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Retina

Macular Pigment Assessment in Indian Population Using Degree of Polarization Threshold: Impact of Diet on Macular Pigment Density

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Purpose: To determine macular pigment (MP) density scores in healthy Indians and examine correlations with demographic and lifestyle variables.

Methods: We observed 484 Indians without an ocular pathology. Body mass index (BMI) and self-reported lifestyle factors (sunglasses usage, physical activity, and smoking) were noted. MP density was assessed as the threshold of perception of the shadow of their macular pigments on their retina using a new MP assessment tool (MP-eye). Lutein and zeaxanthin intake was assessed using a prevalidated questionnaire regionally designed for the Indian diet. Clusters of participants were created for statistical analysis based on MP-eye scores secondarily to detect any relevant effects in very low, low, medium, and high ranges of MPs.

Results: Data analyzed included 235 males and 249 females with mean age of 36.1 $\pm$ 12.9 years (range, 14–72). The median MP-eye score was 6 (range, 0–10, with 10 being high). Most were non-smokers (413, 85.3%) and did not use sunglasses (438, 90.5%), and 314 (64.9%) had low physical activity. Diabetes was present in 62 participants (12.8%) and hypertension in 53 (10.9%). Advancing age ($r = -0.209$; $P < 0.000$) and BMI ($r = -0.094$; $P = 0.038$) had weak negative correlation with MP-eye scores. Hypertension was less prevalent (7/88) in the cluster with the highest median MP-eye score ($P = 0.033$). Dietary intake of MPs and other lifestyle factors did not correlate significantly with MP-eye score overall or when analyzed in clusters.

Conclusions: MP-eye scores of an Indian population were normally distributed. Higher age, high BMI, and presence of hypertension were weakly associated with lower MP-eye scores. The impact of diet on MPs requires further evaluation.

Translational Relevance: This normative regional database enables risk stratification of macular degeneration.

Introduction

The ability of humans to perceive polarized light is a well-described phenomenon known as Haidinger's brushes (HBs).[1–6] It is dependent on xanthophyll carotenoids, including lutein, zeaxanthin, and meso-zeaxanthin, present in the retina, collectively referred to as macular pigments. This entoptic phenomenon is mediated by the absorption of short-wavelength light and radial arrangement of macular pigments in lipid bilayers of retinal axons and Müller cells that

emanate outward from the center of the fovea like spokes of a wheel. This arrangement creates concentric circular alignment of the long axes of diattenuating macular pigment molecules.[7,8] The tissue-specific arrangement limits the perception of polarized light to the macula,[9,10] where it appears as a bow-tie or hourglass-shaped pattern. The phenomenon and its dependency on macular pigments have been utilized by a novel macular pigment (MP) measuring device, the MP-eye (Azul Optics, Bristol, UK). This device uses the threshold at which a subject can just perceive HBs, when the degree (percent) of polarized light is decreased, to quantitively assess macular pigment density (MPD) as the MP-eye score.

MPs strongly absorb high-energy visible (violet–blue) light and have antioxidative, antiinflammatory, and neuroprotective properties.[11,12] Although they have been studied for various macular disorders such as diabetic retinopathy, macular telangiectasia, central serous chorioretinopathy, and retinal dystrophies, most research on MPs is focused on age-related macular degeneration (ARMD).[13,14] ARMD can be a disabling disease and is the leading cause of blindness in Western populations. Prevention of ARMD is important, as there is no available cure, and it is estimated that it will affect 288 million people by 2040.[15] MPs are protective against ARMD, which is supported by an inverse relationship between the incidence of ARMD and (1) macular pigment density (MPD), (2) serum concentration of lutein and zeaxanthin, and (3) dietary intake of lutein and zeaxanthin.[16–19] Recently, 10-year outcomes of the Age-Related Eye Disease Study 2 (AREDS2) suggest that the consumption of lutein (10 mg/d) and zeaxanthin (2 mg/d) offers an additional risk reduction for the progression of ARMD.[20]

The amount of macular pigment in retina is dependent on both physiology and lifestyle. MPs cannot be produced by the body; therefore, they must be ingested. Dietary sources include egg yolks, dark green leafy, and colorful vegetables and fruits. The uptake of MPs is dependent on effective digestion and sequestration, as well as carrier and binding proteins, to ensure that these xanthophyl carotenoids make their way from the gut to the retina.[21] Lutein and zeaxanthin are fat soluble and readily stored in adipose tissue, and body fat content has been found to be inversely correlated with MPD.[22] In addition, because the antioxidant activity of MPs is finite, lifestyle behaviors that increase oxidative stress such as poor diet, smoking,[23,24] and sun exposure[25–27] have been found to be inversely correlated with MPD, whereas physical activity, which can increase the body's ability to deal with oxidative stress, is positively correlated with MPD.[28–32]

