·Clinical Research·

Association of macular pigment optical density with early stage of non-proliferative diabetic retinopathy in Chinese patients with type 2 diabetes mellitus

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Abstract

• AIM: To detect the association between macular pigment optical density (MPOD), which reflects the antioxidant ability of retina, and diabetic retinopathy (DR) and to investigate the correlated factors of MPOD.

• METHODS: Totally 435 subjects of urban Chinese were recruited to the study and divided into 3 groups: non-diabetes mellitus controls (NDM), diabetic patients without retinopathy (DWR), and patients with early stage of non-proliferative diabetic retinopathy (DR). Demographic and lifestyle characteristics were ascertained by questionnaire. A food-frequency questionnaire, general physical and ophthalmic examinations were completed for all participants. MPOD was measured by heterochromatic flicker photometry. Foveal thickness was measured by optical coherence tomography. The difference of MPOD among 3 groups was analyzed by analysis of covariance. The correlation analyses of MPOD with the candidate influence factors were assessed using the generalized estimating equations (GEE) model.

• RESULTS: Of the 435 participants, 34 could not perform the MPOD measurements. Final analysis included 401 subjects, including 48 were in DR group, 134 in DWR group, and 219 in NDM group. MPOD was not significantly different among DR (0.49±0.21), DWR (0.45±0.21), and NDM (0.49±0.17) groups (P=0.24) after adjustment for fasting plasma glycemia, central foveal thickness, green vegetables, Chinese wolfberry, carotene and vitamin E. For all the 401 participants included, MPOD was positively associated with central foveal thickness (E=0.0007, P=0.001), Chinese wolfberry (E=0.0345, P=0.01), and green vegetables (E=0.0596, P<0.001) intake.

• CONCLUSION: The data suggest that MPOD level is not statistically significantly influenced by the onset of diabetes or early stage of DR in the studied population. MPOD level is positively associated with thicker central foveal thickness and higher intake of foods containing carotenoids.

• KEYWORDS: diabetic retinopathy; diet; foveal thickness; macular pigment optical density
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INTRODUCTION

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and the main cause of blindness among the middle-aged populations of developed countries [1]. Since the prevalence of diabetes has been growing at an alarming rate in recent years [2], the number of DR patients has rapidly increased. The incidence and progression factors of DR include hyperglycemia, diabetes duration, hypertension, cholesterol, and insulin usage [3]. Moreover, increasing evidence has emphasized the critical involvement of oxidative stress in the pathogenesis of DR[4].

Macular pigment is constituted by lutein, zeaxanthin and mesozeaxanthin (a synthesis product of lutein), which can filter optical waves shorter than 550 nm and provide antioxidant protection to the human retina by inhibiting the peroxidation of long-chain polyunsaturated fatty acids[5]. High levels of macular pigment may be a protective factor against photo-oxidative damage caused by blue light. The relationship between macular pigment and age-related macular degeneration and other macular diseases, such as Stargardt macular dystrophy, have been investigated in many studies [6-8]. In recent years, several experimental studies also

demonstrated a reduction in retinal oxidative damage after carotenoid supplementation in diabetic rats[9]. However, just a few studies evaluated the association between DR and macular pigment optical density (MPOD), and the results were not consistent. Some studies indicated that diabetic patients with retinopathy had lower levels of macular pigment[10-11]. Conversely, another one implied no difference[12].

Since lutein and zeaxanthin are entirely of dietary origin and cannot be synthesized by the human body, foods rich in those elements, such as green leafy vegetables, corn, squash, Chinese wolfberry, and egg yolks may increase levels of macular pigment [13-14]. Macular pigment levels are also affected by multiple other factors, including genetics, age, gender, smoking status, and body mass index [15-16]. Studies have also shown that the foveal architecture plays a role in the deposition of macular pigment in the retina[17].

We have previously reported that MPOD levels in the Chinese population might be relatively higher than that of other populations [18]. In this study, heterochromatic flicker photometry (HFP) was used to investigate the association between MPOD and diabetes as well as association between MPOD and early stage of non-proliferative DR in a Chinese population. Moreover, the correlation of MPOD with diet and foveal architecture was also investigated.

