The Role of Biochemical Parameters in Prediction of Retinal Diseases and their Relationship to Cataract, Diabetes, and Hypertension, in Ibn Al Haytham Hospital, Baghdad, Iraq

Huda H. Hassan[1], Fayhaa M. Khaleel[2], Khudair Abbas Jassim[3]

[1]Lecturer, in Department of Chemistry and Biochemistry, College of Medicine, University of Al-Mustansiriyah, Baghdad, Iraq, and Student in Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad, Iraq, [2]Assist. Prof., in Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad, Iraq, [3]Assist. Prof., in Ibn Al Haytham Hospital in Baghdad, Iraq, Ministry of Health, Baghdad, Iraq

Abstract

This work summarize multiple protective roles serum Zeaxanthin, and Malondialdehyde, in association with metabolic profiles and as a risk of retinal disorder disease with cataract, diabetes, and hypertension, totally thirty healthy control group, and seventy-five patients group for both genders were studied. Retinal disorder disease subdivided after clinical diagnosis, into three major -subgroups, the first cohort was twenty-five patients suffers of cataract, the second twenty-five of diabetic patients, while the third group is twenty-five hypertensive patients. Lipid-profile, Fasting-insulin-levels, serum zeaxanthin and malondialdehyde, have been done to all groups. Important findings presented in the roles of serum zeaxanthin, and malondialdehyde, by similarities and differences, in retinal diseases with cataract, diabetes, and hypertension. The activity levels of serum zeaxanthin in hypertensive retinopathy patients (32.80±30.56 ng/mL), was non-significantly (P>0.01) reduced compared to healthy control (88.85±139.31 ng/mL), in compression with the same patients for serum malondialdehyde MDA which expressed the highest level of MDA (2.456±2.149 μg/mL) among the rest groups, which was significantly higher (P<0.05) than that in control (0.783±0.937 μg/mL), but the differences were non-significant (P>0.05) compared to diabetic retinopathy group (1.839±1.515 μg/mL).

The activity levels are negatively associated with malondialdehyde levels in retinal disease patients with cataract, diabetes, and hypertension. Retinal disease patients with cataract, diabetes, and hypertension pathogenesis aren't legitimately ensured. But multiple protective roles may be adopted in clinical diagnosis of retina, also in response to higher levels of oxidative stress, including serum malondialdehyde, and zeaxanthin, by fasting at least eight hours pre sophisticated lens surgery.

Key words: *Zeaxanthin, Malondialdehyde, Retinal disorder diseases, Cataract, Diabetes, Hypertension.*

Introduction

The retina is the light-sensitive part of the eye and responsible for converting the focused image into nerve action potentials, which are then relayed to the brain via the visual pathway. There are many layers of nerve cells within the retina, which provide complex connections between the light-sensitive cells located toward the posterior parts of its surface [1]. Lens is external part of the eye, a bright, transparent focuses light on the retina dark part, and refract the

reminder. It is noteworthy that pacity of lens is a direct outcome of oxidative stress, lens cells undergoing oxidation, crosslinking, and insolubilization form lens fibers accumulate in the lens center that *fibrosis* are progressively compressed result in lens nuclear sclerosis leading to opacity. A plaque-like opacity grows in the axial posterior cortical layer in posterior subcapsular cataract. In most patients, over one type of cataract is found [2]. Under hyperglycemic conditions, part of the excess glucose reacts non-enzymatically with proteins or other tissue or blood constituents, leading to the formation of advanced glycation end products. Progressive accumulation of advanced glycation end products in the diabetic lens has been shown to contribute to the acceleration of cataractogenesis in hyperglycemic animals and diabetic humans [3]. The crystalline lens does not require insulin for glucose and other simple sugars to enter into the lens through the capsule. In the case of diabetes, high concentrations of glucose in the aqueous humor can passively diffuse into the lens. The lens aldose reductase enzyme converts excess glucose to sorbitol or galactose to galactitol. These sugar alcohols (polyols—sorbitol or galactitol) cannot passively diffuse out of the lens, and they accumulate inside the lens. The accumulation of polyols inside the lens results in an osmotic gradient, which facilitates diffusion of water from the aqueous humor to the crystalline lens. The water drags sodium with it, and the lens swelling and electrolyte imbalances result in lens fiber disruption and cataract formation. [4]

