·Clinical Research·

Application of Lutein and Zeaxanthin in nonproliferative diabetic retinopathy

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Abstract

• AIM: To compare serum Lutein and Zeaxanthin (L/Z) concentrations between patients with nonproliferative diabetic retinopathy (NDR) and normal subjects, and to explore the effect of L/Z supplementation on serum L/Z level and visual function in NDR patients

• METHODS: Subjects were divided into three groups: 30 NDR patients supplied with Lutein 6mg/d and Zeaxanthin 0.5mg/d for three months (DR Group), 30 NDR patients without L/Z supplementation (DR Control Group) and 30 normal subjects (Control Group). Serum L/Z concentrations were measured by liquid high-resolution chromatography (HPLC). Visual acuity was recorded at baseline, 1 month, 2 months and 3 months post initial supplementation. Serum L/Z concentration were measured at baseline, 1 month and 2 months post initial supplementation. Contrast sensitivity (CS) and fovea thickness were recorded at baseline and 3 months post initial supplementation.

• RESULTS: Mean serum lutein concentrations in DR group were 0.0686±0.0296μg/mL and zeaxanthin concentration was 0.0137 ±0.0059 μg/mL . The L/Z level of DR group was significantly lower compared to the control group (lutein: 0.2302±0.1308μg/mL ,zeaxanthin: 0.0456±0.0266μg/m , P= 0.000). The concentration of lutein and zeaxanthin in the DR control group at base line was 0.0714 ±0.0357μg/mL and 0.0119±0.0072μg/mL, respectively. There was no significant change of L/Z concentration in the DR control group during the study. Serum L/Z concentrations of DR group increased significantly after supplementation (F=109.124, P=0.000; F=219.207, P=0.000).Visual acuity improved significantly after medication. Compared with pre-medication, the average CS values of 1.5cpd, 3cpd and 6cpd after three months increased significantly (P=0.030,0.013,0.008) and the foveal thickness decreased. (P=0.05)

• CONCLUSION: Serum L/Z concentrations in DR patients are significantly lower than those in normal subjects, and L/Z intake can improve the visual acuity, CS and macular edema in DR patients, suggesting that L/Z supplementation might be targeted as potential potential therapeutic agents in treating NDR.

• KEYWORDS: Lutein; Zeaxanthin; liquid high-resolution chromatography; contrast sensitivity; optical coherence tomography; visual acuity; nonproliferative diabetic retinopathy
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INTRODUCTION

Lutein and Zeaxanthin (L/Z) are both carotenoid with antioxidant properties, which are commonly present in many fruits, vegetables, and egg yolk [1]. They are dihydroxy-carotenoids with ionone ring, as the isomer of each other. Almost all tissues of the eye contain carotenoids. However, lutein and zeaxanthin are the only carotenoids existing in the retina and lens [2, 3]. Zeaxanthin is the most important carotenoid in the fovea retina of adults, and lutein is the most important carotenoid in the peripheral retina [4]. A large number of epidemiological surveys and studies have shown that they have some certain effect on the prevention and treatment of a variety of ophthalmic diseases. L/Z has important biological functions in three aspects: acting as antioxidants, filtering out photo-toxic short-wavelength visible light and minimizing the effect of chromatic aberration. Oxidative stress played an important role in the pathogenesis of cataract and age related macular degeneration, and numerous researches focusing on the application of Lutein and Zeaxanthin in cataract, age-related macular degeneration have been conducted. However, the role of L/Z in diabetic retinopathy remains unknown.

In this study, we examined patients with NDR and normal subjects for serum L/Z concentration and the effects of L/Z implementation on visual function in patients with nonproliferative diabetic retinopathy.

MATERIALS AND METHODS

Subjects *Test group:* 34 patients diagnosed as

nonproliferative diabetic retinopathy (NDR) were recruited from July 2008 to April 2009 at Tianjin Medical University Eye Center. One patient quit because of adverse events; three lost follow-up. Ultimately 12 males and 18 females averaging 59.07±14.0 years old (range: 44 to 75) were enrolled. Of the 30 patients, 4 have type 1 diabetes and 26 have type 2 diabetes for a duration of 3 to 28 years with an average of 11.5±6.4 years. The blood glucose level of the group ranged from 4.9 to 15.5mmol/L, with an average of 8.0±2.9mmol/L; body mass index (BMI) ranged from 19.25 to 30.50Kg/m^2, with an average of 24.63±3.68Kg/m^2. The treatment of Lutein 6mg/d and Zeaxanthin 0.5mg/d was administered orally for three months.

