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Association of advanced oxidation protein product, thiobarbituric acid reactive substances and total sulfhydryl groups with retinal blood vessels caliber

Утицај продуката узнапредовале оксидације, супстанци реактивних са тиобарбитурном киселином и укупних сулфхидрилних група на дијаметар крвних судова ретине

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SUMMARY

Introduction/Objective Intensive oxidative stress is proven in patients with DM and important in the development of a microvascular complication of DMT2.

The aim of the study was to investigate the relationship between morphometric parameters of retinal blood vessels in patients with diabetic retinopathy (DR) and the levels of parameters of oxidative stress: advanced oxidation protein product (AOPP), thiobarbituric acid reactive substances (TBARS) and total sulfhydryl groups (SH groups) in blood samples.

Methods Patients were (group with DR and controls) matched by sex and years of life. Glycaemia, HbA1C, total cholesterol and its fractions, and triglycerides were measured in blood samples. AOPP and total SH groups were determined in plasma by specific methods. Modification of TBA method has been used for determinatinon of TBARS.

Number and diameter of retinal blood vessels, as morphometric parameters on digital retinal photography was performed with the ImageJ software. The Student's t-test has been used as statistical method for evaluation of differences between the morphometric and blood test parameters. The significance of differences in morphometric parameters of retinal blood has been establish by one-way ANOVA.

Results Significantly higher levels of parameters of oxidative stress **(**AOPP and TBARS**)** were in group of patients with DR than in controls. This difference was also present among the patients with mild and severe form of DR (AOPP F 77.03, p<0.001) (TBARS F 63.28, p<0.001).

Diameter of retinal blood vessels correlated with levels of AOPP, but only in patients with mild DR.

Conclusion Parameters of oxidative stress, AOPP and TBARS may be important for the follow-up of DR. In early stages in diabetic retinopathy, AOPP can be valuable biomarker.

Keywords: diabetic retinopathy; oxidative stress; retinal vessels

САЖЕТАК

Увод/Циљ Оксидативни стрес налази се основни настанка микроваскуларних компликација у особа са типом 2 дијабетес мелитуса. Циљ нашег рада био је утврђивање везе између нивоа параметара оксидативног стреса, продуката убрзане оксидације протеина (*AOPP*) и тиобарбитурно реактивних супстанци (*TBARS*) и параметра антиоксидативне заштите укупне сулфхидрилне групе (СХ групе) у узорцима крви са морфометријским параметрима код испитаника са дијабетичном ретинопатијом (ДР).

Метод Испитаници су подељени у групу пацијената са ДР и контрола. Лабораторијске анализе крви обухватале су одређивање гликемије наште, *HbA1C*, укупног холестрола, фракција *LDL*, *HDL*, триглицерида. *AOPP* и СХ групе одређивани су у плазми испитаника. *TBARS* одређиван је модификованом методом *TBA*.

За морфометријску анализу крвних судова ретине, број и дијаметар, коришћен је *ImageJ Software* за анализу дигиталне фотографије очног дна. За статистичку анализу биохемијских и морфометријски параметара коришћен је Студентов *t*-test а, *one-way ANOVA* за утврђивање статистички значајне разлике.

Резултати Вредности *AOPP* и *TBARS* биле су статистички значајно више у групи испитаника са узнапредовалом ДР (*AOPP F* 77,03, $p < 0.001$) (*TBARS F* 63,28, $p < 0,001$). Вредности *AOPP* корелирале су са вредностима дијаметра крвних судова.

Закључак Вредности *AOPP* и *TBARS* могу бити параметри праћења развоја ДР, а вредности *AOPP* могу бити биомаркер раног стадијума ДР.

Кључне речи: дијабетична ретинопатија; оксидативни стрес; крвни судови ретине

INTRODUCTION

A vision-threatening microvascular complication of diabetes reported in about one-third of patients is diabetic retinopathy (DR)[1].

