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ORIGINAL DATA

Orbital Doppler Evaluation of Blood Flow Velocities in Patients with Diabetic Retinopathy

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■ Abstract

BACKGROUND: There have been conflicting results in relation to impaired ocular hemodynamics in the orbital vessels of patients with diabetic retinopathy (DR). Clarification of the early signs of retinopathy in diabetic patients is urgently needed. **AIMS**: We aimed to evaluate orbital blood flow velocities using Doppler and gray-scale sonography in patients with DR, and to compare the results with those of their non-diabetic and diabetic peers without retinopathy. **METHODS**: Orbital Doppler and gray-scale sonography were performed in 123 patients aged 29-77 who had been divided into 3 groups: non-diabetic controls (n = 25), diabetes and impaired glucose tolerance with minimal clinical retinopathy (n = 74), and diabetes with untreated non-proliferative retinopathy (n = 24). Retinopathy was diagnosed by an ophthalmologist on the basis of fundoscopic examination. The peak systolic (PSV) and end-diastolic (EDV) blood flow velocities, and the resistivity and pulsatile indices, of the ophthalmic artery, central retinal artery, posterior ciliary artery, and central retinal vein were measured. **RESULTS**: Compared with healthy controls, the age-adjusted resistivity and pulsatile indices of the ophthalmic artery were significantly higher in patients with DR ($p < 0.05$). PSV and EDV of the posterior ciliary arteries were significantly lower in diabetic patients with DR. After further adjustment for age, gender, HbA1c, fasting plasma glucose, blood pressure, BMI, cholesterol, HDL, LDL, and triglycerides, only the resistivity index of the ophthalmic artery and the central retinal vein remained significantly higher in patients with DR compared with healthy controls ($p < 0.005$ after Bonferroni adjustment). **CONCLUSIONS**: Resistivity index alteration of the ophthalmic artery and central retinal vein may be prevalent among patients with early changes in DR.

Keywords: diabetes · diabetic retinopathy · blood flow parameters · Doppler sonography · ocular circulation · ophthalmic artery

Introduction

Diabetic retinopathy (DR) is the most common ocular complication in patients with diabetes [1]. Despite intensive research efforts, the mechanisms of initiation and progression of DR are still not well understood. Vascular changes and subsequent ocular hemodynamic changes are critical events in the pathogenesis of DR. However, the role of hemodynamics in DR has not been clearly defined [2]. There have also been conflicting reports of impaired hemodynamics in the orbital vessels in patients with DR [3-14]. Orbital blood flow velocities were increased in some studies [3], but decreased in others [4, 9-11, 13, 14], or unchanged [15].

Color Doppler imaging is one of the most widely used and well-established techniques for assessing ocular blood flow velocities in the retrobulbar vessels. This is a non-invasive, painless imaging method with highly reproducible procedures. Estimation of orbital blood flow velocity from color

Doppler imaging of the ophthalmic artery, central retinal artery, posterior ciliary artery, and the central retinal vein is a technique offering great potential for the identification of early retinopathy in diabetic patients [16].

However, it is not clear whether impaired hemodynamics in the orbital vessels of patients with diabetes is the decisive factor in the pathogenesis of DR. We therefore examined patients with untreated DR, and compared them with controls regarding the values obtained for orbital blood flow velocity, resistivity index, and pulsatile index in the ophthalmic artery, central retinal artery, posterior ciliary artery, and the central retinal vein. We used color Doppler and gray-scale sonography at a stage before the development of proliferative retinopathy.

Subjects and methods

Subjects

The study population consisted of 123 participants, 57 (46.3%) men and 66 (53.7%) women, including non-diabetic controls (n = 25), subjects with diabetes and impaired glucose tolerance with minimal clinical retinopathy (n = 74), and diabetes patients with untreated non-proliferative retinopathy (n = 24). The patients with type 2 diabetes and their non-diabetic first-degree relatives (non-diabetic controls and subjects with impaired glucose tolerance) were recruited from the Isfahan Endocrine and Metabolism Research Center between August 2008 and May 2009. Cases of diabetes, impaired glucose tolerance, and non-diabetic controls were categorized using the oral glucose tolerance test according to American Diabetes Association criteria [17]. Exclusion criteria were previous laser photocoagulation, and any disease or anomaly of the eye, which may affect blood flow velocity, such as ocular inflammation, trauma, non-diabetic vascular disease, and glaucoma. Pregnant and breast-feeding women were also excluded. No participants had a history of major systemic disease, including cardiovascular disease, arterial hypertension, and hyperlipidemia.

