0.294	0.358
	P	0.001	0.002	0.000
	n	30	30	30

FDA=Folic acid-deficiency anemia, Hb=Hemoglobin, MCV=Mean corpuscular volume, RNFLT=Retinal nerve fiber layer thickness

except in very severe BDA, where an inverse relationship was documented [Figure 4]. In this study, none of the patients required bone marrow examination to be performed, as none was detected to have pancytopenia or features of nonmegaloblastic anemia.

Discussion

The latest National Family Health Survey 4 carried out by the Ministry of Health and Family Welfare, India, reported that the prevalence of anemia was 53.1% and 22.7% among Indian women aged 15–49 years and men aged 15–49 years, respectively.[1]

To the best of our knowledge, most RNFL studies reported on IDA are oriented to the females (with or without conception) or children, whereas no study has yet been reported on BDA or FDA other than handful of studies based only on their subnormal deficient status.[9,11,21-27]

In the present study, in the IDA group, the mean RNFLT in total, superior, inferior, nasal, temporal quadrants of retina was significantly thinner, as compared to the control group ($P < 0.001$). RNFLT studies on adult IDA patients comprising both the genders are limited. A recent, probably the first similar Indian study conducted by Jaiswal *et al*. with SD-OCT reported RNFL-t of mean total and all four quadrants; however, the mean inclusion age was less as compared to this study.[28] From overseas, RNFL studies reported are mostly oriented to adult females with IDA; Acir *et al*. with Cirrus HD-OCT reported insignificant differences of mean total or any four RNFL quadrants, except at four- and five-clock hour quadrant (nasal-inferior); Akdogan *et al*. reported significant RNFL-t in the nasal and inferior quadrants; Cikmazkara and Ugurlu reported RNFL-t of mean total and all three quadrants, except superior; and Eltohamy *et al*. reported thinning of all four including mean total quadrant.[21-24] Türkyilmaz *et al*. reported thinning of superior and inferior quadrants only in children.[25] Differences in criteria for case selection and defining anemia in terms of various hematological parameters such as Hb% cutoff value, type of OCT machine used, and geographical variation in RNFLT might have led to such varying results. The mean total RNFLT of IDA cases in this study was significantly correlated positively with serum Hb%, ferritin, iron, MCV, MCHC, and MCH and negatively with serum TIBC. Although this result was

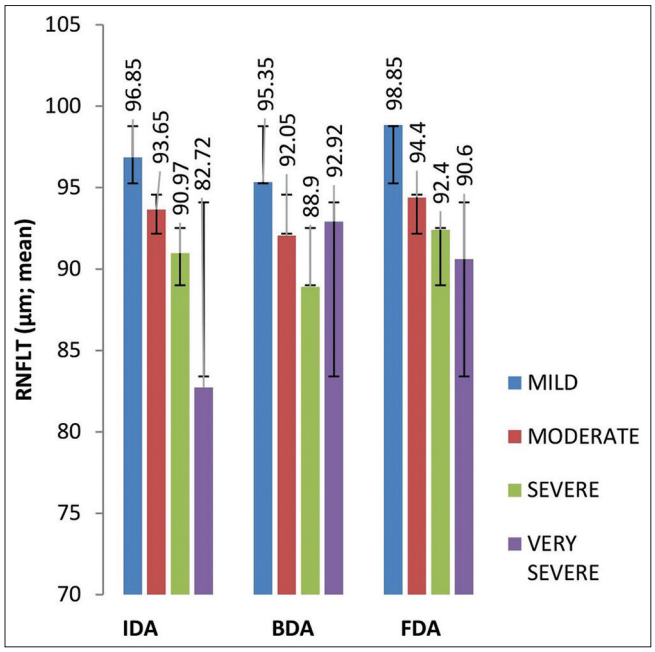

Figure 4: Mean total RNFLT of the IDA, BDA, and FDA groups of patients according to the increasing severity grades of anemia. IDA: Iron-deficiency anemia, BDA: Vitamin B12 deficiency anemia, FDA: Folic acid-deficiency anemia, RNFLT: Retinal nerve fiber layer thickness

fully supported by the result reported by Jaiswal *et al*.[28] and Cikmazkara *et al*.,[22] the correlation established by others differs with the present study (Türkyilmaz *et al*.[27]: mean total with Hb%, Acir *et al*.[23]: clock hour 10 with ferritin, Eltohamy[24]: mean total with serum Hb% and iron, and Akdogan *et al*.[21]: did not find any correlation with any blood parameters).

