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Research Article

Assessment of Smooth Muscle Cell and Endothelial Functions in Branch Retinal Vein Occlusion

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Abstract

Purpose: One of the common retinal vascular disorder is branch retinal vein occlusion (BRVO) that causes visual impairment. The purpose of this study is to evaluate vascular smooth muscle cell and endothelial functions in BRVO patients.

Methods: Thirty 30 BRVO patients and 30 healthy subjects were included in this study. Nitrate-mediated dilatation (NMD) which represent smooth muscle relaxation and flow-mediated dilation (FMD) which represent the endothelium-dependent relaxation of the brachial artery were measured by ultrasonography. Biochemical and laboratory tests including total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, apolipoprotein (Apo)-A1, Apo-B and mean systolic blood pressure (SBP), diastolic blood pressure (DBP) were also considered.

Results: The median NMD value was 15.55 (12.3/25) % in the control group and 12.5 (8.3/18.7) in the BRVO group ($p < 0.001$). The mean FMD value was 9.71 ± 2.19% in the control group and 5.13 ± 2.72 % in the BRVO group ($p = 0.002$). The mean SBP value was 117.17 ± 7.01 in the control group and 140.17 ± 10.38 in the BRVO group ($p = 0.001$). The median DBP value was 75.5 (61/84) in the control group and 89.5 (68/109) in the BRVO group ($p < 0.001$). Also, FMD and SBP were found as an independent risk factor for BRVO ($p = 0.040$ and $p = 0.019$).

Conclusion: Impaired brachial artery NMD and FMD levels may imply that not only endothelial dysfunction also smooth muscle cell dysfunction may play a role in the occurrence of BRVO. Also, these parameters can be used for further analyses of these patients.

Keywords: *Branch Retinal Vein Occlusion; Flow-Mediated Dilation; Nitrate-Mediated Dilatation; Endothelial Dysfunction; Smooth Muscle Cell Dysfunction*

Introduction

One of the common retinal vascular disorder is branch retinal vein occlusion (BRVO) that causes visual impairment in middle aged and elderly people. Various systemic risk factors including thrombophilia, hypercoagulable states, arterial hypertension, low levels of high-density lipoprotein (HDL), diabetes mellitus, hypercholesterolemia and Hyperhomocysteinemia have been documented in BRVO patients [1-8]. However, not all these associations have been consistently reproduced.

It seems that in most of the cases, arterial disease is a predominant pathogenetic mechanism for BRVO. Seitz proposes that the structural changes of vascular layers including venous endothelium and intima media, as a consequence of the compression from overlaying artery, is the basic of the BRVO pathogenesis [9]. Also, Frangieh., *et al.* found similar results. In their study, majority of the patients had hypertrophy at the level of intima media layer [10]. Nowadays, it is possible to evaluate the changes in vascular structures noninvasively by using nitrate-mediated dilatation (NMD) and flow-mediated endothelial-dependent vasodilatory function (FMD).

FMD that present the endothelium-addicted relaxation of the brachial artery, defined by Celermajer., *et al.* contained application of sublingual nitrates to investigate the vasodilating efficacy of an exogenous resource of nitric oxide (NO) [11,12]. NO affects directly at the plane of the arterial smooth muscle and generate an endothelium-independent dilatation reaction. For this reason NMD is used as a control test to make sure that an impaired FMD value is truly a consequence of endothelial damage [13].

Recently, impaired FMD and NMD have been shown in arterial hypertension and diabetes mellitus which are the traditional major risk factors for atherosclerosis, and also associated with vascular disease [13-15].

We conducted this study to assess the vascular smooth muscle cell functions along with endothelial functions noninvasively using nitrate-mediated dilatation (NMD) and flow-mediated dilation (FMD) in BRVO patient.

