C-reactive protein and chitinase 3-like protein 1 as biomarkers of spatial redistribution of retinal blood vessels on digital retinal photography in patients with diabetic retinopathy

Sonja Cekić[1]*, Tatjana Cvetković[2], Ivan Jovanović[3], Predrag Jovanović[1], Milica Pešić[4], Gordana Stanković Babić[1], Svetislav Milenković[5], Dijana Risimić[5]

[1]Clinic for Eye Diseases, Clinical Centre, Faculty of Medicine, University of Niš, Serbia, [2]Department of Biochemistry, Faculty of Medicine, University of Niš, Serbia, [3]Department of Anatomy, Faculty of Medicine, University of Niš, Serbia, [4]Clinic of Endocrinology, Diabetes and Metabolic Disorders, Clinical Centre Niš, Faculty of Medicine, University of Niš, Serbia, [5]Clinic for Eye Diseases, Clinical Centre of Serbia, Faculty of Medicine, University of Belgrade, Serbia

Abstract

The aim of the study was to investigate the correlation between the levels of C-reactive protein (CRP) and chitinase 3-like protein 1 (YKL-40) in blood samples with morpohometric parameters of retinal blood vessels in patients with diabetic retinopathy. Blood laboratory examination of 90 patients included the measurement of glycemia, HbA1C, total cholesterol, LDL-C, HDL-C, triglycerides and CRP. Levels of YKL-40 were detected and measured in serum by ELISA (Micro VueYKL-40 EIA Kit, Quidel Corporation, San Diego, USA). YKL-40 correlated positively with diameter and negatively with number of retinal blood vessels. The average number of the blood vessels per retinal zone was significantly higher in the group of patients with mild non-proliferative diabetic retinopathy than in the group with severe form in the optic disc and all five retinal zones. The average outer diameter of the evaluated retinal zones and optic disc vessels was significantly higher in the group with severe compared to the group with mild diabetic retinopathy. Morphological analysis of the retinal vessels on digital fundus photography and correlation with YKL-40 may be valuable for the follow-up of diabetic retinopathy.

KEY WORDS: diabetic retinopathy, retinal vessels, CRP, YKL-40

Bosn J Basic Med Sci. 2014;14(3):177-184. © 2014 ABMSFBIH

INTRODUCTION

Diabetic retinopathy (DR) is a microvascular complication of diabetes. It is the leading cause of new cases of blindness among adults. There were more than 382 million people suffering from diabetes in 2013 worldwide [1]. The International Diabetes Federation projected that the number of diabetic patients will reach 592 million in 2035 [1]. It has been reported that about one-third of diabetic patients have sings of DR and about one-tenth of them have vision-threatening retinopathy. Total of 60% of all patients with diabetes mellitus type 2 (DMT2) was diagnosed with DR, and 35% of DR patients progress to proliferative DR and severe vision loss in 10 years, respectively [2].

*Corresponding author: Sonja Cekić
Bulevar Dr Zorana Đinđića 48, Niš, Serbia
E-mail: sonjaziv@yahoo.com

Submitted: 13 April 2014 / Accepted: 19 July 2014

DR can be categorized into early non-proliferative diabetic retinopathy (mild NPDR), moderated and severe, or pre-proliferative diabetic retinopathy (PPDR) and proliferative diabetic retinopathy (PDR) [3]. Early pathology is considered to be mild non-proliferative. Clinically, the retinal vascular microaneurysms, and blot hemorrhages may be seen. The middle stages include moderate, severe, and very severe non-proliferative diabetic retinopathies, usually with hard exudates and maculopathy. During this phase, venous changes, retinal capillary loss, retinal ischemia, cotton wool or soft exudates, dot, blot spots, and extensive intraretinal hemorrhages are evident. Finally, advanced disease is called PDR. Clinical signs include neovascularisation, preretinal and vitreous hemorrhages, fibrovascular proliferation, and retinal detachments.The retinal vessels are accessible to noninvasive visualization, providing a unique opportunity to investigate the relationship between structure and pathologic features of the microcirculation and systemic and ocular diseases, such as

glaucoma, cardiovascular diseases, stroke, obesity and dyslipidemia [4-12].

It has been proven that low activity chronic inflammation underlies the development of diabetes mellitus type 2. However, the precise mechanism which is responsible for the development of DR complication has remained unclear. The hypothetical role of inflammation and oxidative stress has been a focus of many different studies recently [12-18].

Some inflammatory biomarkers and risk factor for endothelial dysfunction such as C- reactive protein (CRP), tumor necrosis factor α (TNF-α), interleukin 18 (IL-1β) and serum asymmetric dimethylarginine (ADMA) have been the subjects of new studies [13-19]. Chitinase 3-like protein 1 is a novel biomarker of inflammation and tissue remodelation [20]. It is suggested that it may be considered as a promoter of angiogenesis in neoplasms [21].

The aim of this paper was to investigate the correlation between the levels of CRP and chitinase 3 protein 1 (YKL-40) in blood samples with morphometric parameters of retinal blood vessels, number and diameter, on digital fundus photography, in patients with DMT2 and DR.