Photochemical insult, which is intraocular penetration of light and the consequent generation of reactive oxygen species, such as superoxide and singlet oxygen, and their derivatives, such as hydrogen peroxide and hydroxyl radical, induces damage to the epithelial cell deoxyribonucleic acid (DNA) of the lens, thus triggering a sequence of events leading to cataracts. Potential sources of oxidative stress to the lens include UV light, oxidants in the ocular fluids, endogenous oxidants produced in lens cells, and smoke constituents [4]. Diabetic retinopathy divided into two stages: nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) [5]. Non-proliferative diabetic retinopathy involves basement membrane thickening, pericyte loss, microaneurysms, intraretinal hemorrhages, cotton wool spots, hard exudates, venous beading, venous dilation, capillary acellularity, capillary non-perfusion, and intra-retinal microvascular abnormalities. Early on, capillary basement membrane thickening is seen with increased levels of collagen and laminin. Basement membrane thickening may affect capillary autoregulation as well as interactions with proteins and neighboring pericytes. Pericyte loss is hypothesized to be one of the initial pathologic alterations in DR, and their loss leads to altered microvascular autoregulation, a disrupted blood-retinal barrier, and proliferation of endothelial cells [6].

Proliferative diabetic retinopathy is the advanced type of diabetic retinopathy. When abnormal new blood vessels, (lacking mature endothelial cell tight junctions), began to grow on the surface of the retina and the optic nerve, it would result the onset of Proliferative diabetic retinopathy. If left untreated, proliferation of these vessels (termed retinal neovascularization) can lead to severe vision loss from vitreous hemorrhage and/or tractional retinal detachment [7].

While, for hypertensive retinopathy patients with higher levels of blood pressure, focal ischemic areas of the nerve fiber layer are clinically visible as “cotton wool spots”. Breakdown of the blood-retinal barrier causes secondary exudation of blood and lipid “hard exudates”, both of which are visible on clinical examination. At very high blood pressure levels there may be variable degrees of optic disc swelling [8].

Arachidonic acid is converted by cyclooxygenase, COX, lipoxygenase LOX, and and cytochrome P

CYP pathway into eicosanoids [9,10,11,12]. These lipid mediators can contribute considerably to oxidative stress, inflammation[10,13], and vascular function [14,15]. A type of functional, and bioactive lipid mediators known as Eicosanoids. They derived through long-chain polyunsaturated fatty acids metabolism, and mediated by three types of enzymes including cytochrome enzymes (CYPs), cyclooxygenases (COXs), and lipoxygenases (LOXs).

Patients and Method

Patients are examined for retina disorder conditions by common Diagnostic A-Scan, Fluorescein angiography used to diagnose and monitor the impact of macular degeneration, diabetic retinopathy, and also for Fundus photos. Nerve fiber layer analyzer, and Visual field testhave been done by a ophthalomegalist according according to Amsler grid test, and Fundus Auto Fluorescence (FAF), doctor checked whether patients have the symptoms for retinal disorders diseases with each group of cataract, diabetes or hypertension patients. Biochemical Analysis done for 105 control and patients of both genders, at morning fasting at least for eight hours pre surgery gets started by artificial intraocular len for blood specimens and data were collected directly in Ibn Al Haytham Hospital, stored in dark place and directly analyzed, all the measured individual ages are between (16-65 year), 30 subjects are diagnosed as normal controls, and 25 subjects Cataract, 25 diabetic subjects, and 25 subjects having hypertension. The biochemical tests have been done are serum Zeaxanthin level was determined according to Stahl et al method [16], by HPLC technique. Malondialdehyde, by an enzyme-linked immunosorbent assay, also called ELISA using kit from My Bio Source, its a test that detects and measures blood antibodies. This test can be used to determine if you have antibodies related to certain infectious conditions. ELISA, Fasting Blood Sugar (FBS), insulin resistance test & glucose level we use Cobas c 111 Analyzer for estimation assay, blood pressure by Blood Pressure Monitor, and Blood Picture Film in Ibn Al Haytham Hospital Baghdad Iraq.

Statistical Analysis

Statistical analysis have been done according to SPSS-27 (Statistical Packages of Social Sciences-version 26). The data are simple measurement for frequency, percentage, mean, standard deviation SD, the range (minimum – maximum values). The difference significance of different means (quantitative data) has been tested by Students-t-test for difference between two independent means, or ANOVA test, for difference among more than two independent means. Difference significance of different percentages (qualitative data) were tested using Pearson Chi-square test (c^2-test) with application for Yate's correction or Fisher Exact test if applicable. Statistical significance was considered by P value equal or less than 0.05. Pearson correlation was calculated between two quantitative variables with its t-test for testing significance for correlation.The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. Also, to correlation the r^2 was calculated (The coefficient of determination), i.e., when value of r=0.58, then r^2=0.34, this means that 34% of variation of y may be accounted for by knowing values of x or vice versa. [17-21].