Materials and Methods

Thirty BRVO patients who had variable degrees of macular thickening (≤ 6 months) and 30 healthy age matched control subjects were included in this study. The study was approved by the hospital ethics committee and patients gave their informed consent. Patients with BRVO accompanied by arterial occlusion, and who had previous any ocular surgical operation or laser treatment, intraocular injection were excluded. Patients using medications affecting vascular system, such as angiotensin-converting enzyme inhibitors, calcium antagonists, beta blockers nitrates were also excluded.

To confirm BRVO, complete physical and ocular examinations including fundus photography, fluorescein angiography and optical coherence tomography (OCT) were performed. Patients were routinely consulted by a cardiologist and biochemical analyses were obtained from the patients' hospital files which included *apolipoprotein (Apo)-B,* Apo-A1, LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides. Also mean systolic and diastolic blood pressure were recorded.

Assessment of endothelial function

Endothelial functions was determined according to the procedure described by Celermajer., *et al* [11,12]. Images were taken by one sonographer thereafter an overnight fast in a temperature-controlled place (22°C) in the supine, resting condition. FMD value was achived from patient's right arm. Using 7.5 MHz transducer connected to an Hewlett Packard SONOS 2500 echocardiography system (Andover, MA), brachial artery measurement was made from 5cm distance before antecubital fossa. Baseline brachial artery thickness was assessed two times and every one minute thereafter discharge of five minute upper arm pressure cuff blockage (250 mm Hg). FMD (%) was described as greatest vessel thickness change after cuff discharge/mean control thickness. Thereafter other 10-minute relaxation, sublingually five miligram isosorbide dinitrate was applied and the brachial artery thikness was assessed every one minute thereafter five minute of nitrate application. NMD (%) was described as highest vessel thickness change after nitrate/mean control thickness.

Statistical methods

We used SPSS 25.0 statistical analysis program for data analysis. The normal distribution of the data was assessed by the Shapiro-Wilk test, whereas the homogeneity of variance was assessed by the Levene. Independent qualitative data in the two groups were matched with the independent-samples t-test, while when used along with the Bootstrap results the Mann-Whitney U test with the Monte Carlo simulation technique was employed. When comparing categorical variables testing was done with the, the Pearson Chi-Square test when using Exact results.

To determine the cause and effect relationship of patients and control groups with their descriptive variables, Multiple Logistic regression tests were analyzed with the Enter method. Sensitivity, specificity, positive predictivity and negative predictivity ratios of the relationship between the classification and the actual classification of the cut off value was calculated according to patient and control group variables with the Receiver Operating Curve (ROC) being examined and expressed as a curve-analysis. Quantitative variables are tabulated as mean ± SD (standard deviation) - Minimum/Maximum and median (Minimum/Maximum) and categorical variables were shown as n (%). Variables were evaluated with a confidence interval level of 95% and a P value of 0.05 or less was considered meaningful.

Results

No statistical significance was detected in the control and patient groups according to gender, age, TG, LDL, Apo-A1, Apo-B, total cholesterol and HDL ($p > 0.05$). The mean FMD and median NMD values were statistically significantly lower in the patient group (FMD, $p = 0.002$ and NMD, $p < 0.001$), while the mean SBP and median DBP values were statistically significantly higher (SBP, $p = 0.001$ and DBP, $p < 0.001$) (Table 1).

	Control group (n = 30)	BRVO group (n = 30)	P Value
	n (%)	**n (%)**	
Gender			
Man	14 (46.7)	11 (36.7)	0.601[c]
Women	16 (53.3)	19 (63.3)	
	Mean ± SD	**Mean ± SD**	
Age (years)	57.97 ± 8.99	59.13 ± 9.27	0.625[a]
TG	131.13 ± 43.65	144.97 ± 52.07	0.259[a]
LDL - cholesterol	121.73 ± 45.09	126.37 ± 32.09	0.648[a]
Apo - A1	137.27 ± 12.85	133.80 ± 10.74	0.261[a]
Apo - B	101.23 ± 22.99	110.93 ± 33.62	0.191[a]
FMD	9.71 ± 2.19	5.13 ± 2.72	0.002[a]
SBP (mmHg)	117.17 ± 7.01	140.17 ± 10.38	0.001[a]
	Median (Min/Max)	**Median (Min/Max)**	
DBP (mmHg)	75.5 (61 / 84)	89.5 (68 / 109)	< 0.001[b]
Total cholesterol	187 (128 / 359)	201 (138 / 282)	0.084[b]
HDL – cholesterol	42.5 (22 / 72)	42 (32 / 54)	0.389[b]
NMD	15.55 (12.3 / 25)	12.5 (8.3 / 18.7)	< 0.001[b]