Methods used to measure MPD can be grouped into two categories: (1) *psychophysical*, including heterochromatic flicker photometry,[33–35] motion photometry,[36] chromatic visual evoked potential,[37] and degree of polarization threshold[38,39]; and (2) *optical*, including fundus reflectance,[40] macular pigment reflectometry,[41–44] dual-wavelength autofluorescence,[45–47] resonance Raman spectroscopy, and fluorescence lifetime imaging ophthalmoscopy.[48] Of these, determining the degree of polarization threshold, as implemented by the MP-eye, offers the advantage of being a portable, fast, repeatable, and easy-to-perform nonmydriatic test. Thus, it fits well into regular optometric eye exams and large-scale public health screening for disease prevention.[49,50]

In this study, we provide a normative regional database for assessment of MPD using the MP-eye tool. MP concentrations in the eye depend on the SLAMENGHI factors, which refer to amount and characteristic of MPs ingested; factors affecting their absorption; host factors including nutrition, lifestyle, and genetics; and other interactions.[51,52] Understanding of the role of MPs in health has evolved toward the need to evaluate and supplement MPs in "normal" individuals much before disease manifests, prompting steps such as maternal supplementation of MPs in the prenatal period in large randomized trials.[53] Therefore, in the current study, we also evaluated the impact of diet on MPD by using regionally designed food frequency questionnaires, and we provide the self-reported lifestyle factors and systemic health of our study sample.

Methods

Design

This was a cross-sectional, hospital-based study of Indians without any ocular pathology barring refractive error. The study protocol was reviewed and approved by the LV Prasad Eye Institute Ethics Committee (LEC-BHR-P-07-21-719). The study was registered with the Indian Clinical Trial Registry (CTRI/2021/09/-36892). All of the study procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from each participant.

Participants

Enrollment took place between December 2021 and April 2022 at a tertiary care center in South India. Participants were screened from the comprehensive eye clinic using an electronic medical record system. The

sample size was set as more than 500 to have relevant numbers for each of the subgroups (enumerated later). Ocularly healthy individuals between the ages of 14 and 75 years with best-corrected visual acuity better than 20/25, and without any documented ocular pathology were included. Participants were excluded who were unable to understand the test procedure or were unable to communicate their psychophysical inputs (verbally or by a rotatory finger motion, which was necessary to perform the subjective test task). People with known ocular diseases or suspicious undiagnosed macular findings, high refractive error (myopia, ≤ -6.00 D; hyperopia $>+5.00$ D), or severe convergence dysfunction (inability to form a single binocular test image) were also excluded. Color vision of all participants was assessed prior to recruitment using standard HRR plates. Participants using dietary supplements of any kind were also excluded.

Data Collection

Medical history, demographic details (age, sex, smoking, sunglasses usage, and exercise), body mass index (BMI, standard weight and height ratio in kg/cm^2), dietary intake of lutein and zeaxanthin (LZ), and features of a standard ocular examination were recorded. The participants were asked questions about smoking, sunglasses usage, and physical exercise. The participants assigned a grade to themselves for all of these lifestyle factors. BMI was calculated through a single manual measure using a calibrated analog weighing scale and a standing height scale bar. These measurements were obtained by a single observer (PS) on a single device, which was calibrated daily. Additionally, fundus photographs were acquired for all participants to document retinal status (central 45° using a Visucam 500; Carl Zeiss Meditec, Jena, Germany). Dietary assessment and the MP-eye test were performed as described below.

Procedures

MP-Eye Measurement

MPD testing was done by a single observer (PS) who underwent training with the MP-eye developer (ST). Details of the testing procedure were the same as protocol 3 in Temple et al.,[54] where a single descent variation of the method of limits was used. Prior to testing, the participant was shown a simulation of the HBs phenomenon on an Android tablet screen to familiarize them with the rotation speed, color, and approximate size of the effect as it would appear during the test. Verbal instructions were given on how to perceive the effect and how the test would proceed. During testing the participant looked into the device with both eyes and identified the direction of rotation of HBs at the easiest setting (i.e., highest degree of polarization; 98% for step 1). After correctly identifying the direction of rotation, the participant moved to the next presentation. The first three presentations were at the easiest settings. After that, each step got progressively more difficult to see by reducing the degree of polarization of the stimulus, which made HBs appear fainter. The first presentation was always clockwise and the second always anticlockwise, which enabled the operator to ensure that the participant was confident identifying clockwise and anticlockwise. The third presentation and all subsequent presentations were presented in a pseudorandom order of clockwise and anticlockwise, with software enforcing a maximum of three consecutive sequential and similarly oriented presentations. The operator did not know the rotation direction, which ensured removal of observer bias. The participants were instructed not to guess and to tell the operator when they could no longer determine the direction of rotation. The last step (degree of polarization) for which participants could correctly identify the direction of rotation was their threshold. Each participant was assigned a score out of 10, with 1 being the easiest step (98% polarized) and 10 being the most difficult step (15% polarized). The software permitted only one error to be made during an MP-eye examination. A second error ended the test.