MATERIALS AND METHODS

Subjects

The study included 90 Caucasian patients of whom 60 had DMT2. Thirty patients (17 males and 13 females) suffered from mild NPDR and, 30 patients (18 females and 12 males) had very severe NPDR. The control group included 30 healthy individuals (17 males and 13 females) without any form of glycemia disorder or eye disease previously diagnosed, or any other systemic or inflammatory disease at the moment of study. In addition, they had no family history of diabetes. 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 aim of the study and provided their written informed consent to participate. The study was in keeping with the rules of the Internal Ethic Committee of the Medical Faculty in Niš.

The standard ophthalmic examination was performed in all patients and controls: the best corrected visual acuity, anterior segment examination, tonometry, and posterior segment examination by indirect ophthalmoscopy, photofundus (FF) and fluorescein angiography (FA). Photofundus check-up, color and green mode, as well as fluorescein angiography were performed in all patients with DR, having their pupils dilated; 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 [3]. Patients with any sign of presence of newly formed blood vessels were excluded from the study.

Blood Chemistry Analysis

Blood laboratory examination of all 90 patients included the measurement of glycemia, glycated hemoglobin (HbA1C), total cholesterol, LDL-C, HDL-C, triglycerides, plasma fibrinogen (PF) and CRP with Olympus AU680. Levels of YKL-40 were detected and measured in serum by an enzyme immunoassay method (Micro VueYKL-40 EIA Kit, Quidel Corporation, San Diego, USA). Blood samples were taken in early morning hours, on an empty stomach, and before receiving any treatment.

Morphometric Analysis

Digital retinal photography with pupils dilated, according to an imaging protocol described below, was taken by the same ophthalmologist under the same conditions. We preformed morphometric analysis using the ImageJ software (http://rsbweb.nih.gov/ij/) in controls and all the patients. Both the right and the left eye of each patient were analyzed. System was spatially calibrated for magnification of retinal digital camera (1 pixel = 17.7 μm) according to the manufacturer instructions. 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 the "concentric circles" plugin (http://rsbweb.nih.gov/ij/plugins/concentric-circles.html) in order to divide retinal images into five concentric zones with the center matching the centroid of the optic disc (Figure 1).

The first concentric area was the optic disc and the next one 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 determined as equal and their size in different

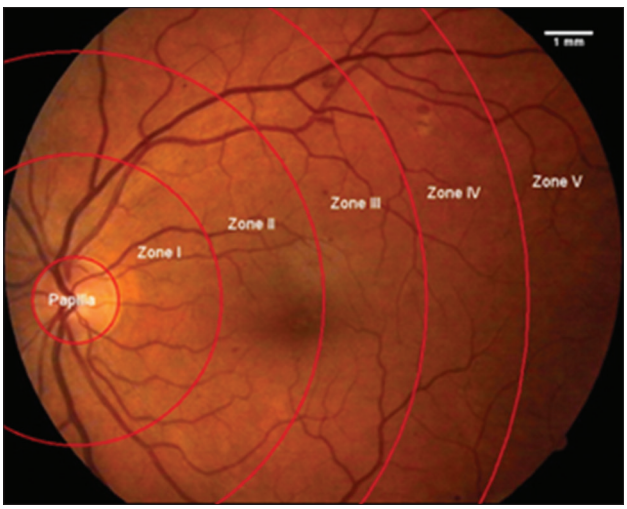

FIGURE 1. Digital fundus photography with concentric zones

patients depended on the optic disc location in the retinal images. Macular region was located in the zone II and zone III in the majority of the retinal images. In some images it occupied some parts of the zone IV which was caused by more medial location of the optic disc and subsequent reduction of the width of the concentric retinal zones determined around it. The number of retinal blood vessels (N) in each retinal zone, including the optic disc, was established with "cell counter" plugin (http://rsbweb.nih.gov/ij/plugins/cell-counter.html). All observed vessels were counted. In the case of blood vessel bifurcations, two newly formed blood vessels were counted as separate vessels, too. The outer diameter of all counted blood vessels (D_{BW}) in one zone was measured at three different locations in each of them and then the mean value was calculated.

Statistical Analysis

Statistical package NCSS PASS 2007 was used for statistical analysis. Mean values of analyzed parameters were compared between the evaluated groups by Kruskal-Wallis One-Way ANOVA test and Dunn's post hoc test. Mann Whitney U test was applied in both groups of patients with DR. Correlations between evaluated parameters in each of the analyzed groups were established by Spearman's rho (ρ).

RESULTS

Indirect ophthalmoscopy, in patients with mild DR, FF and FA showed a small number of stable microaneurysms without leaking, while cotton walls were detected in the group of patients with very severe DR, as well as hemorrhages; microaneurysms were observed in all 4 quadrants of retina, venous bleeding in more than two quadrants and intraretinal vascular abnormalities (IRMAs) in two or more quadrants. These were in accordance with ETDRS classification.