SUBJECTS AND METHODS

Study Participants and Clinical Evaluation Patients with type 2 diabetes mellitus and non-diabetic individuals over 45 years of age were recruited between April 2012 and August 2014 from the Desheng Community of urban Beijing. Subjects that had 20/25 or better best corrected visual acuity were included. Subjects with visible media opacity, other history of ocular disease, surgery except phacoemulsification, or a shallow anterior chamber precluding mydriasis were excluded. The study protocol was approved by the Ethics Committee of Beijing Tongren Hospital and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their enrollment.

Diabetic participants were recognized based on either a history of physician diagnosed type 2 diabetes or undergoing treatment for diabetes. All subjects underwent a standardized evaluation consisting of a questionnaire, ocular and anthropometric examinations, and laboratory tests. The questionnaire elicited basic information (age, sex, ethnicity, income, education), lifestyle information (such as smoking and alcohol intake), health status information (such as the use of insulin therapy and any history of systemic disease), and a 12-item food-frequency questionnaire (Chinese wolfberry, green vegetables, carrot, spinach, egg yolk, corn, red vegetables, yellow vegetables, tea, shrimp, milk, bean curd) with a list of foods rich in lutein and zeaxanthin[19], according to the local dietary habits. The food list ascertained the average frequency of eating corn, egg yolk, Chinese wolfberry, carrots, spinach, tea, bean curd, fish, shrimp, yellow vegetables, red vegetables, black vegetables, and milk per week. Whether or not the subjects took supplements of xanthophyll, carotene, fish oil, vitamin A, vitamin C, vitamin E, vitamin B compound or multivitamins was also recorded. Anthropometric parameters, including body weight and height, waist and hip circumferences, and three measurements of body mass index (BMI, kg/m^2) and waist-to-hip ratio (WHR), were calculated. A comprehensive ophthalmological examination included corrected visual acuity, slit-lamp biomicroscopy, and fundus photography. Seven-field 30° color fundus photographs with stereoscopic images of the optic disc and macula were taken through the dilated pupils of each patient using a digital fundus camera (Zeiss Visucam Pro, Oberkochen, Germany).

The overall retinopathy grade for each eye was determined according to the protocol described in the Early Treatment Diabetic Retinopathy Study (ETDRS)[20]. Patients whose eyes were DR-absent were assigned to the diabetic-without-retinopathy (DWR) group. Patients whose eyes had questionable to moderate non-proliferative DR were assigned to the DR group. If either eye of any patient had worse than moderate non-proliferative DR, macular edema or any other retinopathy, that patient was excluded. Macular edema was diagnosed by optical coherence tomography. Non-diabetic subjects over 45 years of age with no retinopathy were assigned to the non-diabetes mellitus (NDM) group.

Laboratory Assays Fasting blood samples were collected for the measurement of fasting plasma glucose (FPG), C-reactive protein (CRP), creatinine, and a lipid profile including total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, in an automated system with reagents for routine biomarkers[21].

Measurement of Macular Pigment Optical Density MPOD was measured psychophysically with the MPS1000 (Hartest Precision Instruments, Surrey, UK), a computerized device that utilizes the principle of HFP [22-23]. The MPS1000 uses specific wavelengths of blue light (470 nm; maximum absorption of macular pigment is at 460 nm) and green light (540 nm; not absorbed by macular pigment) to gauge a patient's response. The equal luminance points are obtained by presenting the two lights at a series of different intensity ratios of blue and green. The flicker frequency starts at a high rate, where flicker cannot be detected. The blue/green intensity ratio is slowly reduced until the observer sees the flicker, at which point he or she presses the response button. This process is repeated for the central 0.5° parafoveal eccentricity, where the concentration of macular pigment peaks, and for the peripheral 8° parafoveal eccentricity, where macular pigment is considered to be absent. Two

graphs of frequency versus the blue/green ratio are obtained. Each curve has a minimum, which corresponds to the equal luminance point for the blue/green target. The built-in software calculates the difference among these minima to obtain the MPOD.

Optical Coherence Tomography Examination Macular cube scans (512 A-scans ×128 B-scans) of both eyes of all subjects were captured after pupillary dilation using spectral domain optical coherence tomography with the Cirrus HD-OCT 400 (Carl Zeiss Meditec, Dublin, USA). The ETDRS grid was automatically centered on the fovea[24]. The foveal architecture values, including the average center foveal thickness (CFT, central circle within 1 mm diameter), cube average thickness (CAT, square of 6×6 mm), and cube volume (CV, square of 6×6 mm), were assessed automatically by the built-in topographic mapping software, version 6.0. The retinal thickness was calculated as the distance between the internal limiting membrane (ILM) and the retinal pigment epithelium (RPE). A good-quality scan with signal strength of at least 5 was accepted.