If the participant could not perceive HBs at the first step (i.e., they made a mistake in any of the first three presentations), then the device automatically switched the background illumination to blue instead of white. Blue makes the HBs phenomenon much easier to see because MPs strongly absorb violet–blue light. The participant was then presented with up to eight steps of decreasing degree of polarization in blue. However, the final score was 1 out of 10, because the blue mode is much easier than observing HBs under white illumination.

Diet Questionnaires for LZ Intake

We could not find a validated dietary questionnaire for LZ intake based on an Indian diet. To create a weekly recall-based food frequency questionnaire, we worked with local dietitians to assess food commonly consumed by the regional population and created a list of items with high levels of LZ. The LZ values for all of these foods items (per 100 g) were obtained using their individual values as previously reported by Perry et al.[55] and were incorporated into a LZ questionnaire (Tufts-LZQ). A second questionnaire scoring was based on the Indian Food Composition Tables (IFCT-LZQ).[56]

Table 1. Common Foods Rich in Lutein and Zeaxanthin Available and Consumed in India

IFCT (2017) Food List	Lutein + Zeaxanthin (mg/100 g Food)	Perry et al. (2009) Food List	Lutein + Zeaxanthin (mg/100 g Food)
Amaranth leaves (chaulai), green	8561	Kale or spinach, cooked, boiled, drained, with salt	8884
Spinach, cooked	3867.23	Peppers, sweet, orange, raw	1873
Radish leaves	1763.71	Zucchini (courgette)	1355
Watermelon	972.17	Fenugreek (methi) leaves	6603
Tomato, ripe	1180.74	Kale or spinach, raw	6603
Spinach, raw	3867.23	Lettuce, romaine, raw	3824
Field beans (average of all types of green broad beans), cooked	627.65	Broccoli, raw or cooked, boiled, drained, with salt	772
Coriander leaves	6379.3	Avocado (California), skin and seed removed	400
Bengal gram (chickpea), whole	410.74	Beans, snap, green, frozen, cooked, boiled, drained, with salt	306
Peas, dry	497.41	Corn, sweet, yellow, frozen, kernels off cob, boiled, drained, with salt	404
Cluster beans (gawar phali)	559.49	Lima beans, large mature seeds, cooked, boiled, drained, with salt	155
Fenugreek leaves	2303.28	Noodles, egg, spinach, enriched	176
Ladies finger	801.53	Squash, yellow, cooked, boiled, drained, with salt	150
Colocasia leaves (taro leaves), green	5358	Wheat bran	240
Bengal gram (chana dal)	315.05	Grain, cornmeal, yellow, (Quaker)	1532
Papaya, ripe	285.43	Onions, spring or scallions (includes tops and bulb), cooked in oil	2488
Sweet potato	384.5	Snack, pistachio, dry roasted, with salt	1405
Papaya, raw	273.88		
Red chillies	4555		
Potato	131.35		
Bitter gourd	286.32		

Data from Perry et al.[55] and the 2017 Indian Food Composition Tables.[56]

The food items were listed and prioritized based on expected cumulative LZ intake, accounting for both the nutrient density and typical dietary frequency as a multiple. The number of food items on our list was restricted to develop a final questionnaire that could be answered within 5 to 7 minutes so subject recall bias could be negated. Images of the food items were included in the questionnaire that was administered vernacularly. Two separate questionnaires were developed based on US and Indian databases, both of which generated separate daily LZ intake values (Table 1). These were then tested in a pilot feasibility study on 51 subjects by two separate observers (PS, SB). Following the process, these 51 subjects were excluded from the rest of the analysis.

Outcome Measures

The primary outcome measure was the MP-eye score for each participant. Secondarily, the response time to complete the test was recorded. Age, gender, history of systemic diseases, refractive error, daily LZ intake, BMI, smoking, physical activity, and use of sunglasses were independent variables.

Statistical Analysis

First, we looked for correlations between MP-eye scores and the independent variables using Spearman's correlation coefficient (Table 2). Then, because we did not see expected correlations discussed previ-

Table 2. Summary of Participant Characteristics and Parameters Tested in the Study ($N = 484$)