In the BDA group, this study showed significant RNFL-t of the mean total as well as three out of four quadrants (except superior). Currently, there is no supportive study reported worldwide yet. However, working with patients with subclinical subnormal B12 level, Türkyilmaz *et al*. reported significant mean total and temporal RNFL-t as measured by Cirrus HD-OCT in younger B12-deficient adult patients (mean age = 31 years, serum B12 cutoff ≤189 pg/mL); Dogan *et al*. reported insignificant RNFL-t of mean total and superior-temporal as measured by Spectralis OCT in B12-deficient adult patients taking cutoff ≤200 pg/dL; Özkasap *et al*. reported significant superior but insignificant mean total RNFL-t as measured by Cirrus HD-OCT in children (8–16 years of age, serum B12 <200 pg/mL).[9,26,27] Variation in case selection (age, nonanemic patients, duration, and severity of deficiency), criteria for serum B12 cutoff level, and OCT techniques possibly might have led to such differences in their results. In this study, in all BDA patients, mean total RNFL-t was found significantly correlated positively with the serum B12 level and Hb% and negatively with MCV. In contrast, Türkyilmaz *et al*., and Özkasap *et al*. reported a positive correlation of the mean total RNFLT with serum B12 only. Whereas, Dogan *et al*. failed to find any correlation of mean total RNFLT with serum B12 level. Variation in the nature of the study as already explained, as well as geographic and age-related variation in retinal thickness, possibly played a role for such differences. Diagnosis of subclinical BD without anemia is difficult.[9,29] Appearance of overt clinical symptoms may take 5–6 years after the onset of BD, and due to difficulty in laboratory diagnosis, results even from standard laboratories are not reliable due to several reasons.[30,31] Nonspecific neurological features may arise even without hematological manifestation, and the nature of alternate demyelination–remyelination process in persistent B12 deficiency further complicates the diagnostic accuracy.[32]

Distinguishing FDA from BDA is another difficult task, as both have similarity in their etiology and have overlapping clinical and hematological manifestations.[33] The prime reason for lesser number of FDA cases in our study was possibly due to the fact that we excluded combined FDA/BDA cases, and there was only one case under very severe grade. The prevalence of FDA is not as high in Indian population as compared with BDA.[1] In this study, FDA cases showed significant RNFL-t in the mean total, nasal, and inferior quadrants of retina, except temporal and superior quadrant. So far, literature search revealed only one such related study reported worldwide, where Ceylan *et al*. found significant RNFL-t of mean total and all except temporal quadrant using SD-OCT (mean age: 71.71 years, serum FA level <7 nmol/L).[11] We believe that their result differs with this study because of the differences in the mean age, serum FA cutoff level, exclusion of anemic cases, and geographic variation of normal RNFLT. The mean total RNFLT of our FDA cases was significantly correlated positively to that with serum Hb% and FA level and negatively to that with MCV. There is no such study reported yet where all the three parameters were considered simultaneously; however, Ceylan *et al*. reported a positive correlation of FA level with mean total RNFLT.

In this study, except in the very severe form of BDA patients, graded thinning of mean total RNFLT in all other patients of NDA was observed, which was at par with the advancement of their severity. We assume that there may be an existence of inverse relationship between the severity of BDA and degree of neurological features based on alternate degeneration–regeneration process in neuronal cells, as reported by Pandey *et al*. and Healton *et al*. in their study. Further related large-scale studies may certainly throw some light on such paradox.[32,34]

Limitations of the study were: cross sectional nature of the study did not allow us to study the effect of micronutrient supplementation on RNFLT; duration

of anemia could not be ascertained due to lack of information provided by the patient; FDA cases were less in number and tests such as gastrointestinal endoscopy, magnetic resonance imaging spinal cord, methylmalonyl coenzyme test, serum homocysteine estimation, etc., could not be performed because of logistics or nonavailability. Nevertheless, the characteristic changes observed in this study may have enormous clinical significance. Further, being a regional study and also as we excluded children and pregnant females, our study population may not represent the whole general population of our country. Thus, further randomized multicenter trials would be necessary to validate the facts generated from this study.

Conclusion

In the current study, SD-OCT showed a significant peripapillary RNFL-t of the mean total, inferior, superior, nasal, and temporal quadrants of retina in IDA patients; of the mean total, inferior, nasal, and temporal quadrants in BDA patients; and of the mean total, inferior, and nasal quadrants in FDA patients, as compared to their age- and sex-matched healthy nonanemic control. Further, in all NDA patients, the mean total RNFLT significantly correlated with their corresponding hematological parameters and showed a graded thinning response at par with progression of severity of anemia other than very severe grade of BDA. Thus, SD-OCT may be a valuable tool to detect RNFL-t in NDA patients at its earliest in preventing further irreversible damage and may prove informative for differentiating from other nonglaucomatous optic neuropathies, such as common neuro-ophthalmic and neuropsychiatric disorders, and may lay down preventive therapeutic strategies for high-risk patients. Since the prevalence of NDA is very high, the RNFL evaluation should be done in all the cases of anemia which would act as indirect evidence and also as a marker for the severity of anemia. This would in turn aid the graded diagnosis and guide the subsequent management of the patients accordingly. Further similar prospective, multicenter, randomized studies with bigger cohort are warranted to better understand and characterize the outcomes of this study.

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Patient's informed consent

Informed consent was obtained from all individual participants included in this study. No identifiable patient information was used (patient photograph or images related).

Compliance with ethical standards

Although this is an observational study, the Institutional Ethics Committee was received prior to the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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