Statistical Analysis All data were double entered and validated with the EpiData program, version 3.1 (the EpiData Association, Odense, Denmark). Statistical analysis was performed using the R statistical software package, version 2.11.0. (http://www.r-project.org/). The χ^2 test was used to compare categorical data of the three groups. The Shapiro-Wilk test was performed to assess the distribution type. Parametric variables were compared between groups by analysis of variance. Nonparametric data were compared by using the Kruskal-Wallis test. Analysis of covariance was used to analyze the difference of MPOD among three groups (right eye of each subject was included). The correlation analyses of MPOD with the candidate influence factors were assessed using the generalized estimating equations (GEE) model. Both eyes of each subject were included as paired samples. The controlled variables were finally included according to quasi-likelihood under the independence model criterion (QIC). The level of significance was set as $P<0.05$.

RESULTS

A total of 435 subjects participated in the study and 34 could not perform the MPOD measurements. Final analysis included 401 subjects, including 48 in DR group, 134 in DWR group, and 219 in NDM group. The characteristics of the 401 subjects included for the final analysis are given in Table 1.

The mean ages of NDM, DWR, DR groups were 63.6±7.4, 63.5±8.0 and 61.4±6.4y, respectively ($P=0.17$). Type 2 diabetes patients with or without DR had a higher prevalence of systemic hypertension and hyperlipidemia ($P<0.001$). The percentage of insulin usage was higher in the DR group than in the DWR group ($P=0.001$). Sex, smoking status, BMI, and WHR showed no statistical differences among the three groups. On the laboratory testing, FPG, cholesterol, HDL and LDL were significantly different among groups ($P<0.05$). The diet constructions of the three groups were different. Patients in the DR group consumed Chinese wolfberry, carrot, and green vegetables more frequently than DWR and NDM groups ($P<0.05$). DWR and DR group had more multi-vitamin B and carotene than NDM group ($P<0.05$).

Paired t-test showed no statistically significant difference in MPOD between right eyes and left eyes ($P=0.14$). MPOD in NDM, DWR, and DR groups were 0.49±0.17, 0.45±0.21, and 0.49±0.21, respectively ($P=0.24$). After adjustment of covariates, including fasting plasma glycemia, central foveal thickness, green vegetables, Chinese wolfberry, carotene and vitamin E, there is still no significant difference in MPOD among the groups ($P=0.44$). The diopter of spherical power in NDM, DWR, and DR groups were 0.87±2.00, 0.19±3.06, and 0.60±1.63, respectively ($P=0.03$). The thickness and volume of the macula (CFT, CV, and CAT) among NDM, DWR, and DR groups were not statistically significant ($P>0.05$).

The association of characteristics with MPOD is shown in Table 2. CFT, intake of Chinese wolfberry (E=0.0345, $P=0.01$) and green vegetables (E=0.0596, $P<0.001$) were positively associated with MPOD. However, FPG (E=-0.0076, $P=0.02$), vitamin E (E=-0.0791, $P=0.004$) and carotene (E=-0.2062, $P=0.003$) were negatively associated with MPOD. The duration of diabetes was not significantly associated with MPOD before or after adjustment of other factors ($P>0.05$).

DISCUSSION

In the present study, the association of MPOD with diabetes, early stage of non-proliferative DR, and other factors were investigated. The data show no significant difference in MPOD levels among DR, DWR, and NDM groups before or after adjusting for covariance including demographic data, laboratory values, diet, and ocular conditions. In addition, MPOD levels were found to be positively associated with central fovea thickness, the intake of Chinese wolfberry and green vegetables, and negatively associated with hyperglycemia.

Macular pigment, which is mostly located in Henle's fibers at the fovea and in the inner nuclear layer in the perifoveal area, is considered to be an antioxidant in the retina [25]. Previous studies showed diabetic patients had significantly lower MPOD values than that in the control subjects [10-12]. In addition, serum lutein and zeaxanthin concentrations, which were shown to be positively related to MPOD [26], have been found to be significantly lower in diabetic patients compared with normal controls [27]. In the present study, we found that DWR had a slightly lower MPOD level (0.45±0.21) when compared with controls (0.49±0.17), however, the difference was not statistically significant before or after adjustment of other factors.