Variable	Unit of Measure	Overall	Cluster 1 ($n = 77$) Very Low Scores	Cluster 2 ($n = 126$) Low Scores	Cluster 3 ($n = 170$) Medium Scores	Cluster 4 ($n = 111$) High Scores	Intercluster P (<0.05)	
Age (y)	Mean (range)	36.1 (14–72)	40.9 (15–66)	37.4 (17–65)	34.8 (14–72)	33.1 (16–64)	<0.001 (Kruskal–Wallis test)	
	r_s (P)[a]	**−0.209**[b] **(0.000)**	−0.207 (0.07)	−0.049 (0.59)	−0.034 (0.66)	−0.144 (0.13)		
Gender, n	Male/female	235/249	35/42	56/70	89/81	55/56	0.537 (χ^2)	
MP-eye score	Median (range)	6 (0–10)	2 (0–3)	5 (4–5)	6 (6–7)	8 (8–10)	—	
LZQ dietary intake (μg/d)								
IFCT score (μg)	Mean (range)	1754 (249.5–5817.4)	1622.4 (249.5–5797.5)	1774.5 (242.9–5817.4)	1706.5 (195–5499.6)	1894.8 (297.7–5137)	0.226 (Kruskal–Wallis test)	Between Tufts and IFCT LZQ **<0.01** ($r = 0.793$[c]) (paired t-test)
	r_s (P)[a]	0.059 (0.19)	0.204 (0.08)	0.015 (0.868)	−0.097 (0.208)	−0.009 (0.924)		
Tufts score (μg)	Mean (range)	902 (0–5518.37)	832.7 (0–5129.34)	794.8 (0–5455.35)	941.5 (0–5290.5)	1011.4 (0–5518.37)	0.568 (Kruskal–Wallis test)	
	r_s (P)[a]	0.062 (0.17)	0.086 (0.46)	0.062 (0.49)	−0.022 (0.77)	0.001 (0.99)		
BMI (kg/m^2), n	Low (<18.5), medium (18.5–24.9), high (>25)	27, 210, 247 (237/247)	5, 28, 44 (33/44)	5, 56, 65 (61/65)	7, 73, 90 (80/90)	10, 53, 48 (63/48)	0.0234 (Kruskal–Wallis test)	
	BMI assigned value, r_s (P)[a]	**−0.109**[b] **(0.017)**	**−0.122**[b] **(0.289)**	0.71 (0.430)	**−0.205**[c] **(0.007)**	**−0.210**[b] **(0.027)**		
	BMI actual value, r_s (P)[a]	**−0.094**[b] **(0.038)**	−0.166 (0.314)	0.00 (0.996)	**−0.242**[c] **(0.001)**	−0.164 (0.085)		
Smoking, n	Yes/no	71/413 (14.7%)	12/65 (15.6%)	20/106 (15.9%)	18/152 (10.6%)	21/90 (18.9%)	0.225 (χ^2)	
Exercise, n	Yes/no	170/314 (35.1%)	22/55 (28.6%)	41/85 (32.5%)	72/98 (42.4%)	35/76 (31.5%)	0.096 (χ^2)	
Sunglasses use, n	Yes/no	46/438 (9.5%)	05/72 (6.5%)	16/110 (12.7%)	14/156 (8.2%)	11/100 (9.9%)	0.450 (χ^2)	
Co-conditions or H/o systemic disease, n	Yes/no	119/365 (24.6%)	24/53 (31.2%)	37/89 (29.4%)	35/135 (20.9%)	23/88 (20.7%)	0.127 (χ^2)	
DM, HTN, dyslipidemia, and other		62, 53, 7, 43	11, 15, 1, 7	20, 15, 3, 13	20, 16, 3, 13	11, 7, 0, 13	0.530, **0.033**, 0.469, 0.734	

[a] r_s (P) values were calculated with the overall MP scores and within a cluster Spearman rank correlation.
[b] Correlation is significant at the 0.05 level (two-tailed).
[c] Correlation is significant at the 0.01 level (two-tailed).

ously,[51,52] we divided the MP-eye score data into four clusters using unsupervised learning *k*-means algorithms in an attempt to gain more statistical power. The elbow method was used to determine the optimal number of clusters in the *k*-means clustering, and e1071 and factoextra in R (R Foundation for Statistical Computing, Vienna, Austria) were used for the cluster analysis. All of the statistical analysis and data management were performed using R. The χ^2 test was used for comparing categorical variables, and the Kruskal–Wallis test was used for comparing continuous variables among the four clusters (Table 2). $P < 0.05$ was considered significant.

Results

Distribution

Of the 560 participants screened, 484 were included in the creation of a generalized normative database. Fifty-one participants were removed from the analysis, as their data were used to refine the diet questionnaire during the pilot phase (see Methods section), and another 25 were removed from the main trial because they did not meet the study criteria due to an inability to comprehend the MP-eye test or due to suspicious fundus photograph findings. MP-eye scores were normally distributed, with a median MP-eye score of 6, ranging from 0 to 10. More than half of the participants had MP-eye scores between 5 and 8 with an interquartile range (IQR) of 3 (Fig. 1a, Table 2). Mean uncorrected visual acuity was found to be 20/30 (0.2 logMAR; IQR, 0–0.4) with the best corrected visual acuity of >20/25 in each case. Mean age was 36 years (range, 14–72), and there were 235 males and 249 females. Most participants were in the third or fifth decade of life (Fig. 1b). More than half ($n = 247$) of the participants had a high BMI (≥25 kg/m^2), and only 27 (5.6%) had low BMI (<18.5 kg/m^2). Most participants were non-smokers ($n = 413$), had reduced physical activity ($n = 314$), and did not regularly wearing sunglasses ($n = 438$) (Table 2). Diabetes ($n = 62$) and hypertension ($n = 53$) were the most commonly reported systemic diseases. Mean time to complete the MP-eye test was 74 seconds (SD = 33 seconds) (Fig. 1).