The mean age of examined group of patients is given in Table 1. Results of the median values of evaluated parameters of blood in the analyzed groups are also given in Table 1. Median duration of DMT2 in the group of patients with mild NPDR was 10 years (95% CI: 8 – 11 years), and in the group of patients with very severe NPDR 11.5 years (95% CI: 9 – 14 years) which statistically did not differ (Z=1.89, p=0.06). Levels of glycemia were significantly higher in patients with DR (95% CI: 8.2 mmol/L) than in controls (95% CI: 5.0 mmol/L), but without statistical significance among the two different groups with different form of DR and HbA1C. Levels of HbA1C in the group with very severe form were significantly higher than the same in the group with mild NPDR (Z=2.26, p=0.03).

Non-specific markers of inflammation significantly differed between the evaluated groups (PF: H=56.07, df=2, p<0.0001; CRP: H=57.46, df=2, p<0.0001). The values for PF and CRP were significantly higher in the groups with mild, as well as very severe NPDR in relation to the controls (p<0.05) (Table 1). However, in case of PF and CRP, the value of the group with very severe form was higher than the value in case of the mild NPDR, but this difference was not significant (p>0.05) (Table 1).

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

Parameter	N	Controls	Mild NPDR	Very severe NPDR	p
Age (years)	30	50.7±6.9	50.6±7.4	52.6±7.7	NS
Glucose (mmol/L)	30	5.04±0.44	8.48±2.26[a]	8.9±2.43[a]	0,05
HbA1C (%)	30	5.07±0.49	7.45±0.95[a]	8.46±1.68[a,b]	0.05
Cholesterol (mmol/L)	30	4.41±0.69	5.92±1.39[a]	5.51±0.96[a]	0.05
HDL-C (mmol/L)	30	1.61±0.69	2.00±1.39	1.26±0.26 [a,b]	0.05
LDL-C (mmol/L)	30	2.80±0.97	4.03±0.99[a]	3.88±0.96[a]	0.05
Triglycerides (mmol/L)	30	1.21±0.31	1.91±0.72[a]	1.90±0.67[a]	0.05
BMI (kg/m^2)	30	24.37±3.87	28.57±4.82[a]	27.37±5.65	0.001
PF (g/L)	30	3.99±0.40	7.83±3.10[a]	7.37±2.38[a]	0.001
CRP (mg/L)	30	2.34±0.60	3.66±1.82[a]	6.02±4.71[a]	0.05
YKL-40 (ng/mL)	30	64.27±12.11	115.91±12.93[a]	231.98±73.35[a,b]	0.05

a vs. control; b vs. mild NPDR

Finally, the values of YKL-40, as specific marker of inflammation, were significantly different between the evaluated groups (H=76.13, df=2, p<0.0001). Using Dunn's post hoc test we showed that its value was significantly higher in the group with very severe NPDR in relation to the group with the mild form of the disease and controls (p<0.05). Additionally, the average value of the YKL-40 in the group with mild NPDR group was significantly higher than the average value in the control group (p<0.05).

Markers of the lipoprotein profile: total cholesterol (H=25.83, df=2, p<0.0001), LDL-C (H=20.12, df=2, p<0.0001), HDL-C (H=10.98, df=2, p=0.004) and triglycerides (H=25.32, df=2, p<0.0001) significantly differed between the evaluated groups. Using Dunn's post hoc test we showed that total cholesterol levels were significantly higher in the groups of patients with DR (patients with mild and very severe NPDR) than in controls (p<0.05), but their values were not significantly different. The same trend of statistical significance was found for the LDL-C and triglycerides' levels (Table 1). However, the values of HDL-C were significantly higher in controls and the group with mild non-proliferative DR in relation to the group with very severe form of the disease (p<0.05).

According to the Kruskal-Wallis One-Way ANOVA, the values of BMI (H=9.88, df=2, p=0.007) were significantly different between the analyzed groups. As far as BMI was concerned, the using Dunn's post hoc test we showed that its values in the groups with mild, and very severe NPDR were significantly higher than in controls (p<0.05) (Table1). Average value of BMI in the group with mild NPDR was higher than

the one in the patients with very severe form of the disease, but this difference was not significant (p>0.05) (Table 1).

Using correlation analysis, we demonstrated that there was a significant positive correlation between BMI and CRP (ρ=0.53, p=0.02) in the control group. As for non-specific inflammatory markers, a significant positive correlation was found between the CRP and HDL-C (ρ=0.43, p=0.018). Apart from the abovementioned significant correlations of HDL-c, a significant positive correlation between the total plasma cholesterol and YKL-40 (ρ=0.43, p=0.018) was established.

In the group of patients with mild NPDR, as in the control group, BMI was significantly positively correlated with CRP (ρ=0.37, p=0.042), and, in this group, with total plasma cholesterol as well (ρ=0.40, p=0.027). Among non-specific inflammatory markers, CRP and PF were significantly positively correlated (ρ=0.53, p=0.003).