According to American Diabetes Association and Diabetic Retinopathy Guidelines, DR can be categorized as early non-proliferative diabetic retinopathy (mild NPDR), moderated and severe, or pre-proliferative diabetic retinopathy PPDR) and proliferative diabetic retinopathy (PDR) [1]. Microaneurysms and blot hemorrhages are clinical sings of mild non-proliferative DR. In the middle stages NPDR hard exudates, maculopathy, venous changes, retinal capillary loss and ischemia, cotton wool or soft exudates, dot, blot spots, and extensive intraretinal hemorrhages are present [2]. Neovascularization, preretinal and vitreous hemorrhage, fibrovascular proliferation, and retinal detachments are present in patients with of PDR [2].

Retinal tissue is rich in poly unsaturated fatty acids (PUFAs), direct exposed to UV radiation and high demand for energy which makes it prone to oxidative stress. Oxidative stress is proven in patients with DM and is also important in the development of a microvascular complication of DMT2 [3-6].

The aim of this paper was to investigate the correlation of number and diameter of retinal blood vessels as morphometric parameters and oxidative stress parameters - oxidation protein product (AOPP), thiobarbituric acid reactive substances (TBARS) and total sulfhydryl groups (SH groups), as parameter of antioxidative defense in patients with diabetic retinopathy.

METHODS

Subjects

The study included 51 Caucasian patients. Seventeen patients (9 males and 8 females) were with mild NPDR and nine patients (4 females and 5 males) had PPDR. The control group included 25 healthy individuals (14 males and 11 females). Family history of diabetes was negative. Excluding criteria were intraocular inflammatory diseases (scleritis, uveitis), glaucoma and age-related macular degeneration, smoking, use of angiotensin receptor

blockers, antioxidants or mineral supplements, any previous ophthalmic surgical or laser interventions. The study was performed at the Clinic for Eye Diseases, in the Center for Biochemical Research of the Clinical Centre Niš and Department of Biochemistry, Faculty of Medicine, University of Niš, Serbia. All patients were informed about the methods and the aim of the study, and provided their written informed consent to participate. The study was performed in agreement with the rules and was approved by the Internal Ethic Committee of the Faculty of Medicine in Niš.

In all subjects the ophthalmic examination obtains: best corrected visual acuity, tonometry, anterior segment and posterior segment examination by indirect ophthalmoscopy, photo fundus (FF) and fluorescein angiography (FA). Photofundus and FA were done in all patients with DR, under the same conditions, by the same digital fundus camera and by the same ophthalmologist. ETDRS classification was used for the staging of the DR [2].

Blood chemistry analysis

Glycaemia, HbA1C, total cholesterol and its fractions (LDL-C and HDL-C), and triglycerides were measured in blood samples with Olympus AU680. The samples were collected in early morning on an empty stomach.

AOPP was determined by in plasma using the method of Witko-Sarsat etal.[7]. Concentration of AOPP groups is expressed in μmol/L.

Total -SH concentration was determined by using5-5'-dithio-bis (2-nitrobenzoic acid) -(DTNB)[8].Absorbance were measured at 412 nm against blank samples without and expressed as mmol/l. Concentration of SH groups are expressed in μmol/L.

TBARS were determined by modification of TBA metoda [9]. The concentration of TBARS is expressed in μmol/L.

Morphometric analysis

Morphometric analysis of digital fundus photography was preformed using the ImageJ software in all examined participants. Both eyes in each patient were analyzed (figure 1 and

2). According to the manufacturer instructions, spatially calibration for magnification of retinal digital camera (1 pixel = 17.7 μm) was used. In the first phase of the morphometric analysis, the optic disc Ferret's diameter (D_F), circularity and centroid were measured. Then, in the second phase we applied "concentric circles" plugin in order to divide retinal images into five concentric zones which center was the centroid of the optic disc (Figure 1). The first concentric area was the optic papilla and next to it was marked as the first zone (zone I). Other zones (zone II, zone III, zone IV and zone V) were marked according to the gradual increase of their distance from the optic disc. Zones were constructed as equal and their size in different patients depended on the optic disc location in the retinal images. Macular region was located in the zones II and III. The number of retinal blood vessels (N) in each retinal zone, including the optic disc was established with "cell counter" plugin. In the case of blood vessel bifurcations, two newly formed blood vessels were counted as separate vessels. The outer diameter of all counted blood vessels (D_{BW})in one zone was measured at three different localizations in each of them, and then the mean value was calculated. The same method has been used in the study by Cekić et al. [10].