Control group: 60 healthy subjects, aged from 43 to 70 with an average of 55±9 years old, including 24 males and 36 females were included. The selection criteria of normal subjects were: patients with no complaint about eye discomfort, best corrected visual acuity ⩾1.0, refractive error ⩽3.00DS, no abnormal eye examination records (including intraocular pressure, slit-lamp microscopy, direct and indirect ophthalmoscopy), and no chronic systemic diseases such as diabetes, hypertension.

DR Control group: 33 patients diagnosed as nonproliferative diabetic retinopathy (NDR) were recruited from July 2008 to April 2009 at Tianjin Medical University Eye Center. Two were lost during follow-up. Altogether 17 males and 14 females aged from 42 to 76, with an average of 60±15 years old were included. Of the 31 patients, 6 have type 1 diabetes and 25 have type 2 diabetes for a duration of 2 to 32 years with an average of 13.58±5.491 years. The blood glucose level of the group ranged from 4.7 to 18.1mmol/L, with an average 8.1±3.0mmol/L; body mass index (BMI) ranged from 18.93 to 29.75Kg/m^2, with an average of 25.12±3.82 Kg/m^2.

Selection criteria included 1) Nonproliferative Diabetic retinopathy with macular edema. It has been reported that[5,6] the average thickness of neuroepithelium at fovea centralis was about 150μm. Considering the factors of age and refractive change, the maximum thickness was no more than 200μm. In this research, 200μm was taken as the upper limit of normal macular foveal thickness, 201-300μm as that of mild edema, 301-400μm as moderate edema, above 400μm as severe edema; 2) No previous Lutein and other anti-oxidants replacement therapy; 3) Patients are willing and able to provide a written consent and authorization; 4) Over 40 years old; and 5)Pseudophakic eyes (single focal lens). The Exclusion criteria included 1) Associated with eye disease or systemic infection or inflammation associated with active ocular disease; 2) A history of glaucoma or age-related macular degeneration; 3) Active hepatitis, severe liver disease, kidney failure, cancer, a history of poor control hypertension, thrombosis disease (including cerebral infarction, myocardial infarction, *et al*) and other systemic diseases. The study have been approved by the Ethics Committee of Tianjin Medical University Eye Centre..

Methods Patients in DR Group were supplied with Lutein 6mg/d and Zeaxanthin 0.5mg/d for 3 months, while none of the other subjects received any supplement of L/Z. Serum L/Z concentrations were measured by liquid high-resolution chromatography (Shimadzu Instruments) at baseline (pre-medication), 1 month and 2 months post supplement Best Corrected. Visual acuity were measured at baseline, 1 month, 2 months and 3 months post supplement, and were converted to logMAR equivalents for statistical calculations Contrast sensitivity (CS) Using the Functional Acuity Contrast Test (STEREO OPTICAL Instruments, United States) and foveal thickness demonstrated by OCT (Zeiss-Humphrey) were measured at baseline and 3 months post supplement. Retinal structure and retinal thickness were measured as described previously by Brown *et al*[5,6].

Statistical Analysis Results are expressed as mean±SD and the *P* values were determined by independent-sample *t* test, paired-sample *t* test, correlation coefficients were determined by repeated measures ANOVA. $P<0.05$ was regarded as statistically significant.

RESULTS

At baseline ,the mean lutein and zeaxanthin concentration was 0.0686±0.0296μg/mL and 0.0137±0.0059μg/mL in the DR group, and was 0.2302±0.1308μg/mL and 0.0456±0.0266μg/mL in the control group. The lutein and xeaxanthin concentration of the DR control group was 0.0714±0.0357μg/mL and 0.0119±0.0072μg/mL, respectively. Compared with control group, serum L/Z concentrations in DR group were significantly lower. In DR group, serum L/Z concentrations increased significantly after L/Z supplementation. After 3 months of supplementation of L/Z, the serum concentration of L/Z in DR group increased to 0.5409±0.1807μg/mL and 0.2816±0.0731μg/mL. In DR Control Group, the serum L/Z concentrations after the experimental period was 0.0701±0.0280μg/mL and 0.0224±0.0091μg/mL , and was of no statistical significance compared to the baseline concentration.

The BCVA improved significantly after medication compared to base line BCVA(F=18.698, P=0.000, Table 1). Compared with baseline, the average CS values of 1.5cpd, 3cpd and 6cpd at 3 months post medication increased significantly. The correlation coefficient between serum Lutein concentration and CS of 3cpd was 0.569 (P=0.027). The correlation coefficient between serum Zeaxanthin concentration and CS of 12cpd was 0.969 (P= 0.031). Compared

Table 1 The visual acuity increased significantly at different time before and after medication

	Baseline	post-1 month	post-2 month	post-3 month	*F*	*P*
BCVA	0.38±0.298	0.41±0.321	0.44±0.335	0.45±0.334	18.698	0.000

with baseline (mean 318.9±157.38), the foveal thickness at 3 month post L/Z supplementation was significantly decreased (mean 286.50±134.185, P=0.05). Serum Zeaxanthin concentration and foveal thickness were negatively related (r = - 0.560, P= 0.030).