The results of the morphometric analysis (the number of observed blood vessels, and their mean outer diameter in each of evaluated retinal zones) were used for cluster analysis (k-means method) and mean values are given in Table 2 and Figure 2 A and B. These tables also present the results of the Student's t – test.

The average number of the blood vessels per retinal zone was significantly higher in patients with mild NPDR than in patients with very severe NPDR in the optic disc and all five retinal zones on the right side (Table 2). Opposite

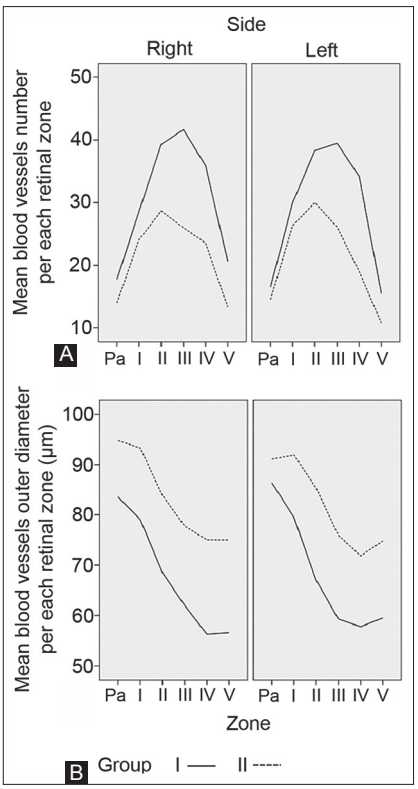

FIGURE 2. Mean retinal blood vessels number and diameter per each retinal zone. I Group - patients with mild NPDR, II Group - patients with very severe NPDR. Pa, Papilla

to the previous results, the average outer diameter of the evaluated retinal zones and optic disc vessels of this side was significantly higher in the group of patients with very severe NPDR than in the mild NPDR (Table 2, Figure 2 B). The same morphometric parameters showed a similar trend on the left side. However, the average number of observed blood vessels in the optic disc, zone I and zone V was higher in the group with mild than in the group with very severe form of disease, however, this difference was not significant. This parameter was significantly higher in the group with mild than in the group with very severe form of disease in the zones II, III and IV (Table 2, Figure 2A). The average blood vessels outer diameter of this side was significantly higher in the group with very severe form of diseases than in the group with mild form in all retinal zones, except in the optic disc in which this difference was not significant (Table 2, Figure 2 B).

Using one way ANOVA we showed that the average number of observed blood vessels was significantly different between optic disc and each of all five retinal zones on the right eye in the group of mild NPDR patients ($F(5,96)=29.44$, $p<0.0001$, $d=2.05$) and very severe NPDR patients ($F(5,48)=6.24$, $p<0.0001$, $d=2.58$) and, on the left eye, $F(5,96)=29.61$, $p<0.0001$, $d=2.07$ and $F(5,48)=12.63$, $p<0.0001$, $d=1.89$, in both groups, respectively. Generally, the values of this parameter increased from the optic disc towards the zone III, in which its value was the highest and, then decreased gradually toward the zone V on both sides and in both groups of examinees. Using Games Howell post hoc test we showed that the average number of retinal blood vessels in the right eye of the group of patients with mild NPDR was significantly higher ($p<0.05$) in the zone II, zone III and zone IV than in the optic disc, zone I and zone V. This parameter was higher in the zone IV than in the zone I, but this difference was not significant. The average number of zone II, zone III and zone IV blood vessels was not significantly different, according to this test. Tukey's post hoc test showed that in the group of patients with very severe NPDR the average number of blood vessels was significantly higher ($p<0.05$) in the zone II and zone III than in the optic papilla and zone V, as well as, in zone I than in zone V. The values of this parameter were not significantly different between all other zones of this side in this group. The average number of blood vessels per each zone showed a similar trend on the left side in the group of patients with mild form of disease, while in the group with very severe NPDR 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. Therefore, these findings might suggest that the reduction of blood supply in patients with very severe form of disease is more pronounced in the macular region than in other zones, in both eyes.

TABLE 2. Number and diameter of retinal blood vessels on optic disc and different retinal zones on both eyes