Statistical method

Statistical package NCSS PASS 2007 was used for the statistical analysis. Kruskal–Wallis one-way ANOVA test and Dunn's post-hoc test were used to compare median values between groups, while Mann–Whitney U test was used in case of two groups.

Correlations between parameters were established by Spearman's rho (ρ).

RESULTS

The patients were classified into two groups according to changes detected by indirect ophthalmoscopy, FF and FA.ETDRS classification was used. In patients with mild DR, a small number of microaneurysms was detected showed. Microaneurysms, different form of hemorrhages (dot, blot spots, and intraretinal hemorrhages); and cotton walls, venous bleeding and, intraretinal vascular abnormalities (IRMAs) in two or more quadrants in the group of patients with PPDR.

The mean age of examined group of patients and results of the median values of evaluated parameters of blood are presented in Table 1. Median duration of DMT2 in the two groups of patients was not statistically different (Z=1.89, p=0.06) (Table 1). Levels of HbA1C were higher in the group with very severe form were significantly higher than the same in the group with mild NPDR (Z=2.26, p<0.001).

The values for AOPP and TBARS as biomarkers of oxidative stress, and total SH groups as parameter of antioxidative defense are presented in Table 1. The values for SH group were higher in group with mild NPDR than in controls and group of patients with PPDR (F 24.08, p<0.001).

Levels of AOPP were significantly higher in group of patients with DR than in controls, as well as among the two different groups with different form of DR (F 77.03, p<0.001) as well as levels of TBARS (F 63.28, p<0.001).

The results of the morphometric analysis were used for cluster analysis (k-means method) and mean values are given in Table 2. These tables also present the results of the Student's t – test.

The values of average number of observed blood vessels increased from the optic disc towards the zone III, and, then decreased gradually towards the zone V. The average number of blood vessels per each zone showed a similar trend on the left side in the group of patients with mild NPDR.In the group with PPDR in zones I, II and III there was a significantly higher average number of blood vessels than in the optic disc and the zones IV and V.

The average blood vessel outer diameter decreased from the optic disc towards the zone V and, this decrease was significant on the right side in the mild NPDR and in the PPDR.

The outer diameter of the blood vessels in the zones III, IV and V was significantly (p<0.05) lower than in the optic disc. This parameter showed a similar trend in mild NPDR and PPDR. On the both eyes and in both groups of examined patients, NPDR and PPDR the outer diameter of blood vessels decreased from the optic disc towards the zone V.

Finally, correlation analysis revealed that outer diameter positively correlated with levels of AOPP (Table 3). This correlation was present on optic disc and in zone I, zone II and zone III and only for patients with early or mild form of NPDR. The levels of SH groups

also had similar correlation with morphological parameters of blood vessels but not in all zones and only in group of patients with PPDR (Tables 4). This correlation was not present for levels of TBARS (Tables 3 and 4).

DISCUSSION

Oxidative stress is proven in patients with DM and in pathogenesis of microvascular complication [11,12]. The present study has a role to investigate the correlation between the levels AOPP and TBARS with severity of disease and morphometric parameters of retinal blood vessels.

Significant higher levels were present for levels of AOPP and TBARS, in plasma of examined patients with DR (Table 1). The levels of AOPP and TBARS correlated positively with progression of DR (Table 1). Correlation analyze revealed that AOPP and diameter of retinal blood vessels correlated positively in patients with mild, early stage of DR (Table 3). According to this results AOPP maybe a biomarker of early changes in DR.

In diabetes, the formation of AOPP is induced by intensified glycoxidation processes, oxidant-antioxidant imbalance, and coexisting inflammation. The role of AOPP in pathogenesis of DR could be explained by its structurally and biological similarity with advanced glycation product (AGE)[13]. Also, it is proposed that AOPP express proinflammatory activities [13, 14].

AOPP accumulation contributes to DR thought direct tissue, damage effects well as thought the activation of specific AGE receptors (RAGE) [13,14,15]. RAGEs activation induces permeability of microvascular endothelial cells and productions of ROS. Endothelial damage due to accumulation of AGE, activation of PKC, increased expression of VEGF and intracellular adhesion molecule (ICAM-1), and increases in reactive oxygen species (ROS) leads to expression of endothelial nitrit oxid sintetasis NOS. RAGE activation subsequently evokes fibrogenic reaction[11].Thickening of the basement membrane coupled with its increased permeability, loss of pericytes leading to diminished vessel wall tone, and development of protruding microaneurysms, as well as proliferation of mesangial and causing obstruction and obliteration of capillaries are results of all of these processes.