Table 1 Demographic characteristics and clinical findings of the study participants *n* (%), $\bar{x} \pm s$

Parameters	NDM group *n*=219	DWR group *n*=134	DR group *n*=48	*P*
Age	63.6±7.4	63.5±8.0	61.4±6.4	0.17[c]
Male	77 (35.2)	48 (35.8)	19 (39.6)	0.85[d]
Hypertension	65 (29.7)	73 (54.5)	30 (62.5)	<0.001[d]
Hyperlipidemia	39 (17.8)	75 (56.0)	22 (45.8)	<0.001[d]
Current smoker	20 (9.1)	16 (11.9)	3 (6.3)	0.47[d]
Past smoker	40 (18.3)	22 (16.4)	11 (22.9)	0.61[d]
BMI (kg/m^2)	25.4±3.5	25.9±4.1	26.4±3.8	0.14[c]
WHR	0.9±0.1	0.9±0.1	0.9±0.1	0.08[c]
Diabetic duration:median (range)	-	7.3 (4.2, 13.1)	7.1 (3.0, 12.0)	0.59[e]
Insulin use	-	18(13.4)	17(35.4)	0.001[d]
FPG (mmol/L)	5.1±1.0	7.3±2.1	8.4±2.1	<0.001[c]
TG (mmol/L):median (range)	1.5 (1.0, 2.0)	1.6 (1.0, 2.1)	1.3 (1.0, 1.8)	0.36[e]
Cholesterol (mmol/L)	5.4±1.0	5.1±0.9	5.5±1.4	0.02[c]
HDL (mmol/L)	1.3±0.3	1.2±0.3	1.3±0.3	<0.001[c]
LDL (mmol/L)	3.2±0.8	3.0±0.7	3.2±0.9	0.003[c]
MPOD[a]	0.49 ±0.17	0.45±0.21	0.49±0.21	0.24[c]
CFT[a] (μm)	246.6±26.6	248. 6±41.1	245.3±24.7	0.78[c]
CV[a] (μm)	9.8±0.5	9.8±0.9	9.9±0.4	0.60[c]
CAT[a] (μm)	274.8±15.1	273.3±25.3	276.4±11.4	0.60[c]
DS[a] (D)	0.87±2.00	0.19±3.06	0.60±1.63	0.03[c]
DC[a] (D)	-1.00±0.87	-1.03±0.82	-0.71±1.00	0.08[c]
Chinese wolfberry[b]	63 (28.8)	40 (29.9)	20 (41.7)	0.02[d]
Green vegetable[b]	174 (79.5)	122 (91.0)	47 (97.9)	<0.001[d]
Carrot[b]	122 (55.7)	81 (60.4)	29 (60.4)	0.04[d]
Vitamin B compound[b]	6 (2.7)	22 (16.4)	6 (12.5)	<0.001[d]
Xanthophyll[b]	1 (0.5)	0	0	0.66[d]
Carotene[b]	0	0	1 (2.1)	0.03[d]

NDM: Non-diabetes mellitus; DWR: Diabetes without retinopathy; DR: Diabetic retinopathy; FPG: Fasting plasma glucose; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; MPOD: Macular pigment optical density; CFT: Center foveal thickness; CV: Cube volume; CAT: Cube average thickness; DS: Diopter of spherical power; DC: Diopter of cylindrical power. [a]Data were collected from righ eyes; [b]Intake at least one time every week within the latest month; [c]One-way analysis of variance; [d]χ^2 test; [e]Kruskal-Wallis test.

Table 2 Association of characteristics with MPOD levels in multiple regression analysis using GEE

Variable	E	Standard error	*P*
FPG	-0.0076	0.0032	0.02
CFT	0.0007	0.0002	0.001
Chinese wolfberry	0.0345	0.0139	0.01
Green vegetables	0.0596	0.0071	<0.001
Vitamin E	-0.0791	0.0274	0.004
Carotene	-0.2062	0.0692	0.003

MPOD: Macular pigment optical density; CFT: Center foveal thickness; FPG: Fasting plasma glucose; GEE: Generalized estimating equations.