Table 1*: Demographic and clinical characteristics of the study and control groups.*

TG: Triglycerides; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; FMD: Flow-Mediated Vasodilation; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; NMD: Nitrate Mediated Dilatation; a: Independent Samples T Test(Bootstrap); b: Mann Whitney U Test(Monte Carlo); c: Pearson Chi-Square Test; SD: Standard Deviation.

The optimal cut off value for FMD was 60% sensitive for 5.2, and 71.4% specificity, 100% positive predictive, 100% negative predictive and the area below the curve (AUC) value was 0788 (SE: 0.068) and this cut off was statistically significant between the patient and control groups ($p < 0.001$) (Table 2).

	Control group	BRVO group	AUC (SE)	P Value
	n (%)[1] (%)[2]	**n (%)[1] (%)[3]**		
FMD				
> 5.2	30 (71.4)[npv] (100)[sp]	12 (28.57) (40.0)	0.788 ± 0.068	< 0.001
≤ 5.2	0 (0.0) (0.0)	18 (100)[ppv] (60.0)[ss]		
SBP				
≤130	30 (85.7)[npv] (100)[sp]	5 (14.29) (16.7)	0.953 ± 0.027	< 0.001
> 130	0 (0.0) (0.0)	25 (100)[ppv] (83.3)[ss]		
DBP				

≤82	29 (76.3)^{npv} (96.7)^{sp}	9 (23.68) (30.0)	0.855 ± 0.049	< 0.001
> 82	1 (4.5) (3.3)	21 (95.45)^{ppv} (70)^{ss}		
NMD				
> 12.7	28 (68.3)^{npv} (93.3)^{sp}	13 (31.71) (43.3)	0.806 ± 0.056	< 0.001
≤ 12.7	2 (10.5) (6.7)	17(89.47)^{ppv} (56.7)^{ss}		

Table 2: *Evaluation of performance of the control and patient groups according to the analysis of the statistically significant FMD, SBP, DBP and NMD values.*

FMD: Flow-Mediated Vasodilation; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; NMD: Nitrate Mediated Dilatation; ROC: Receiver Operating Curve Analysis (Honley & Mc Nell - Youden index J); AUC: Area Under the ROC Curve; SE: Standard Error; SS: Sensitivity; SP: Specificity; ppv: Positive Predictive Value; npv: Negative Predictive Value; 1 Row n %; 2 Column n %.

Similarly, the cut off values for SBP, DBP, and NMD for sensitivity values were respectively; 130, 82, 12.7; for specificity values respectively; 83.3%, 70%, 56.7%; positive predictive values respectively; 100%, 96.7%, 93.3%; for negative predictive values respectively 100%, 95.45%, 89.47%; and the remaining area under the curve (AUC) values respectively; 85.7%, 76.3%, 68.3% [respectively (AUC ± SE); (0.953 ± 0.027), (0.855 ± 0.049), (0.806 ± 0.056)] while the cut offs were statistically significant when comparing the patient and control groups (all p values are < 0.001) (Table 2). The fact that FMD is less than or equal to 5.2 and NMD is less than or equal to 12.7 and because SBP is greater than 130 and DKB is greater than 82, the patient groups are distinguished from one another.

In univariate analyses, data included in the model was not significant for gender, TG, LDL-cholestrol, HDL-cholestrol, total cholestrol, Apo-A1 and Apo-B. FMD and SBP were found to be statistically significant (FMD, p = 0.040 and SKB, p = 0.019) (Table 3). The decrease of FMD in patient groups was 1.359 times (95%: 1.014-1822), with the height of the SBP being higher than the control group value of 1.397 (95%: 1.058-1.846) (Table 3). This statistically significant (p < 0.001) model estimates the 96.7% of the control group, 96.7% of the patient group, and 96.7% of all patients.

