Research Article

The effect of lutein and zeaxanthin administration on reactive oxygen species levels in blood serum and contrast sensitivity changes in patients with non-proliferative diabetic retinopathy

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ABSTRACT

Background: Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus. The high levels of reactive oxygen species (ROS) in diabetes can cause pericyte loss and endothelial damage, which causes disturbances in visual function. This study aims to investigate the effect of lutein and zeaxanthin (L/Z) administration on ROS levels in blood serum and contrast sensitivity in patients with non-proliferative DR (NPDR). **Materials and Methods:** This study used a pre- and post-test control design. All subjects were NPDR patients. ROS levels in blood serum were measured using ELISA and contrast sensitivity was examined using the Pelli-Robson chart, before and after administration of 6 mg of lutein and 0.5 mg of zeaxanthin (Cendo-TGF®) for 30 days. Correlations were performed to examine the relationship between changes in ROS levels and contrast sensitivity. **Results:** The mean ROS level before L/Z administration was 15.512 ng/ml and was reduced to 6.707 ng/ml after L/Z administration ($P < 0.05$). The mean contrast sensitivity before L/Z administration was 1.286 and was increased to 1.425 ($P < 0.05$). There was a positive correlation between ROS serum level and contrast sensitivity, but this was not significant ($P > 0.05$). **Conclusion:** L/Z administration is able to decrease ROS levels in blood serum and increase contrast sensitivity scores in patients with NPDR. The reduction in ROS level is positively correlated with increasing contrast sensitivity score, but this correlation is not statistically significant.

KEY WORDS: Contrast sensitivity, Lutein, Non-proliferative diabetic retinopathy, Reactive oxygen species, Zeaxanthin

BACKGROUND

Diabetes mellitus (DM) is a metabolic disorder marked by a chronic state of hyperglycemia caused by a relative or absolute deficiency of insulin, or tissue resistance to insulin. Globally, the prevalence of DM is reported to be 2.8% in every age group and is predicted to experience a two-fold increase by 2030. Research from the department of health in 2008 showed that the national prevalence of DM was 5.7%.[1,2]

Diabetic retinopathy (DR) is the main microvascular complication of DM, marked by microangiopathy derived from retinal microvascular obstruction and leakage. The Wisconsin epidemiological study of DR (WESDR) found that the prevalence of DR was very high (67–98%) in patients who had been suffering from DM for 20 years but was decreased by controlled metabolic function.[3-5]

There are four biochemistry pathways that play a role in the development of DR : An increase in the polyol-sorbitol pathway, advanced glycation end product (AGE) production, protein kinase isoforms (PKC) activation, and the hexosamine pathway. In addition, a chronic state of hyperglycemia can cause a rise in oxidative stress which leads to an increase in reactive oxygen species (ROS) production. ROS overproduction plays a role in the development and progressivity of endothelial and pericyte dysfunction in DR.[5-9] One of the complications of retinopathy is disturbances to visual function. Contrast sensitivity is one of the visual function components which is more sensitive for detecting abnormalities in retinal ganglion cells in patients with DM, despite an absence of microvascular abnormalities.[3-5]

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Today, antioxidants are widely used as adjunctive therapy in DR. Antioxidants can work through several paths By inhibiting ROS production or fighting against free radicals, or by increasing the ability of the antioxidant defense enzyme.[10,11]

Lutein and zeaxanthin (L/Z) are carotenoids which possess antioxidant properties. The only carotenoids contained in the retina and lenses are L/Z. L/Z are widely used to prevent cataract progressivity in patients with macular degeneration, but their use in patients with DR is still relatively unexplored.[12,13]

The purpose of this study was to examine the effect of L/Z administration on changes in ROS levels in blood serum, and changes in contrast sensitivity, in patients with non-proliferative DR (NPDR).

MATERIALS AND METHODS

This experimental study used a pre- and post-test control design. Fourteen subjects diagnosed with NPDR (aged between 50 and 79 years old) were included in this study. Each subject signed an informed consent form before participating in this study. Ethical clearance was approved and published by the Ethical Committee of the Saiful Anwar Hospital, Malang.

Inclusion criteria included Patients with a diagnosis of NPDR retinopathy and who agree to participate in this study; aged 50–79 years old; systolic blood pressure of ≤140 mmHg and diastolic blood pressure of ≤90 mmHg; do not have significant turbid refraction media; do not have any history of intraocular surgery or intraocular inflammation; do not have any history of laser therapy; do not have acute infection, chronic inflammatory disease (inflammatory bowel disease, osteoarthritis, rheumatoid arthritis, chronic hepatitis, gout, and asthma bronchial), cardiovascular disease (angina pectoris, acute myocardium infarct, and cerebral infarct) or history of malignancy; currently, do not use steroids, immunosuppressants, statins, aspirin, angiotensin-converting enzyme inhibitor, or hormone therapy; do not take routine antioxidant supplements; and follow a special diet for DM patients as advised by a dietitian.