According to previous reports, different mechanisms could lead to reduced MPOD in diabetic patients, such as hyperglycemia and oxidative stress [12-13], body fat and its distribution [16], and serum HDL [28]. Consistent with previous studies [12-13], we found in the present study that MPOD was negatively associated with FPG. However, no association was found with BMI, WHR, and serum HDL. Hyperglycemia could result in the generation of reactive oxygen species, which ultimately lead to increased oxidative stress in the retina of diabetic patients [4]. A previous study showed an inverse relationship between HbA1c levels and MPOD, which suggested that poor glycemic control may contribute to the lower MPOD levels in diabetic patients because of oxidative stress. Macular pigment carotenoids were known to accumulate in adipose tissue [29], and primarily transported by HDL in plasma[28]. Thus, previous study supposed BMI, WHR and serum HDL were associated with MPOD [12]. However, the hypothesis has not been verified by our study. The similar results were also acquired from a south Indian population study, in which lack of association were reported between MPOD and various types of obesity[30].

There are only a few studies having evaluated the relationship between DR and MPOD. In contrast to our study, Davies and Morland [11] found in a study with 26 diabetic subjects that patients with diabetic maculopathy grade 2 (modified Airlie House classification) had

significantly lower pigment density than those with no maculopathy by using a different instrument (Wright tristimulus colorimeter) and color matching technique. Another study, in which the MPOD were measured in 29 diabetic participants by dual-wavelength autofluorescence imaging, showed mean MPOD averaged in a 2° -diameter circle around the fovea was significantly lower in DR patients when compared with DWR patients [10]. However, it should be noted that both of those studies did not include diet or macular thickness to adjust the results. Since different diet habits and foveal region structures might influence the MPOD, those could in part explain the differences in the results. In addition, both of the studies had relatively small sample size as compared to our study. Therefore, significant selection bias might exist in previous studies. In one recently published study, in which MPOD was determined by using HFP methods in a relatively larger sample size of 102 diabetic patients, the result showed no significant association between MPOD and DR after adjustment for dietary carotenoids intake [12], similar to the results as our current study.

Macular pigment concentration peaks at the foveal center, where the highest density of macular pigment is found in the receptor axon layer, but it declines rapidly with increasing eccentricity to low, relatively constant levels within 1 mm retinal eccentricity [31]. Consistent with this distribution, we found average retinal thickness within 1 mm diameter of central circle (CFT) was positively significantly correlated with MPOD, that is consistent as previous studies [17,32]. However, in our study, the foveal thickness of DR did not differ significantly from that of normal subjects in the condition of excluding patients with maculopathy, and this result was consistent with previous study [33]. Since retinal structure, especially CFT, was significantly associated with MPOD, our result that no significant difference was found between DR and control group in MPOD, could be partly explained by no difference in CFT.

Humans cannot synthesize macular pigment, but must absorb lutein and zeaxanthin from the diet. Many studies [34-35] have demonstrated that diet, especially carotenoid intake, influences macular pigment levels, which were also proved in our study. Ford *et al* [36] found that serum levels of macular carotenoids in diabetic patients were significantly lower than normal subjects, implying a deficiency of lutein and zeaxanthin in diabetic diet or poor absorption from the gut in diabetic patients. In contrast, another study showed no significant difference in serum levels of lutein and zeazanthin between diabetic and non-diabetic groups [37]. Those findings may relate to different diet habits in the two study populations. In our study, patients in DR group were found to consume more carrot, Chinese wolfberry, and green vegetables, which are rich in carotenoids, especially lutein and zeaxanthin [38]. It is possible that the dietary habit may interact with other factors such as hyperglycemia, influencing the MPOD levels, which could partially explain the results of no association between DR and MPOD found in our study.

Several limitations of this study exist. First, MPOD was only measured in central 0.5° parafoveal in our research. It was reported that central MPOD levels are only poorly correlated with the total amount of macular pigment, and the total amount of macular pigment cannot be reliably predicted from only central attenuation [39]. Topographical variations display in macular pigment is required for calculating total macular pigment content [40], which definitely provides a more complete and accurate representation of macular pigment levels and may enable the correlation of distribution with developing pathology. Second, the patients with more severe levels of DR, who may be more likely to have lowered MPOD scores because oxidative stress is higher in the later stages of the disease, were not included in this study. Finally, the diet conditions were obtained by using a semi-quantitative food frequency questionnaire in this study, which could not accurately analyze the intake of lutein and zeaxanthin. The influence of those factors should be considered when interpreting our results.

In conclusion, we find that MPOD levels are not associated with diabetes or with early stage of non-proliferative DR in the studied Chinese population. MPOD is found to be associated with thicker central foveal thickness and higher intake of Chinese wolfberry, and green vegetables. Further studies to verify the relationship between MPOD and the development of DR, in particular severe non-proliferative DR or proliferative DR, are warranted.

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