Dietary Intake of LZ

The mean IFCT-LZQ score was higher than the mean Tufts-LZQ score (1750 vs. 902 µg/wk) (Table 2). The MP-eye scores were distributed similarly in both the dietary intake evaluations, and both IFCT- and Tufts-based LZQ scores correlated well ($r = 0.793$; $P < 0.05$) (Fig. 2). However, the overall correlation between the LZQ scores and the MP-eye scores was low (less than 0.2) and not statistically significant (Table 2). When analyzed by clusters of MP-eye scores, there was a positive trend toward increasing MP-eye scores with increasing LZ intake for both IFCT-LZQ and Tufts-LZQ scores (Fig. 2).

BMI and Other Lifestyle Factors

A weakly negative correlation ($r = -0.1$) was detected between MP-eye score and BMI, which was statistically significant ($P = 0.017$). Similar results were noted across most clusters (discussed later). However, no significant association was observed between MP-eye score and smoking or physical activity or sunglasses

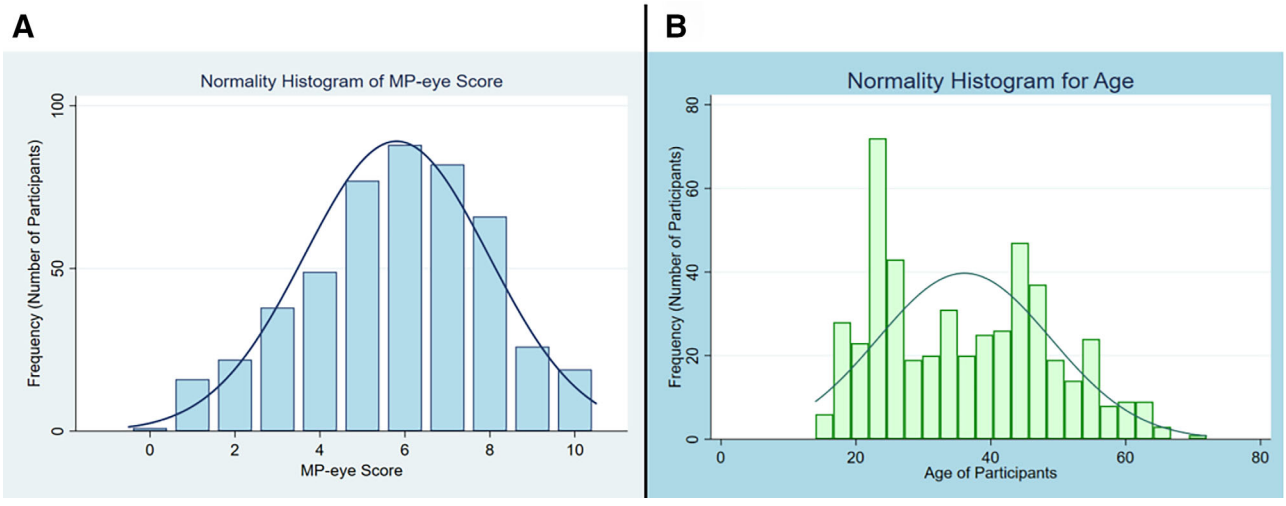

Figure 1. Histograms showing MP scores (**A**) and age distribution (**B**) of the sample participants.