Zone	Parameter	Group	N	Right eye±SD $\bar{X}$±SD	p	Left eye±SD $\bar{X}$±SD	p
Optic disc	Number	Controls	30	18.64±3.22		18.48±3.15	
		Mild NPDR	30	17.76±3.80	NS	16.53±386[a]	0.05
		Very severe NPDR	30	14.00±3.39[a,b]	0.05	14.56±3.13[a,b]	0.05
	D_{bw}(μm)	Controls	30	74.89±10.35		76.37±8.14	
		Mild NPDR	30	83.56±6.66[a]	0.05	86.37±9.19[a]	0.05
		Very severe NPDR	30	94.75±12.28[a,b]	0.05	91.05±14.46[a,b]	
Zone I	Number	Controls	30	29.88±6.610		30.000±4983	
		Mild NPDR	30	28.59±5.112[a]	0.05	30.000±6.982[a]	0.05
		Very severe NPDR	30	24.11±4.106[a,b]	0.05	26.333±4.472[a,b]	0.05
	D_{bv}(μm)	Controls	30	74.287±8.317		76.579±7.563	
		Mild NPDR	30	79.043±6.660[a]	0.05	79.435±8.581[a]	0.05
		Very severe NPDR	30	93.300±10.643[a,b]	0.05	91.894±12.422[a,b]	0.05
Zone II	Number	Controls	30	43.760±8.828		40.68±8.097	
		Mild NPDR	30	39.294±2.289[a]	0.05	38.29±8.380[a]	0.05
		Very severe NPDR	30	28.667±7.382[a,b]	0.05	30.00±7.106[a,b]	0.05
	D_{bw}(μm)	Controls	30	64.194±8.052		64.316±7.266	
		Mild NPDR	30	68.537±7.768[a]	0.05	66.974±8.266[a]	0.05
		Very severe NPDR	30	83.859±11.612[a,b]	0.05	85.282±14.973[a,b]	0.05
Zone III	Number	Controls	30	44.04±7.080		42.96±10.826	
		Mild NPDR	30	41.65±11.096[a]	NS	39.47±9.362[a]	NS
		Very severe NPDR	30	26.00±11.325[a,b]	0.05	26.11±9.033[a,b]	0.05
	D_{bw}(μm)	Controls	30	59.389±8.591		60.269±10.146	
		Mild NPDR	30	61.994±8.231	NS	59.273±7.568	NS
		Very severe NPDR	30	77.758±10064[a,b]	0.05	75.198±15.229[a,b]	0.05
Zone IV	Number	Controls	30	36.44±7.932		31.36±7.999	
		Mild NPDR	30	35.88±8.371[a]	0.05	34.18±10.979[a]	0.05
		Very severe NPDR	30	23.56±11.26[a,b]	0.05	19.00±7.382[a,b]	0.05
	D_{bw}(μm)	Controls	30	55.62±6.683		57.677±11.357	
		Mild NPDR	30	56.276±4.519[a]	0.05	57.765±10.154[a]	0.05
		Very severe NPDR	30	75.043±8.860[a,b]	0.05	71.844±14.084[a,b]	0.05
Zone V	Number	Controls	30	21.40±5.099		16.76±6.023	
		Mild NPDR	30	21.65±5.766	NS	15.47±6135	0.05
		Very severe NPDR	30	13.33±4.243[a,b]	0.05	10.78±4.764[a,b]	0.05
	D_{bw}(μm)	Controls	30	56.114±6.466		61.805±15.052	
		Mild NPDR	30	56.575±6.722	NS	69.487±10.324	0.05
		Very severe NPDR	30	75.014±11.394[a,b]	0,05	74.795±17.345[a,b]	0.05

a P<0.05 vs. Controls; b P<0.05 vs. RDNP

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 ($F(5, 96)=48.22$, $p<0.0001$, $d=2.78$) and in the very severe NPDR ($F(5,48)=6.04$, $p<0.0001$, $d=1.26$). Using Tukey's post hoc test we showed that in the first group this parameter's decrease was significant ($p<0.05$) in the zones I and II than in the optic disc. 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, zones I and II, but its values did not significantly differ between these zones. In the very severe NPDR this parameter showed a similar trend with exception that its decrease was significant ($p<0.05$) in the zones III, IV and V in relation to the optic disc and zone I. The difference of this parameter between all other zones was not significant. The outer diameter of blood vessels in the left eye decreased from the optic disc towards the zone V. Similarly to the right eye in the patients with mild NPDR, using Tukey's post hoc test we showed that the outer diameter decreased significantly ($p<0.05$) in the zones II, III, IV and V in relation to the optic disc and zone I. Its decrease was significant on this side in the patients with very severe NPDR, too. However, using Games Howell post hoc test we showed that blood vessels' outer diameter was significantly ($p<0.05$) higher in the zone I than in zone IV.

Correlation analysis revealed that the outer diameter retinal blood vessels positively correlated with levels of YKL-40 in all zones and in both eyes, and negatively correlated with the number of retinal vessels (Table 3 and 4). The levels of CRP also had a positive correlation with outer retinal blood vessels diameter and a negative correlation with the number of retinal vessels. This correlation was not present in all zones, and not on the optic disc of the left eye. This correlation was not significant as in case of YKL-40 (Table 3 and 4).