The results of morphological changes in our examined patients have showed the outer diameter of blood vessels decreased significantly with progression of DR (Table 2). The same results are presented in study by Cekić et al. [10]. The remodulation and regression of vascular net in DR has been in focus of many different studies [12,13]. Formation of peroxiynitrite due to reaction between ROS and nitric oxide further causes endothel dysfunction. Increased apoptosis of retinal capillary cells is result of damage of the mitochondrial lipid membrane by ROS. Increased nitrate stress in retinal vascular cells, via activation of nuclear transcriptional factor, NF-kB by AGE, leads to apoptosis of retinal pericytes [12,16]. Our results have shown that levels AOPP correlate with severity of DR.

Levels of TBARS are elevated in both groups of patients with DR and correlate with severity of disease (Table 1). But, levels of this parameter of oxidative stress have not shown correlation with retinal blood vessels in our study. Similar results are presented in the study conducted by Rui et al. [17]. TBARS serve as potential biomarkers for DR.

The antioxidant status of diabetic patient has an important role in producing oxidative stress and development of vascular complications in patients with DM. The reports of antioxidants and antioxidant enzymes in DR patients are contradictory [4,5,6,18]. The total thiol levels as marker of antioxidant status in diabetics has shown to be significantly deceased in patients with DR. In our study levels of total SH group in serum were higher in NPDR, and significantly lower in PPDR. An inverse relation between the level of HbA1C and total SH groups in patients with moderated form of DR indicate reduction in antioxidant status in poorly controlled patients. Sharma et al. (2015) has been demonstrated that decreased GSH levels, in patients with PDR patients, are associated with in vivo structural changes of retina (19). These results correlated with ours, but the precise mechanisms are still unclear. Therefore, levels of total SH groups could be predictive for development of DR and its progression.

CONCLUSION

These findings suggest that AOPP and TBARS can be used as a biomarker for DR and its progression. Levels of AOPP correlate with diameter of retinal blood vessels in early stage of DR and AOPP may be a parameter of early stage of DR.

Limitations factors of this study that should be noted are as follows: this study included only Caucasian patients, the influence of local and ocular factors on retinal blood vessel caliber could not be avoided. More precise medical imaging and correlation with studied parameter are needed.

Conflict of interest: None declared.

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Table 1. Mean values of measured parameters in blood of evaluated groups

Parameter	Group	n	Mean	SD	F	p
Age	Control	25	52.12	6.19	2.65	n.s.
	Mild NPDR	17	57.71	7.90		
	PPDR	9	55.78	11.61		
HbA1c (%)	Control	25	5.08	0.52	39.52	<0.0001
	Mild NPDR	17	7.99	1.52		
	PPDR	9	8.64	2.07		
SH	Control	25	300.96	63.52	24.08	<0.0001
	Mild NPDR	17	401.83	50.18		
	PPDR	9	267.89	27.04		
AOPP	Control	25	31.11	4.06	77.03	<0.0001
	Mild NPDR	17	47.51	10.82		
	PPDR	9	87.09	22.92		
TBARS	Control	25	12.08	1.77	63.28	<0.0001
	Mild NPDR	17	16.15	1.03		
	PPDR	9	20.71	3.68		

a – Control vs. I, p <0.0001;
b – Mild NPDR vs. PPDR, p<0.0001;
c – control vs. II, p<0.000

Table 2. Number and diameter of retinal blood vessels on right and left eye in examined zone