	B (SE.)	P Value	Odds Ratio	95% C.I. for Odds Ratio	
				Lower Limit	Upper Limit
FMD (↓)	-0.307 (0.150)	0.040	1.359	1.014	1.822
SBP (↑)	0.335 (0.142)	0.019	1.397	1.058	1.846
DBP (↑)	0.085 (0.108)	0.431	1.089	0.881	1.346
NMD (↓)	-0.824 (0.567)	0.146	2.280	0.750	6.929
Constant	-34.638 (13.525)	0.010			

Table 3: *Logistic regression analysis modeling according to FMD, SBP, DBP and NMD.*

Estimated accuracy rates: Control group (96.7%) - Patient group (96.7%) - General (96.7%)/P (Model) < 0.001.

Multiple Logistic Regression (Method Enter); C.I: Confidence Interval; B: Regression Coefficients; SE: Standard Error.

Discussion

For the first time, in this study, we used the NMD to assess the peripheral vasodilatation in BRVO patients. These results demonstrated that the NMD was decreased in BRVO patients compared with healthy group but impaired NMD value was not an independent risk factor for BRVO occurring. However FMD was found as an independent risk factor. In a previous study, it has been suggested that a decreased FMD value was an independent risk factor for BRVO occurring [16]. However, they have not evaluated the NMD which should be used as a control test for ensuring that impaired dilatation capacity was a result of endothelial impairment and not due to an accompanying smooth muscle damage [17]. So, in that study, it remained unclear that the impaired FMD in BRVO patients was a consequence of endothelial dysfunction or smooth muscle cell pathology or both. In this study, we found that NMD and FMD values measured noninvasively by

brachial ultrasonography were significantly impaired in BRVO patients. These results showed that besides endothelial impairment, smooth muscle pathologies including structural changes, impaired isosorbide dinitrate bioconversion to NO or impaired bioavailability of NO might be possible causes for BRVO. Furthermore, in addition mechanical forces and NO levels, adenosine, prostaglandin I2, endothelium-derived hyperpolarizing factor (EDHF) which all known to affect FMD, and endothelin 1 which is responsible for vasoconstriction in atherosclerotic process should be taken into consideration [18-21] . Also, in this study, the cut off value of FMD and NMD for prediction of BRVO were 5.2 and 12.7 from ROC curve. It may be emphasized that patients with impaired FMD and NMD values deserves more attention than patients with normal values.

Many reports have evaluated the role of atherosclerotic risk factors including hypertension and dyslipidemia in the etiopathogenesis of BRVO [1-6,22,23]. Although there has been much controversy whether these parameters affect the occurrence of BRVO, hypertension had been usually identified as a risk factor for BRVO [1-3]. Similarly, this study demonstrated a significant association between SBP, DBP and BRVO. Moreover, we found SBP as an independent risk factor for BRVO. The cut off value of SBP and DBP for prediction of BRVO were 130 mmHg and 82 mmHg. However, we could not detect any association between the serum lipid levels and BRVO.

This study was limited by a relatively small sample size. Furthermore, low NMD reflect smooth muscle function and impaired smooth muscle function can affect FMD. So, it is probably difficult that FMD in patients with low NMD can be examined. Nevertheless, this study could reveal NMD and FMD impairment in BRVO patients. Further studies are required to determine the clinical impact of this observation.

Conclusion

The noninvasively detected impaired NMD and FMD levels may imply that BRVO might be a consequence of smooth muscle cell pathology besides endothelial functions or both. In the assessment of BRVO, not only endothelial system also smooth muscle cell and factors affecting the vascular system should be considered. Furthermore, these parameters can be used for further analyses of these patients.

Conflicts of Interest

All authors certify that they have no affiliations with or involvement in any organization with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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