Result and Discussion

This is the first study are demonstrated that early clinical diagnosis based on determination of biochemical concentrations for serum zeaxanthin, and malondialdehyde to gain successful treatments and theraphy may lead to preserve the retina and macula from degradation, also as a risk of various eye diseases, especially for retinal disease patients with diabetes miletus, cataract, and hypertension,

and starving patients at least eight hours also pre lens surgery. All age related retinal diseases, especially cataract development is associated with degradation then loss transparency of crystalline lens related to interactions between concentrations for serum zeaxanthin, malondialdehyde and environmental factors.

In general, serum Zeaxanthin, and Malondialdehyde concentrations as a risk of retinal disorder disease with cataract, diabetes, and hypertension, are determined in associated with sample collection of Iraqi volunteers, so a direct collection for blood specimens done data were collected, and directly analyzed, to avoid sample components destruction by light depending on the risk of male sex, older ages, in Ramadan and out of Ramadan time, at morning fasting at least for eight hours before lens replacement surgery gets started by artificial intraocular lens, for blood specimens collection, fasting glucose concentrations at baseline, antihypertensive drug use location of residence. Table 1 shows the level of MDA in µg/mL expressed as mean ± SD (range). Patients with hypertensive retinopathy have expressed the highest level of MDA (2.456±2.149) among the rest groups, which was significantly higher (P<0.05) than that in control (0.783±0.937), but the differences were non-significant (P>0.05) compared to diabetic retinopathy group (1.839±1.515), and cataract group (2.026±2.367). Furthermore, both of diabetic retinopathy group, and cataract group have shown significant (P<0.05) higher levels of MDA than control group. The results are confirmed with Ateset al (2010), Gönenç et.al. (2013), Kumar et al.(202020) , Sanz-González et (2012), and And Kaliaperumal et (2020).

Table 1: The level of MDA in µg/mL in patients and control.

	Malondialdehyde (µg/ml)	P value compared to		
		Control	Cataract	Diabetes
Hypertension (n=25)	2.456±2.149 (0.080-8.920)	0.0001#	0.505	0.247
Diabetes (n=25)	1.839±1.515 (0.083-4.962)	0.003#	0.741	-
Cataract (n=25)	2.026±2.367 (0.125-8.916)	0.011#	-	-
Control (n=30)	0.783±0.937 (0.026-4.231)	-	-	-
-Data were presented as Mean±SD (Range) #Significant difference between two independent means using Students-t-test at 0.05 level.				

The ocular lens which is constantly exposed, to light and atmospheric oxygen, is vulnerable to the risk of photo-oxidative damage which results, in a cataract. The ROS seem to cause an impairent to the lens crystallins which result, in its aggregation and precipitation, forming opacities, and also, to the proteolytic enzymes, which involved, in the elimination, of the damaged proteins. Oxidation is known, to be a quite early, or initiating step in the comprehensive process in the sequantial steps which lead, to cataractogenesissuggested that it involved in cataractogenesis. [22] MDA is a form of lipid peroxidation, which has been have suggested that it involved in cataractogenesis, mainly because its cross-linking ability. According to Cekic *et al* (2010), The MDA lens may result from lipid peroxidation of the membranes of the lens cells or may be the result of its migration from the readily peroxidized retina or the central body compartment.[23] Kaur *et al* (2016), have suggested that the increase of MDA concentration

in senile cataract patients is a probable indicate to the redox imbalance (in the privilege of oxidative stress), and generating cataracts [24]. Drumond *et al* (2017), have found that serum MDA level is increased in patients with hypertensive, and diabetic retinopathy compared to control, and this increasing was comparable between patients with hypertensive retinopathy, and those with diabetic retinopathy. [25]

In the diabetic state, oxidative stress plays a crucial role. Via multiple interacting pathways and reactive oxygen species ROS generation, oxidative stress harmfully affects the activity of insulin. This could degrade the β-cell islets of the pancreas, resulting in reduced insulin release. Furthermore, free radical creation by non-enzymatic protein glycation, oxidation of glucose, and increasing of lipid peroxidation leads to enzymes damage, sabotage the machinery of cell injury, cell membrane changes, and increased resistance to insulin that is at risk for diabetes .[26] Hypertensive retinopathy is an important condition and a potential source of damage to the target organ due to hypertension. Autoregulation of the retinal circulation is known to fail when blood pressure increases above a critical level. However, the rise in blood pressure alone does not completely account for the degree of hypertensive retinopathy. [27] Hypertension, oxidative stress, resulted either from enhances in ROS production or decreases in antioxidant defenses, is associated with increase in blood pressure, endothelial dysfunction and vascular remodeling.[28]