TABLE 3. Correlation between number and diameter of blood vessels and YKL-40, CRP on the right eye

Parameter		OD_{nbv}	OD_{Dbv}	I_{nbv}	I_{Dbv}	II_{nbv}	II_{Dbv}	III_{nbv}	III_{Dbv}	IV_{nbv}	IV_{Dbv}	V_{nbv}	V_{Dbv}
YKL- 40 (ng/ml)	R	–0.34	0.49	–0.29	0.57	−0.45	0.56	–0.45	0.50	–0.34	0.63	–0.47	0.58
	p	0.013	0.000	0.040	0.000	0.001	0.000	0.001	0.000	0.014	0.000	0.001	0.000
CRP (mg/L)	R	–0.27	0.48	–0.23	0.30	–0.31	0.39	–0.34	0.36	–0.23	0.26	–0.27	0.34
	p	0.028	0.000	0.108	0.031	0.030	0.005	0.013	0.009	0.008	0.050	0.056	0.014

TABLE 4. Correlation between number and diameter of blood vessels and YKL-40, CRP on the right eye

Parameter		OD_{nbv}	OD_{Dbv}	I_{nbvS}	I_{Dbv}	II_{nbvS}	II_{Dbv}	III_{nbv}	III_{Dbv}	IV_{nbv}	IV_{Dbv}	V_{nbv}	V_{Dbv}
YKL- 40 (ng/ml)	R	–0.29	0.43	–0.23	0.42	–0.40	0.53	–0.47	0.37	–0.37	0.33	–0.30	0.26
	p	0.036	0.001	0.105	0.002	0,004	0.000	0.001	0,008	0,009	0.018	0.033	0.066
CRP (mg/L)	R	–0.06	0.12	–0.01	0.17	–0.17	0.17	–0.13	0.11	–0.19	0.13	–0.16	0.22
	p	0.676	0.004	0.971	0.040	0.042	0.020	0.061	0.030	0.187	0.078	0.,277	0.027

DISCUSSION

Recent advances in retinal photographic imaging techniques have allowed objective and precise measurement of subtle vascular characteristics in the retina, including the retinal vessel caliber (diameter). Data from population-based and clinic-based studies show that changes in retinal caliber are associated with systemic vascular risk factors and might reflect early microcirculatory alterations in people with diabetes prior to the onset of clinically significant complications, such as DR. Prospective studies suggest that in people with type 1 diabetes, wider retinal venules are associated with progression of mild to more severe levels of retinopathy, including PDR [8]. Studying retinal vascular caliber changes might offer the potential to improve our understanding of the early pathophysiological pathways of diabetic retinopathy, potentially allowing the development of novel therapies.

Some of the theories have postulated that in diabetic retina, hyperglycemia and hypoxia initiate retinal blood vessel vasodilatation, leading to hyperperfusion [7,8,11]. Hyperperfusion in turn interferes with autoregulation, resulting in further vasodilatation [9,10]. In our study, the average outer diameter of the evaluated retinal zones and optic disc vessels was significantly higher in the group of patients with very severe diabetic retinopathy than in the group with mild non-proliferative diabetic retinopathy (Table 2, Figure 2B). Retinal vessel occlusion and degeneration is in the focus of other theories [11,12,13]. Occlusion and degeneration of retinal vessels are typical features of DR and also cause further progression of DR, which starts with the loss of two cellular components of retinal capillaries, pericyte and endothelial cell [13]. The exact sequence of loss has not been established in humans. The average number of the blood vessels per retinal zone was significantly higher in the group with mild NPDR than in the group with very severe NPDRwith respect to the optic disc and all five retinal zones (Table 2, Figure 2A). In the group with very severe NPDR, zones I, II and III had significantly higher average number of blood vessels than the optic disc and zones IV and V. Therefore, these findings might suggest that the reduction of blood supply in the case of the group with very severe NPDR is more pronounced in the macular region in relation to the other zones, on both sides.

Mechanisms leading to capillary degeneration may involve inflammatory cytokine- induced endothelial cells death since inflammatory cytokines are also known to increase caspase 3 activity and strongly induce endothelial cell apoptosis [13,14,15]. This theory is also known as the vasoregression theory. In our study, we measured the levels of CRP and YKL-40 in blood samples of the examinees.

CRP is one of the best-studied biomarkers of inflammation. It has always been considered to be a marker of acute and chronic inflammation. New studies have investigated CRP as it predicts fatal and/or nonfatal coronary events, stroke and peripheral arterial disease [16, 22, 23]. Our results have shown statistical significance between the examined groups of patients with DMT2 and controls CPR (Table 1).However, there was no statistical significance between the two groups with different stages of DR. In the study of Flosom et al. [24] and Schram et al. [25] a strong relationship between DMT2 and CRP was confirmed. Streja et al. [22] and Hung et al. [23] in their study concluded that CRP reflects the process of atherosclerosis and vulnerability of plaques, and metabolic syndrome. Muni RH et al. [16] studied highly sensitive CRP (hs-CRP), and concluded that it had predicted high risk of clinically significant macular edema and macular and retinal hard exudates. The role of CRP in development of DR can be explained by higher levels of intracellular adhesion molecule 1(ICAM-1) and break of blood-retinal barrier. The results of our study have shown that the correlation between the levels of CRP and retinal blood vessels, diameter and number, was not present in all examined zones of the retina (Table 3 and 4). In zones where a correlation is present, it positively correlates with diameter and negatively with number of retinal blood vessels. A possible role of this biomarker in retinal vascular disorders in DR needs more investigation.