Zone	Parameter	Group	n	Right eye	p	Left eye	p
				$\bar{x}$± SD		$\bar{x}$± SD	
Optic disc	Number	Control	25	18.34±3.33		18.44±3.15	
		Mild NPDR	17	17.77±3.77[a]	0.05	16.33±3.66[a]	0.05
		PPDR	9	14.01±3.49[a,b]	0.05	14.56±3.18[a,b]	0.05
	DBV (μm)	Control	25	74.89±10.35		74.33±8.04	
		Mild NPDR	17	83.66±5.66[a]	0.05	85.33±9.17[a]	0.05
		PPDR	9	94.77±12.28[a,b]	0.05	93.05±14.17[a,b]	0.05
Zone I	Number	Control	25	29.87±5.610		29.989±4.982	
		Mild NPDR	17	27.95±5.111[a]	0.05	30.000±6.922[a]	0.05
		PPDR	9	24.11±4.106[a,b]	0.05	26.333±4.472[a,b]	0.05
	DBV (μm)	Control	25	74.287±8.317		76.579±7.563	
		Mild NPDR	17	79.043±6.660[a]	0.05	79.455±8.551[a]	0.05
		PPDR	9	93.300±10.643[a,b]	0.05	91.888±12.211[a.b]	0.05
Zone II	Number	Control	25	43.760±8.828		40.67±8.079	
		Mild NPDR	17	39.294±2.289[a]	0.05	38.29±8.308[a]	0.05
		PPDR	9	28.667±7.382[a,b]	0.05	30.00±7.104[a,b]	0.05
	DBV (μm)	Control	25	64.194±8.052		64.312±7.166	
		Mild NPDR	17	68.537±7.768[a]	0.05	66.964±8.262[a]	0.05
		PPDR	9	83.959±11.610[a,b]	0.05	85.284±14.971[a,b]	0.05
Zone III	Number	Control	25	44.42±7.070		42.97±10.725	
		Mild NPDR	17	41.65±11.096[a]	0.05	39.75±9.333	NS
		PPDR	9	26.01±11.342[a,b]	0.05	25.11±9.033[a,b]	0.05
	DBV (μm)	Control	25	59.389±8.591		60.269±10.111	
		Mild NPDR	17	62.994±8.321[a]	0.05	59.733±7.566	NS
		PPDR	9	77.766±10.042[a,b]	0.05	76.198±13.220[a,b]	0.05
Zone IV	Number	Control	25	36.54±7.832		31.32±7.966	
		Mild NPDR	17	35.86±8.377[a]	0.05	34.17±10.979[a]	0.05
		PPDR	9	23.45±11.22[a,b]	0.05	19.00±7.882[a,b]	0.05
	DBV (μm)	Control	25	55.60±6.683		57.676±11.333	
		Mild NPDR	17	56.376±4.518[a]	0.05	57.767±10.152[a]	0.05
		PPDR	9	75.043±8.860[a,b]	0.05	71.884±14.044[a,b]	0.05
Zone V	Number	Control	25	21.40±5.991		16.76±6.023	
		Mild NPDR	17	21.55±5.666	NS	15.47±6.135[a]	0.05
		PPDR	9	13.33±4.242[a,b]	0.05	10.78±4.764[a,b]	0.05
	DBV (μm)	Control	25	56.110±6.406		61.805±15.052	
		Mild NPDR	17	56.555±6.731	NS	59.487±10.324[a]	0.05
		PPDR	9	75.012±11.334[a.b]	0.05	74.795±17.345[a,b]	0.05

[a]p<0.05 *vs*. controls;
[b]p<0.05 *vs* mild NPDR

Table 3. Correlation between number and diameter of retinal blood vessels and AOPP. SH groups and TBARS in mild NPDR patients