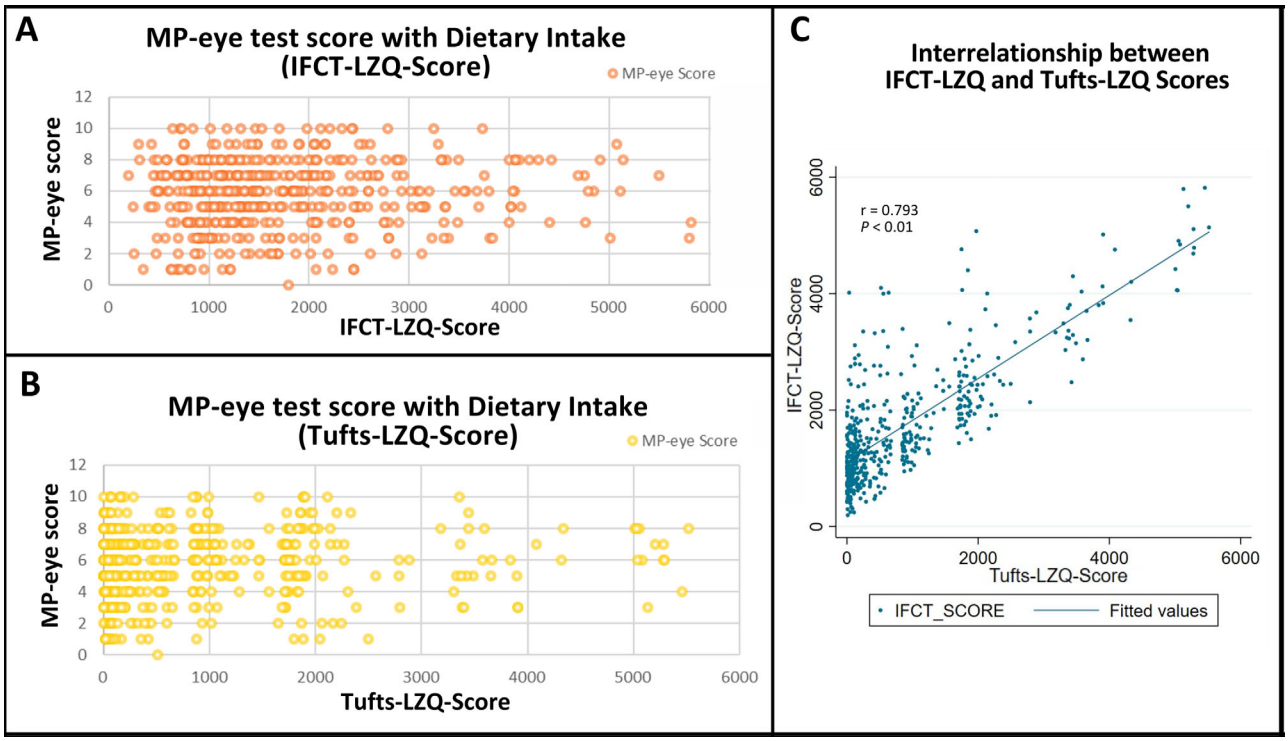

Figure 2. Scatterplots depicting the variation of dietary scores and relation between the two food frequency questionnaires.