YKL-40 is a heparin-, chitin-, and collagen-binding lectin produced by immunologically active cells such as macrophages and neutrophils, vascular smooth muscle and endothelia cells, arthritic chondrocytes, cancer cells, and embryonic and fetal cells [21, 26]. High plasma concentrations of YKL-40 are found in patients with diseases characterized by inflammation or increased tissue remodeling or with cancer [21,27]. We presumed that YKL-40 is valid and independent biomarker of inflammation in peripheral blood, which can be used for the follow-up of DR development. The levels of YKL-40 in the peripheral blood samples were statistically higher in the group of patients with DMT2 and DR than in controls. Also, there was statistical significance in the levels of plasma concentration of YKL-40 among two groups of patients with DMT2 and different forms of DR. Rathcke et al. have shown an elevation of serum YKL-40 in type 2 diabetes [27]. Further, Nielsen et al. have proved that plasma YKL-40 was identified as an obesity independent marker of DMT2 related to glycemia and plasma IL-6 levels [28]. We correlated the results of plasma levels of YKL-40 with PF, CRP and BMI. There was no correlation among the examined parameters. The role of YKL-40 in development of DR can be explained by its influence on metabolic processes. It also activates the protein kinase C that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration, and phosphoinositide-1,3 kinase signaling pathway, leading to antiapoptosis. Moreover, it has a role in angiogenesis and role in the innate immune response [21]. Recent studies have identified YKL-40 as a promoter of angiogenesis in neoplasms, including activating the mitogen activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) pathway in endothelial cells [21]. YKL-40 promotes chemotaxis, cell attachment, spreading, and migration of vascular endothelial cells [21]. YKL-40 also modulates the vascular endothelial cell morphology by promoting the formation of branching tubules, indicating a role of YKL-40 in angiogenesis by stimulating the migration and reorganization of vascular smooth muscle cells [22]. The results from our study have shown that the levels of YKL-40 strongly correlate with diameter and number of retinal blood vessels (Table 3 and 4). Sakamoto et al. [29] have found no association between hs-CRP and albuminuria, as a marker of endothelial dysfunction. These results are similar to ours and may reflect the fact that serum YKL-40 levels increase more sensitively to focal inflammation and endothelial dysfunction as compared to serum CRP levels.

The presented data may have clinical and therapeutic implications. A quantitative measurement of caliber of retinal blood vessels may provide information on further progression of DR[12]. The correlation between measurement of retinal blood vessels and biomarkers as CRP and YKL-40 may also support the value of targeting the microcirculation in treatment of systemic disease. Certain pharmacological agents (antiplatelet therapy, glucocorticoides, aldose reductase inhibitors, protein kinase C inhibitors, advanced glycosylation end product inhibitors, statins, cannabinoids, nitrat therapy, antioxidants) as well as anti-TNF agents have been suggested to have direct beneficial effects on microvessel structure and function and may therefore have added therapeutic value in the management of patients with changes, narrowing of retinal blood vessels and vasoregression [15,17]. Statins have a capacity to inhibit inflammation. Since statins reduce inflammation, higher levels of YKL-40 would be expected in patients without statins in therapy. According to our data, 51 patients with DR (85%), 24 with mild and 27 with very severe NPDR, were treated with statins. The data on duration of therapy in our patients were not available. In our study, according to presented data, a similar number of patients were treated with statins. Therefore, the effect of statins on inhibition of inflammation cannot be observed as relevant. Similar results are presented in the study conducted by Rodbjerg et al. [30].

Limitation factors of this study that should be noted are: this study included only the white population, and it is unclear how local and ocular factors can influence the vascular caliber.

DECLARATION OF INTEREST

The authors declare no conflict of interest for this study.

REFERENCES

[1] International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation, 2013.
[2] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2013;33:67-74.
[3] Diabetic Retinopathy Guidelines. 2012, The Royal College of Ophthalmologists.
[4] Wong TY, Kamineni A, Klein R, Sharrett AR, Klein BE, Siscovick DS, Cushman M, Duncan BB. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons. The cardiovascular health study. Arch Intern Med 2006;166:2388-94.
[5] Mitchell P, Leung H, Wang JJ, Rochtchina E, Lee A, Wong TY, Klein R. Retinal vessel diameter and open-angle glaucoma: The Blue Mountains Eye Study. Ophthalmology2005;112(2):245-50.
[6] Wong TY, Shankar A, Klein R, Klein BE, Hubbard LD. Retinal arteriolar narrowing, hypertension, and subsequent risk of diabetes mellitus. Arch Intern Med 2005;165:1060-5.
[7] Klein R, Klein BE, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes: XXI: The Wisconsin epidemiologic study of diabetic retinopathy. Ophthalmol 2007;114(10):1884-92.
[8] Cheung N, Rogers SL, Donaghue KC, Jenkins AJ, Tikellis G, Wong TY. Retinal arteriolar dilation predicts retinopathy in adolescents with type 1 diabetes. Diabetes Care 2008;31(9):1842-6.
[9] Nguyen TT, Wong TY. Retinal vascular changes and diabetic retinopathy. Curr Diab Rep2009;9(4):277-83.
[10] Nguyen TT, Islam FM, Farouque HM, Klein R, Klein BE, Cotch MF, Herrington DM, Wong TY.Retinal vascular caliber and brachial flow-mediated dilation: the Multi-Ethnic Study of Atherosclerosis. Stroke 2010;41(7):1343-8.