Exclusion criteria included The occurrence of any dangerous adverse effects during the study and loss to follow-up. L/Z (Cendo-TGF®) were given at a dose of 3 mg of lutein and 0.25 mg of zeaxanthin, two tablets once daily for 30 days.

All patients underwent tests for blood pressure, height and weight measurements, glycemic status, and ophthalmologic status. ROS level in blood serum and contrast sensitivity score was examined before L/Z administration and 30 days after L/Z was given.

Examination of ROS level in blood serum was performed using a human ROS Elisa Kit (Elabscience Biotechnology Co., Ltd.) and contrast sensitivity tests were performed with a Pelli-Robson contrast sensitivity chart (distributed by Clement Clarke International, ref 7002250, Metropia Ltd., 1988). The same observer performed the contrast sensitivity test for each subject.

Measurement of ROS

100 µL venous blood serum was taken in the morning after patients underwent a 10 h fasting period. The serum obtained was added into the micro Elisa plate well and incubated for 1 h at 27°C. After mixing with horseradish peroxidase conjugate and washing 5 times, the substrate and stop solution were added, and optical density was measured using a microplate reader with a wavelength of 450 nm. Results of the measurements were converted to ng/ml.

Contrast Sensitivity Test

Contrast sensitivity tests were performed using a Pelli-Robson contrast sensitivity chart with best-corrected visual acuity. Patients stood 1 m from the chart and continued to read the chart until they could no longer read any more letters.

Statistical Analysis

Descriptive data analysis was used to examine the demographic distribution. The Shapiro–Wilk test was used to examine the normality of the data. A Wilcoxon test was performed for statistical analysis, and a Spearman's correlation test was used to examine the correlation between changes in ROS levels and changes in contrast sensitivity.

RESULTS

Basic characteristics of the subjects are presented in Table 1. There were no subjects lost to follow-up. There were more female patients (57.14%) than male patients (42.86%). In total, 57.14% of subjects were overweight body mass index (BMI) 25–30 kg/m^2 and 42.86% subjects were normal weight (BMI 18-25 kg/m^2). All subjects were in an uncontrolled hyperglycemia state (hemoglobin A1c [HbA1C] > 6.5%) [Table 1].

The mean ROS level before L/Z administration was 15.512 ng/ml. After administration of L/Z for 30 days, there was a decrease in mean ROS level to 6.707 ng/ml. Statistical tests revealed that this was a significant decrease with $P = 0.001$ [Tables 2 and 3].

Contrast sensitivity tests were performed before and after L/Z administration. The mean measurement before administration was 1.286, and this was increased to 1.425 after L/Z administration for

Table 1: Characteristics of subjects

No./Gender	Age (years)	Body mass index (kg/m^2)	HbA1C (%)	BCVA
1. M	58	22.05	12.8	1.0
2. F	54	25.92	9.7	1.0
3. M	70	26.98	7.8	0.6
4. F	53	20.82	12.4	0.7
5. M	60	24.58	10.1	1.0
6. F	50	24.60	8.0	0.15
7. F	53	23.70	7.7	0.7
8. F	65	26.56	9.4	0.4
9. M	68	23.87	7.2	0.7
10. F	51	26.44	10.3	1.0
11. M	69	25.00	10.8	0.9
12. F	70	25.00	8.6	0.8
13. F	50	26.60	14.2	1.0
14. M	60	22.68	13.8	1.0

Hba1c: Hemoglobin A1c, BCVA: Best Corrected Visual Acuity, M: Male, F: Female

Table 2: ROS levels pre and post lutein and zeaxanthin administration

Subject No.	ROS level (ng/ml)	
	Pre	Post
1	9.978	8.404
2	21.091	11.182
3	8.808	8.636
4	10.667	6.859
5	21.293	12.303
6	11.404	4.263
7	17.950	10.970
8	17.455	4.253
9	9.616	4.960
10	25.040	5.061
11	10.960	3.939
12	28.232	4.828
13	12.748	3.990
14	11.929	4.253
Mean	15.512	6.707

ROS: Reactive oxygen species

Table 3: Mean comparative test result of ROS levels pre and post lutein and zeaxanthin administration

ROS	Mean	Standard deviation	*Z*	*P*	Result
Pre	15.512	6.317	−3.296	0.001	Significantly different
Post	6.707	3.019			

ROS: Reactive oxygen species

1 month. The increase in contrast sensitivity score was significant at $P = 0.002$ [Figure 1, Tables 4 and 5].