DISCUSSION

At baseline ,there were no significant differences in sex(x^2= 3.121),age(F= 0.510), duration of diabetes (F= 0.921), the blood glucose level (F= 0.781), body mass index (BMI) (F= 0.672)among the three groups(P>0.05).

The variations of serum Lutein and Zeaxanthin concentration in patients with NDR In this study, we found that serum Lutein and Zeaxanthin concentrations were significantly lower in NDR patients than those in normal subjects. Bone *et al* [7] reported that the serum Lutein concentration was 0.08-0.35μg/mL in normal subjects. Our experiment obtained the similar results. It showed that the serum L/Z concentration in DR group were significantly lower than those in normal subjects. The following reasons were considered: 1) The NDR patients' BMIs are generally higher than the normal standard, suggesting a unhealthy diet with low L/Z; 2) Hyperglycemia may have negative effects on absorption of L/Z; 3) Long-standing hyperglycemia may decreases L/Z accumulation in the retina by destructing local microcirculation. By supplementation of Lutein 6mg/d, Zeaxanthin 0.5mg/d, the serum L/Z concentrations in DR group were significantly higher than those in the normal subjects after one month. Kostic *et al* [8] reported that the serum Lutein could reach maximum concentration 16 hours after 10mg Lutein intake, with serum Lutein concentration reaching 1.4μg/mL after 18 days. Kostic D *et al* also observed that L/Z density in retina also increases following serum Lutein elevation[9,10].

The changes of CS in patients with NDR Compared with pre-medication, the average CS values after 3 months of L/Z supplementation increased significantly. Previous studies also indicated that Lutein and Zeaxanthin not only had an antioxidant effect, but also could improved visual acuity. They could enhance the transmission of information among cells through the post-transcriptional mechanisms, which was often accompanied with the increased level of mRNA [11]. Stahl *et al* [12] demonstrated that Lutein and Zeaxanthin played a special role in synaptic connections by enhancing connectivity of cells in the nervous system, which led to their direct or indirect effects on binding protein gene expression. Theoretically, high density of pigments may improve signal transduction in the visual system. The connection, in turn, is also improved between photoreceptors and the higher visual cortex. As a result, the contrast sensitivity function could be improved.

The correlation coefficient between serum Lutein concentration and CS of 3cpd was r= 0.569 (P= 0.027). The correlation coefficient between serum Zeaxanthin concentration and CS of 12cpd was r= 0.969 (P= 0.031). Because Zeaxanthin is the most important carotenoid in the fovea retina [4] and macular lesions is related to high spatial frequency contrast sensitivity[13], Zeaxanthin and high spatial frequency contrast sensitivity was highly positively correlated. On the other hand, we also found that Lutein and the middle-low spatial frequency contrast sensitivity were positively correlated. Since Lutein is the most important carotenoid in the peripheral retina, we hypothesized that the lesions of peripheral retina might be related to middle-low spatial frequency contrast sensitivity. Kvansakul *et al* [14] also proved that supplementation with the carotenoids lutein or zeaxanthin improved human visual performance. Supplementation with L or Z increases MPOD at the fovea and at 2.5 degrees, and that supplementation can improve CATs at high mesopic levels and hence visual performance at low illumination.

Foveal thickness in patients with NDR before and after medication Compared with pre-medication, the average foveal thickness decreased and the foveal thickness decreased in 83% of patients three months after medication. The correlation coefficient between serum Zeaxanthin concentration foveal thickness was -0.560 (P=0.030). We proposed that L/Z, especially Zeaxanthin may play a role in diabetic macular edema. L/Z can reduce vascular permeability, inhibit vascular leakage, and protect the integrity of blood vessels; the macular organizational structure can maintain adequate blood supply and ameliorate the macular retinal hypoxia without reperfusion. Futhermore, they can also prevent free radicals from combining with retinal collagen and strengthen the structure of retinal collagen.

Adverse Reactions One patient had a headache after administration of L/Z, and medication was ceased. The possible explanation was allergy to L/Z. However, the definite reason is still unknown. There have been a report about the cytotoxic effects of carotenoid-derived aldehydes

from beta-carotene, Lutein and Zeaxanthin on human retinal pigment epithelial cells (ARPE-19). Kalariya *et al* [14] study demonstrated that carotenoid-derived aldehydes could exacerbate the oxidative stress in ARPE-19 cells by elevating reduction-oxidation state levels. This suggests that overdosed carotenoid supplementation for treatment of AMD should be used with caution.

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