For Zeaxanthin the level of zeaxanthin in serum of hypertensive retinopathy patients (32.80±30.56 ng/mL), diabetic retinopathy patients (86.38±40.81 ng/mL), and cataract patients (52.58±5.05 ng/mL) was non-significantly (P>0.01) reduced compared to healthy control (88.85±139.31 ng/mL), all demonstrated at Table 2. Dherani *et al* (2008), have found that serum zeaxanthin level is inversely correlated with age-related cataract.[29] Also, Kappi et al (2012), have reported significant decrease of plasma zeaxanthin concentration in ARC patients compared to healthy control, suggesting that zeaxanthin is a protective agent against cataract.[30]

In Liu *et al* (2014), meta-analyses study they have reported significant inverse correlation between blood zeaxanthin and nuclear cataract, as well as blood zeaxanthin is associated with lowering the risk of cortical cataract and sub-capsular cataract.(31) Jiang *et al* (2019), have reported that the increase of zeaxanthin consumption by 10mg per day has significantly decrease the risk of age- related cataract by 26%.[32] The results of zeaxanthin in the current study DR subjects are agreed with She *et al* (2017), who have found a comparable non-significant difference in serum zeaxanthin concentration between DR patients and healthy nondiabetic control. [33] Yet the study of Giehrat and Kowluru (2006), have revealed that supplementation of zeaxanthin is significantly inhibited the development of retinal oxidative damage in diabetic rats and could represent a supplemental therapy to inhibitthe development of retinopathy in diabetes [34] Hozawa *et al* (2009), have found significant negative relationship between serum zeaxanthin level and hypertension, whereas as blood pressure rise up the level of zeaxanthin reduced significantly. [35]

Table 2 : The level of zeaxanthin distributed on age groups and gender.

Groups		Zeaxanthin (ng/mL)			
		Hypertension (n=25)	Diabetes (n=25)	Cataract (n=25)	Control (n=30)
Age (years)	20---29	-	-	53.63±2.87	72.19±98.72
	30---39	-	79.04±0.0	52.67±2.63	88.86±85.81
	40---49	36.93±21.42	69.62±33.73	52.01±2.44	95.60±111.8
	50---59	25.19±8.83	116.72±42.16	50.91±2.10	94.61±106.21
	60---69	35.50±19.66	83.36±49.85	53.61±2.22	91.83±0.0
	P value	**0.313**	**0.213**	**0.274**	**0.996**
Gender	Male	38.85±19.83	83.91±43.93	52.44±2.68	103.87±101.33
	Female	27.02±14.40	88.42±48.46	52.51±2.46	73.56±102.5
	P value	**0.072**	**0.792**	**0.939**	**0.422**

-Data were presented as Mean±SD (Range)
#Significant difference between two independent means using Students-t-test at 0.05 level.
^Significant difference among means by using one way ANOVA.

Table 2 shows the mean ± SD of zeaxanthin distributed on age categories for each of the study cohorts. The variations of zeaxanthin level was non-significant (P>0.05) neither among age categories nor between males and females in all of the study cohorts. Thus age does not consider as risk factor for zeaxanthin in hypertensive, diabetic, cataract, or control subjects of the present study.

In the present study, these biochemical parameters are risk of various eye diseases, especially zeaxanthin. It is a nutritional, not synthesized during development, present in the external membrane of macula and retina cells at high concentrations, act as ocular antioxidants for retardation of age-related cataracts and macular degeneration. These carotenoids are found in the human lens, retinal pigment epithelium/choroid (RPE/choroid), the macula, the iris, and the ciliary body.[36-37] The substituted beta carotene with oxygen as hydroxyl group produce xanthophyll rich with oxygen to cross blood-ocular and blood-brain barriers, as in zeaxanthin. Other carotenoids (β-carotene and lycopene) contain only carbon and hydrogen atoms and do not cross the blood-brain or ocular barriers, and Zeaxanthin also inhibits the proliferation and induces apoptosis in human.[38-39]

Conclusion

The study concludes the followings:

1. Malondialdehyde antioxidant concentrations are significantly high levels with low levels for normal individuals, invers relation for diabetes related to starvation for more than eight hours and increased oxidative stress for metabolic disorder pre surgery resulting in this state, causing inflammations and soreness.

2. Increased concentration of serum Malondialdehyde antioxidant for hypertension shows decreased liver storage of vitamin A, this seems to be very clear for low levels of Zeaxanthin concentration leading to development of cardiovascular diseases, advance stages for liver diseases affecting development and adult tissue regeneration.

3. These results suggest that improvement of Zeaxanthin and Malondialdehyde antioxidant concentrations as important diagnostic risks factor of potential usefulness for all retinal diseases with related states as hypotheses for further study, and a monitor for these cases also.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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