A correlation test was performed between ROS level and contrast sensitivity score. Spearman's correlation test revealed no significant correlation between ROS level decreases, and contrast sensitivity score increases ($P = 0.882$) [Table 6].

DISCUSSION

Among the participating subjects, eight were female (57.14%) and six were male (42.86%). Statistically, there were no differences between the genders in terms of the prevalence of DR visual disturbances. A study

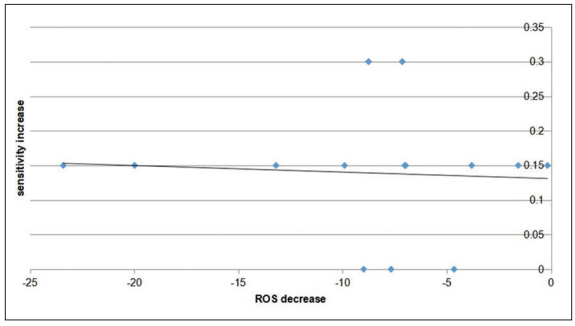

Figure 1 : Correlation between reactive oxygen species level and contrast sensitivity score

Table 4: Contrast sensitivity score pre and post lutein and zeaxanthin administration

Subject No.	Contrast sensitivity score	
	Pre	Post
1	1.35	1.50
2	1.50	1.65
3	1.35	1.50
4	1.20	1.35
5	1.35	1.35
6	0.90	1.20
7	1.20	1.35
8	1.05	1.20
9	1.35	1.35
10	1.35	1.50
11	1.35	1.50
12	1.35	1.50
13	1.35	1.65
14	1.35	1.35
Mean	1.286	1.425

by Al-Amer *et al* also found that gender was not a risk factor for DR. Participants in this study were aged between 50 and 70 years. A published literature review reported that there were no significant differences in the prevalence of DR between age groups, but one survey found that the prevalence of DR was higher among those aged 50–69 years old. Thus, 50 years of age were chosen as the lower limit in this study.[14]

In this study, six subjects (42.86%) were normal weight and eight subjects (57.14%) were overweight. In some studies, nutritional status is considered to be a risk factor for DM DM. Obesity has a role in the occurrence of DM through two pathways; it induces insulin resistance and beta-pancreatic cell dysfunction. Obesity is thought to have several effects on cells, such as disturbing inflammation signals, endoplasmic reticulum stress, ROS overproduction, mitochondrial dysfunction, triglyceride and/or intermediate fatty acid accumulation, and serine-threonine kinase activation. These mechanisms induce each other, resulting in multilevel damage.[15]

Uncontrolled hyperglycemia (HbA1C >6.5%) was observed in all subjects. A chronic uncontrolled hyperglycemic condition causes acute and recurrent change at the cellular level and also causes increases

Table 5: Mean comparative test result of contrast sensitivity score pre and post lutein and zeaxanthin administration

Sensitivity	Mean	Standard deviation	*Z*	*P*	Result
Pre	1.286	0.153	−3.127	0.002	Significantly different
Post	1.425	0.141			

Table 6: Spearman's correlation test result between reactive oxygen species level and contrast sensitivity

Correlation	*P*	Result
−0.044	0.882	Not significantly correlated

in inflammation or oxidative stress. In chronic hyperglycemic conditions, basement membrane thickness and blood flow changes, pericytes and capillaries are also decreased; this results in pericyte ghost, acellular capillaries, and ratio of endothelpericyte <41. Aside from that, a chronic hyperglycemic condition also stimulates increases in oxidative stress, which plays a role in complications of DM occurring through multiple pathways, such as neuropathy, nephropathy, myocardial injury, and retinopathy.[16]

In normal conditions, there is a difference between the ROS level in capillary blood serum and that in venous blood serum. Parmigiani *et al*., stated that the normal ROS level in term babies is 117 ± 58 UF or 3.42 ± 1.51 ng/ml.[17] In this study, mean ROS level obtained from the vein was 15.512 ng/ml.