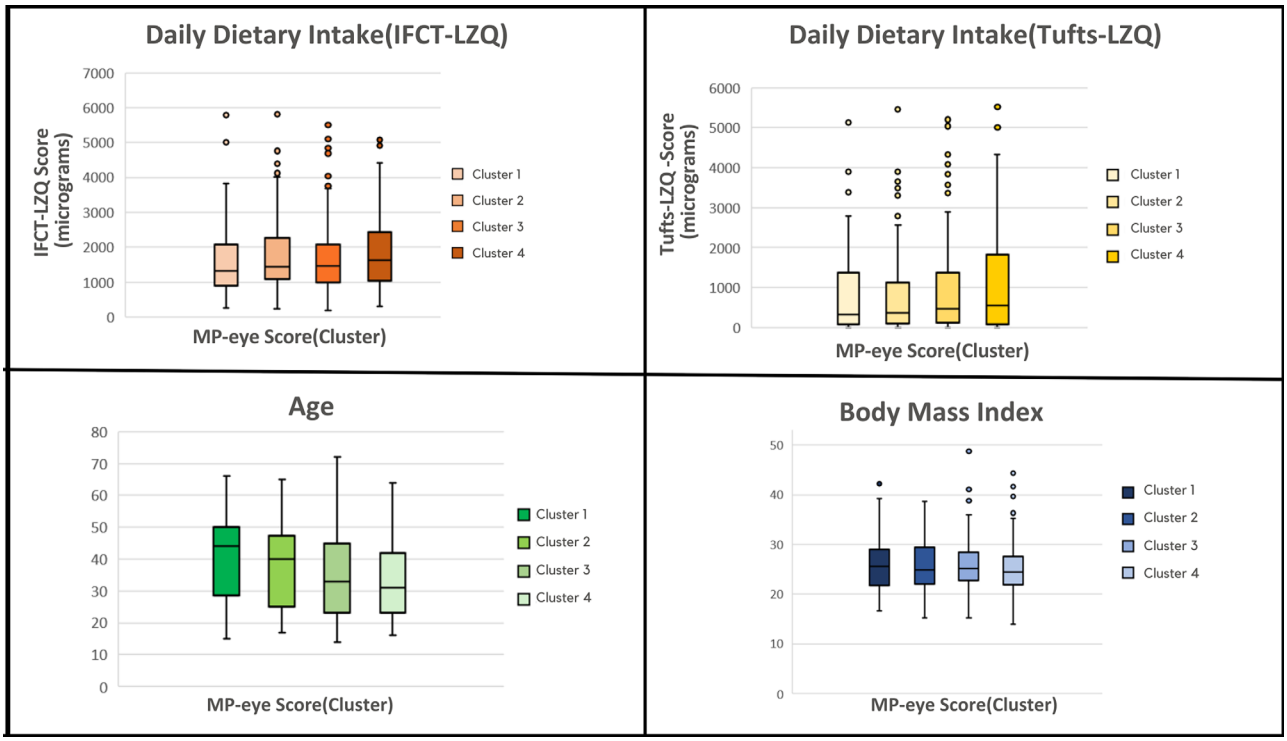

Figure 3. Box plots presenting features of the four clusters and the MP-eye score intercluster relationship with age (**A**), BMI (**B**), IFCT-LZQ (**C**), and Tufts-LZQ 529 (**D**).

usage. Among the systemic diseases, hypertension was significantly ($P = 0.03$) least prevalent among those with high MPD (cluster 4, 7.9%) and most prevalent among those with low MPD (cluster 1, 28.3%).

Clusters

Overall, age had a weakly negative ($r = -0.2$) and significant ($P < 0.001$) relation with MP-eye scores. This, however, could not be reproduced across all of the clusters even though the mean age showed a consistent decreasing trend as the MP-eye score cluster increased (Fig. 3). Apart from the borderline significant relation for IFCT-LZQ ($r = 0.2$; $P = 0.07$) and Tufts-LZQ ($r = 0.5$; $P = 0.06$) scores in cluster 2, there was no significant relation with any of the other clusters with MP-eye score and diet. Mean LZ intake scores were high among those with high MPD (cluster 4 compared to cluster 1), but the difference was not statically significant ($P = 0.19$ and $P = 0.17$, respectively). BMI showed a weakly negative relation ($r = -0.2$) with MP-eye scores in cluster 3 ($P = 0.007$) and cluster 4 ($r = -0.2$; $P = 0.027$), even though the clusters did not compare differently in terms of BMI. All clusters were balanced in terms of gender.

Discussion

By measuring the perceptual ability to detect HBs as the degree of polarization decreases (degree of polarization threshold), the MP-eye provides a score that can be used to assess and compare MPD. Our study has generated a normative dataset for MP-eye scores in an Indian population. The median MP-eye score for this population was 6 (degree of polarization = 55%), which is higher than the mean score of 4 (reported as degree of polarization threshold = 75%) detected in a UK population by Temple et al.[38] A minimum of 30 samples is required for the central limit theorem to be applicable, and a sample of around 100 allows collection of normative data.[57] As we tested five variables, we aimed for a sample of 500 healthy individuals and ultimately analyzed 484, achieving a normative MP score curve (Fig. 1, Table 2).[57]

The measurement made by the MP-eye is not directly comparable to other measures of MP that are typically reported as MPD. Even the different ways of measuring MPD are not directly comparable, as some measure at specific eccentricities while others integrate across some volume/area. A central measure from heterochromatic flicker photometry (HFP) is different from a volume measure made with dual-wavelength fundus autofluorescence (e.g., SPECTRALIS; Heidelberg Engineering, Heidelberg, Germany) or reflectometry (Zeiss Visucam 500).[37,43] HFP typically measures MPD only centrally at 0.5° eccentricity (e.g., MPS II; Elektron Eye Technology, Cambridge, UK), reflectance typically measures specific eccentricity points and/or central volume (e.g., Zeiss Visucam 500), and dual-wavelength fundus autofluorescence is capable of measuring at specific eccentricity points and total volume (e.g., Heidelberg SPECTRALIS). The MP-eye makes an entirely different kind of measure dependent on the ability of the observer to perceive the rotation of HBs, which requires a large portion of the macula and therefore more closely correlates with total MP volume.[37] The total volume of MP or MP optical volume has been considered to be a more relevant measure than measures at specific eccentricities, because it represents the total amount of MP in the macula and takes into account the distributions of all three macular carotenoids.[45] Because the pattern of MP distribution across the retina varies,[58] a central measure at 0.5° will not consistently provide a good indication of total amount/volume of MP.[38,45] In addition, there is no known health benefit or disease linked to differences in retinal distribution of MPD as described by Sharifzadeh et al.,[58] so currently there is no benefit of measuring the pattern/profile of MPD across the retina, and a single indicator of total volume is currently adequate.