RIGHT EYE														
Parameter		RPC	RPD_F	RPN_{BV}	RPD_{BV}	$RZ1N_{BV}$	$RZ1D_{BV}$	$RZ2N_{BV}$	$RZ2D_{BV}$	$RZ3N_{BV}$	$RZ3D_{BV}$	$RZ4N_{BV}$	$RZ4D_{BV}$	$RZ5N_{BV}$	$RZ5D_{BV}$
SH	R	0.143	0.252	-0.513	0.074	-0.08	0.27	-0.256	-0.285	-0.035	-0.257	0.116	-0.162	0.216	0.022
	p	0.585	0.33	0.035	0.777	0.76	0.294	0.321	0.268	0.893	0.32	0.658	0.535	0.404	0.933
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17
AOPP	R	-0.509	0.287	-0.135	-0.492	-0.198	-0.493	0.016	-0.181	0.475	-0.151	0.153	-0.372	0.072	-0.084
	p	0.037	0.265	0.605	0.045	0.445	0.045	0.953	0.047	0.054	0.043	0.557	0.142	0.784	0.749
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17
TBAS	R	-0.342	0.37	-0.193	-0.231	0.374	0.032	0.135	-0.104	0.222	0.058	-0.302	0.128	-0.258	0.104
	p	0.178	0.144	0.459	0.373	0.139	0.904	0.607	0.692	0.391	0.826	0.238	0.625	0.317	0.69
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17
LEFT EYE															
Parameter		LPC	LPD_F	LPN_{BV}	LPD_{BV}	$LZ1N_{BV}$	$LZ1D_{BV}$	$LZ2N_{BV}$	$LZ2D_{BV}$	$LZ3N_{BV}$	$LZ3D_{BV}$	$LZ4N_{BV}$	$LZ4D_{BV}$	$LZ5D_B$	$LZ5N_{BV}$
SH	R	0.086	0.009	-0.241	0.108	-0.166	0.238	-0.351	0.369	-0.361	0.412	-0.374	0.507	-0.237	0.153
	p	0.743	0.972	0.352	0.681	0.525	0.358	0.168	0.145	0.155	0.1	0.139	0.038	0.359	0.556
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17
AOPP	R	-0.565	0.512	-0.286	-0.044	-0.362	0.402	-0.34	0.333	-0.06	-0.285	0.103	-0.007	-0.067	0.143
	p	0.018	0.036	0.266	0.05	0.153	0.01	0.182	0.052	0.82	0.267	0.693	0.979	0.798	0.585
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17
TBARS	R	-0.548	0.409	-0.141	0.093	0.028	0.052	-0.44	0.053	-0.368	-0.206	-0.327	0.131	-0.255	0.309
	p	0.023	0.103	0.59	0.723	0.914	0.844	0.077	0.839	0.146	0.428	0.2	0.617	0.324	0.228
	N	17	17	17	17	17	17	17	17	17	17	17	17	17	17

Table 4. Correlation between number and diameter of retinal blood vessels eye and AOPP. SH groups and TBARS in PPDR patients

RIGHT EYE															
Parameter		RPC	RPD_F	RPN_{BV}	RPD_{BV}	$RZ1N_{BV}$	$RZ1D_{BV}$	$RZ2N_{BV}$	$RZ2D_{BV}$	$RZ3N_{BV}$	$RZ3D_{BV}$	$RZ4N_{BV}$	$RZ4D_{BV}$	$RZ5N_{BV}$	$RZ5D_{BV}$
SH	R	0.654	0.093	0.186	-0.008	0.457	0.111	0.281	0.378	0.18	0.268	0.396	-0.327	-0.131	0.587
	p	0.056	0.812	0.631	0.984	0.216	0.777	0.464	0.316	0.644	0.485	0.292	0.391	0.737	0.097
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
AOPP	R	0.032	0.151	0.332	-0.095	-0.436	0.441	-0.585	0.319	-0.496	-0.089	-0.531	0.482	-0.326	0.231
	p	0.935	0.697	0.383	0.807	0.241	0.234	0.098	0.402	0.174	0.82	0.141	0.189	0.392	0.55
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
TBARS	R	-0.068	0.151	0.367	-0.32	0	0.059	-0.335	0.281	-0.458	0.385	-0.309	0.362	-0.41	0.284
	p	0.861	0.699	0.331	0.401	1	0.879	0.379	0.464	0.215	0.307	0.418	0.339	0.273	0.459
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
LEFT EYE															
Parameter		LPC	LPD_F	LPN_{BV}	LPD_{BV}	$LZ1N_{BV}$	$LZ1D_{BV}$	$LZ2N_{BV}$	$LZ2D_{BV}$	$LZ3N_{BV}$	$LZ3D_{BV}$	$LZ4N_{BV}$	$LZ4D_{BV}$	$LZ55N_{BV}$	$RZ5D_{BV}$
SH	R	0.36	-0.083	0.687	-0.63	0.657	-0.632	0.395	-0.764	0.67	-0.82	0.45	-0.69	-0.024	-0.296
	p	0.342	0.832	0.041	0.069	0.055	0.068	0.293	0.017	0.048	0.007	0.225	0.04	0.951	0.439
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
AOPP	R	0.281	-0.307	0.001	-0.011	-0.392	-0.036	-0.215	0.184	-0.389	0.388	-0.27	0.007	-0.001	-0.184
	p	0.464	0.422	0.997	0.977	0.297	0.926	0.578	0.635	0.301	0.302	0.483	0.987	0.999	0.636
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9
TBARS	R	-0.232	0.241	0.224	-0.234	-0.3	-0.325	-0.291	-0.039	-0.263	-0.112	0.079	-0.208	-0.232	-0.409
	p	0.548	0.533	0.563	0.544	0.433	0.393	0.448	0.921	0.495	0.774	0.839	0.591	0.548	0.275
	N	9	9	9	9	9	9	9	9	9	9	9	9	9	9