In patients with chronic hyperglycemia, there is an increase in oxidative stress. Oxidative stress can cause ROS overproduction of mitochondria in endothelial cells, in both small and big vessels. Abundant ROS activates five pathways for the pathogenesis of diabetic complications Polyol pathway flux, increase in AGEs production and AGEs receptor expression, PKC activation, and excessive activity of the hexosamine pathway. With the activation of these pathways, pro-inflammatory reactions increase. Aside from a hyperglycemic condition, ROS level increases can also be affected by obesity and dyslipidemia, in which the condition increases fatty acid oxidation that leads to increases in ROS production by increases in nicotinamide adenine dinucleotide phosphate (NADPH).[8,10]

After the administration of drugs, ROS levels in serum decreased to 6.707 ng/ml. Statistical analysis revealed that this was a statistically significant decrease with $P = 0.001$. L/Z are antioxidants in the xanthophyll group. As antioxidants, L/Z work by decreasing retinal oxidative stress, decreasing PKC activation, decreasing production of nitric oxides, and decreasing retinal cell loss. L/Z was also thought to be scavengers for ROS. Research by Sasaki *et al*., showed that lutein administration could cause a decrease in ROS level, marked by a decrease of dihydroethidium staining fluorescence in the retinal cells of endotoxin-induced uveitis rats.[18,19]

Various factors can affect ROS level in blood serum, such as obesity, chronic inflammation, cardiovascular disease, and malignancy. In this study, possible confounding factors were minimized using inclusion and exclusion criteria. Regulation of food intake was performed to control blood glucose levels, to prevent the subjects falling into a more severe hyperglycemic state than before administration.[8,10]

Contrast sensitivity test results revealed an increase in contrast sensitivity score by as much as 0.139. Wilcoxon test analysis showed a significant increase in contrast sensitivity score after L/Z administration, as compared to before administration.

The decrease in contrast sensitivity score in DR patients was thought to stem from abnormalities in the neural area at the inner retina, because of errors in information processing in the synapse/neuronal area due to vascular dysfunction. Chronic hyperglycemic conditions cause intracellular hyperglycemia that results in microvascular complications.[13,20]

In addition to their antioxidant roles, L/Z are also able to increase information transmission between cells through a post-transcriptional mechanism, accompanied by an increase in mRNA level. Research by Stahl *et al*. showed that L/Z play a role in increasing synapse connections by increasing cell connectivity in the nervous system. In addition, L/Z also increase the pigment density of the fovea area, thus increasing the transduction signal of the visual system. Connectivity between photoreceptors and the visual cortex also increases, resulting in an increase in contrast sensitivity function[13,20]

In three subjects, contrast sensitivity score did not increase after L/Z administration, as compared to pre-administration scores. In some diabetes patients with particular epigenetics, a hyperglycemic memory condition occurs, in which the progressivity or damage occurs continuously or does not change despite controlled hyperglycemic status. This condition is thought to occur because of the persistent epigenetic change from superoxide production in mitochondria in a hyperglycemic condition[20,21]

A Spearman correlation test was performed to analyze the correlation between ROS level and contrast sensitivity score. There was no significant correlation

between the decrease in ROS level in blood serum and the increase in the contrast sensitivity score. The correlation coefficient was −0.044 which means the correlation between ROS level and contrast sensitivity score was very weak.[22]

Patients with DM and uncontrolled chronic hyperglycemic status will have an increase in intercellular glucose level, especially in endothelial cells. High intercellular glucose level causes an increase in superoxide production in mitochondria and stimulates various inflammation pathways, one of which is excessive oxidative stress. This results in an increase in ROS. In a study by Sasaki *et al.*, it was reported that increases in ROS will decrease retinal function in diabetic model rats. The increase in ROS also causes a reduction in synaptophysin and brain-derived neurotrophic factor level at the synapse area, causing decreases in visual function that study administered lutein for 6 months. Lutein administration can decrease ROS level and also increase retinal function/activity.[18,23]

In the current study, there was a weak, statistically non-significant correlation between decreases in ROS level and increases in contrast sensitivity. It was believed that contrast sensitivity score increases not only because of decreases in ROS level but also because of other mechanisms. Other mechanisms that may occur include increasing information transmission between cells, increasing cell connectivity in the nervous system, and increasing pigment density of the fovea area, which simultaneously results in increasing the transduction signal of the visual system. Connectivity between photoreceptors and the visual cortex will also increase, resulting in an increase in contrast sensitivity.[20]

There are some inevitable limitations in this study. The subjects in this study were outpatients; therefore, there is a difficulty in ensuring patients adheres to the same diet to control glycemic status, antioxidant-containing food intake, and obedience in consuming antidiabetic drugs or the antioxidants given in this study. The administration of antioxidants over a short amount of time, though it resulted in a significant value, was not able to show a strong correlation between ROS levels and contrast sensitivity scores.

CONCLUSION

It can be concluded that there is a significant decrease in blood serum ROS level and an increase in contrast sensitivity score in patients with NPDR after L/Z administration. There was a positive, but not significant, correlation between the decrease in ROS level in blood serum and the increase in contrast sensitivity score in the patients.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally in the data collection and the drafting of the manuscript. All the authors read and agreed to the final manuscript.

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