[11] Ding J, Ikram MK, Cheung CY, Wong TY. Retinal vascular calibre as predictor of incidence and progression of diabetic retinopathy. Clin Exp Optom 2012;95(3):290-296.

[12] Ikram MK, Cheung CY, Lorenzi M, Klein R, Jones TLZ, Wong TY. NIH/JDRF Workshop on Retinal Biomarker for Diabetes Group. Retinal vascular caliber as a biomarker for diabetes microvascular complications. Diabetes Care 2013;36(3):750-9.

[13] Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP. A central role of inflammation in the pathogenesis of diabetic retinopathy. FASEB J 2004;18(12):1450-2.

[14] Nguyen TT, Alibrahim E, Islam FM, Klein R, Klein BE, Cotch MF, Shea S, Wong TY. Inflammatory, hemostatic, and other novel biomarkers for diabetic retinopathy: the multi-ethnic study of atherosclerosis. Diabetes Care 2009;32(9):1704-9.

[15] Rangassamy S, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. Middle East Afr J Ophthalmol. 2012 Jan;19(1):52-9.

[16] Muni RH, Kohly RP, Lee EQ, Manson JE, Semba RD, Schaumberg DA. Prospective study of inflammatory biomarkers and risk of diabetic retinopathy in diabetes control and complications trials. JAMA Ophthalmol 2013;131(4):514-521.

[17] Zorena K, Raczyńska D, Raczyńska K. Biomarkers in diabetic retinopathy and the therapeutic implications.Mediators Inflamm 2013;2013:193604.

[18] Tomić M, Ljubić S, Kaštelan S, Gverović Antunica A, Jazbec A, Poljičanin T. Inflammation, haemostatic disturbance, and obesity: possible link to pathogenesis of diabetic retinopathy in type 2 diabetes. Mediators Inflamm 2013;2013:818671.

[19] Začiragić A, Huskić J, Mulabegović N, Avdagić N, Valjevac A, Hasić S. et al. An assessment of correlation between serum asymmetric dimethylarginine and glycated hemoglobin in patients with type 2 diabetes mellitus. Bosn J Basic Med Sci 2014;14(4):21-24.

[20] Kozakova MH, Sarafian VS. YKL-40--a novel biomarker in clinical practice? Folia Med(Plovdiv) 2009;51(1):5-14.

[21] Johansen J. Study on serum YKL-40 as biomarker in disease with inflammation, tissue remodeling, fibrosis and cancer. Dan Med Bull 2006;53:172-209.

[22] Streja D, Cressey P, Rabkin SW. Associations between inflammatory markers, traditional risk factors, and complications in patients with type 2 diabetes mellitus. J Diabetes Complications 2003;17(3):120-127.

[23] Hung MJ, Hsu KH, Hu WS, Chang NC, Hung MY. C-reactive protein for predicting prognosis and its gender – specific association with diabetes mellitus and hypertension in development of coronary artery spasm. PLoS One. 2013;8(10):e77655.

[24] Folsom AR, Wu KK, Rasmussen M, Chambless LE, Aleksić N, Nieto FJ. Determinants of population changes in fibrinogen and factor VII over 6 years: the Atherosclerosis Risk in Communities (ARIC) Study. ArteriosclerThromVascBiol 2000; 20(2):601-606.

[25] Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes: EURODIAB Prospective Complications Study. Diabetologia 2005;48:370-378.

[26] Johansen JS, Lottenburger T, Nielsen HJ, Jensen JE, Svendsen MN, Kollerup G et al. Diurnal, weekly and long–time variation in serum concentration of YKL-40 in healthy subjects. Cancer Epidemiol Biomarkers Prev 2008;17(10):2603-2608.

[27] Rathcke CN, Johansen JS, Vestergaard H. YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistence. Inflamm Res 2006;552(2):53-59.

[28] Nielsen AR, Erikstrup C, Johansen J, Fischer CP, Plomgaard P, Krogh-Madsen R et al. Plasma YKL-40: a BMI–independent marker of type 2 Diabetes. Diabetes 2008;57(11):3078-3082.

[29] Sakamoto F, Katakami N, Kaneto H, Yasuda T, Takahara M, Miyashita K, Kuroda A, Matsuhisa M, Kosugi K, Shimomura I. Association of serum YKL-40 levels with urinary albumin excretion rate in young Japanese patients with type 1 diabetes mellitus. Endocr J 2013;60:73-9.

[30] Røndbjerg AK, Omerovic E, Vestergaard H.YKL-40 levels are independently associated with albuminuria in type 2 diabetes. CardiovascDiabetol 2011;10:54-9.