Initially, we performed statistical analysis with the entire study sample as a single sampling unit. As we could not detect meaningful correlations with variables previously reported to have such relations, we employed cluster analysis as a secondary statistical approach (details in the Methods section). This approach has been utilized previously to assess correlations with macular antioxidants in diseases such as glaucoma,[59] retinal dystrophies,[60] and ARMD,[61] as well as for anthropometric analyses[62] and evaluating serum antioxidants in ARMD.[63] Cluster analysis increases the statistical power, allowing for the MP scores at extremes to be analyzed as separate sample units and thus estimating correlations for these groups with higher statistical accuracy.[64]

We observed a weak correlation between age and MP-eye scores, overall and across all clusters. In previous studies, the results have varied, such that no age-related decline in macular pigments was reported in ocularly healthy subjects,[65] psychophysical MPD measurement techniques reported weak negative correlation, and other studies have reported no strong effect of aging on macular pigment density.[66–68] Psychophysical tests can be influenced by aging, decreased

oxygenated blood flow to retina, and cognition.[65] In contrast, most participants in the current study were below 50 years of age, when cognition is generally normal. Interestingly, MP-eye scores were significantly lower in participants with hypertension, which is in agreement with the study of Raman et al.,[17] which reported that hypertension is a risk factor for ARMD.[65] BMI also showed weak correlations to MP-eye scores, both when the full dataset was analyzed and when using BMI was used as a ranked variable (Table 2).[69–71] The storage of MP in adipose tissue is thought to explain why individuals with higher BMI tend to have lower MPD.[22,72,73]

We found a trend for increased MPD with increased dietary intake of lutein and zeaxanthin, but the correlation was not significant. Lutein and zeaxanthin accumulation in the retina is primarily dependent on dietary intake. There are various recall and calculation methods for dietary monitoring of lutein, zeaxanthin, and other dietary carotenoids[74–76] apart from plasma concentrations concomitant to a 7-day dietary record.[75] However, none of the previous methods was suitable for an Indian diet. The nutritive value of a food item and routine dietary lifestyle vary around the world. Earlier studies have used 24-hour recall methods (e.g., NHANES,[77] AREDS FFQ,[78] or food diary records[79]), and approximate scores were assigned.[76,80] We tested our questionnaires in a pilot phase and removed the data of those study participants at the time of the analysis. IFCT 2017[56] nutritive concentrations were taken as a standard for IFCT-LZQ in this study, and it showed strong agreement with the Tufts-LZQ (Fig. 2). However, many participants (~39%, $n = 188$) scored less on the Tufts-LZQ than the minimum reported on the IFCT-LZQ (i.e., <195 µg/day). Thus, the Tufts-LZQ underestimated the high LZ consumption by Indian subjects.

LZ sequestration, transport, and deposition in the retina involve complex metabolic pathways.[81] Systemic diseases, lipid function, and BMIs of individuals are reported to influence these pathways.[73,82] Consideration of these parameters is important to note while participants are being screened. An average American adult is reported to consume 1 to 2 mg of lutein per day,[83,84] and the average intake of adults above the age of 50 years is 2 mg of lutein and zeaxanthin per day.[85] From our study, an average Indian is estimated to eat 1.8 mg of lutein and zeaxanthin per day. Dietary intake of ≥6 mg/d is shown to be associated with a decreased risk of ARMD.[86] The lack of a significant correlation between MP-eye score and LZ intake in this study may be due to the general bias with recall methods or limitation of our study sample itself. Prospective long-term diet assessment is impractical. Our findings on the impact of diet on MPD requires verification with a population-based field study. This is important, as it is believed that LZ dietary intake and increased plasma concentrations of LZ reduce the risk of sight-threatening macular degeneration and progression from moderate to severe stages.[20,63] Although we have assessed diet, the data collected by us cannot account for physiological variance in the transfer of pigments to the macula from the gut, which is an important variable affecting MPD.[21]

Our study is limited by lack of objective measures of body fat and physical activity and the measurement of serum LZ levels. These were beyond the scope of the current study. Another concern when measuring MP is that yellow pigmentation of an aging sclerotic lens may interfere with the perception of HBs, as it does for other psychophysical and optical techniques. To test this, we included participants with early nuclear sclerosis in our study and assessed the MP-eye score in these individuals separately. We found that the mean MP-eye score for those with early nuclear sclerosis was 5.6 in 41 participants, which was not different from the overall mean of the sample (5.8), indicating that there was no or minimal impact of nuclear sclerosis on MP-eye scores. This agrees with previous literature showing that corneal opacities do not interfere with the perception of HBs.[87]

The speed and ease of use make the MP-eye an effective tool for assessing MP density in large-scale studies. To our knowledge, this is the first report of assessing MPD using degree of polarization thresholds in a large group of Indians. The frequency distribution and mean scores reported here will be useful in future comparisons of regional differences in MP-eye scores. Our study supports the feasibility of using the test for risk stratification of ARMD. With rising numbers of people developing ARMD, the MP assessment should be considered as a part of widespread preventative measures, as it enables people to be informed about this risk factor long before damage has occurred, giving them time to implement lifestyle changes such as quitting smoking, losing weight, exercising more, improving their diet, or avoiding the sun, which can reduce the risk of developing ARMD.

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