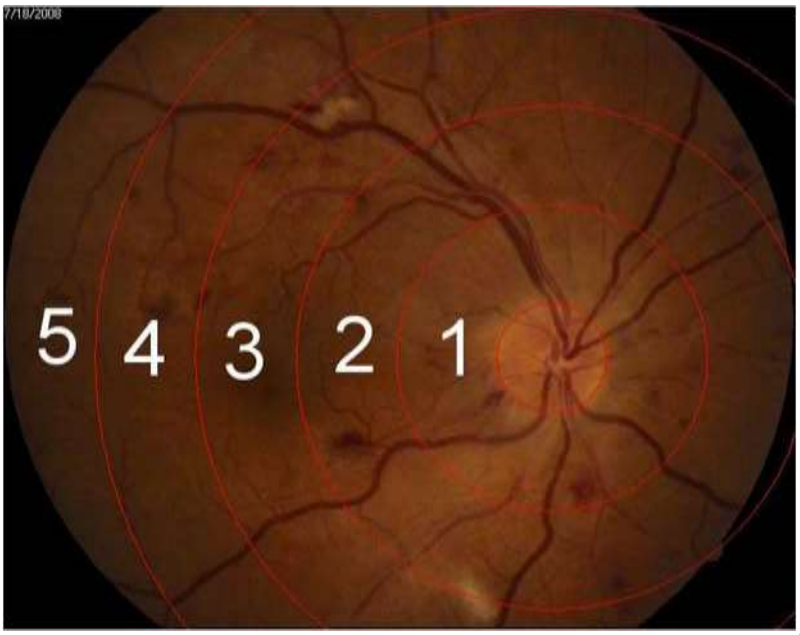

Figure1. Digital fundus photography with concentric zones – right eye

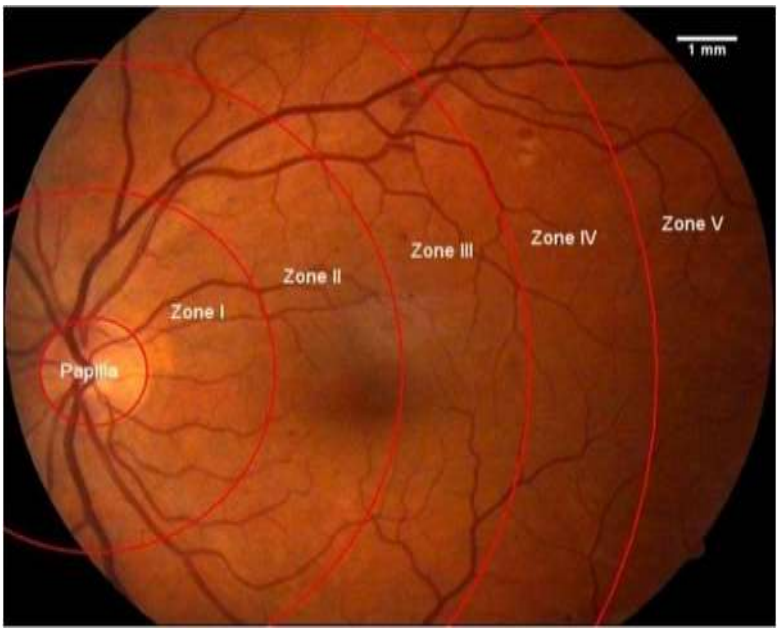

Figure2. Digital fundus photography with concentric zones – left eye