Abbreviations:
ANOVA - analysis of variance
BMI - body mass index
DR - diabetic retinopathy
EDV - end-diastolic velocity
HbA1c - glycosylated hemoglobin A1c
HDL - high-density lipoprotein
LDL - low-density lipoprotein
PSV - peak systolic velocity
SE - standard error
SPSS - statistical package for the social sciences

Assessment of diabetic retinopathy

DR was diagnosed by an expert ophthalmologist (AD). All patients with diabetes underwent dilated fundoscopy using the biomicroscopic indirect ophthalmoscope. Cases of DR were identified according to the modified Airlie House system [18, 19]. Patients without retinopathy had no clinically observable retinopathy. Background retinopathy was defined by the presence of microaneurysms, hemorrhage, and hard exudates, according to retinopathy level 2-3 in the Airlie House classification. Pre-proliferative changes consisted of background retinopathy lesions plus two or more of the following impairments:

- Venous beading or reduplication
- Intra-retinal microvascular abnormalities
- Deep intra-retinal hemorrhage
- Multiple cotton wool spots qualifying for retinopathy level 4-5

Patients with proliferative retinopathy were excluded because they were few in number and had started treatment.

Systolic and diastolic blood pressure, measured during blood flow velocity investigations, did not exceed values of 130 mmHg and 85 mmHg, respectively. Estimation of intrabulbar pressure did not exceed 22.0 mmHg.

Color Doppler and gray-scale sonography

Retrobulbar blood flow velocity was assessed using orbital Doppler and gray-scale sonography. Color Doppler imaging of the eye was performed in all individuals by two masked expert sonographers (KK and AK) using a color Doppler unit and a 7.5-10-MHz linear-array transducer (model G-60; Siemens, Germany). Hemodynamic measurements had intra- and inter-sonographer reliability coefficients of 0.913 and 0.934 (using the kappa coefficient). The patients were examined in the supine position to avoid any pressure on the eye. Sterile coupling gel was applied to closed eyelids, with the examiner's hand resting on the orbital margin to minimize pressure on the globe, and real-time gray-scale and color-flow images were obtained [20].

Angle correction was applied to the pulsed Doppler recordings to minimize errors in the

Table 1. Age, age-adjusted mean, and characteristics of participants with and without diabetic retinopathy

Characteristic	Total	Control	Diabetes and IGT (minimal retinopathy)	Diabetic retinopathy
Number (%)	123 (100)	25 (20.3)	74 (60.2)	24 (19.5)
Age (yr)	52.2 ± 0.7	49.9 ± 1.5	51.1 ± 0.9	58.1 ± 1.5**a,b
Body mass index (kg/m^2)	27.9 ± 0.3	28.1 ± 0.7	27.8 ± 0.4	28.0 ± 0.7
Duration of diabetes (yr)	-	-	5.6 ± 0.7	12.6 ± 1.1**b
Fasting plasma glucose (mg/dl)	120.8 ± 3.0	95.0 ± 5.9	123.4 ± 3.4	139.6 ± 6.3**a,b
HbA1c (%)	6.5 ± 0.1	5.0 ± 0.3	6.5 ± 0.1	7.8 ± 0.3**a,b
Total cholesterol (mg/dl)	191.3 ± 3.5	206.9 ± 7.6	187.7 ± 4.4	186.1 ± 8.2
HDL (mg/dl)	46.5 ± 1.3	43.9 ± 2.9	47.8 ± 1.7	45.2 ± 3.2
LDL (mg/dl)	111.8 ± 2.9	126.0 ± 6.4	107.3 ± 3.6	111.4 ± 6.7*b
Triglycerides (mg/dl)	164.0 ± 6.5	166.3 ± 14.5	167.0 ± 8.3	152.6 ± 15.2
Systolic blood pressure (mm Hg)	118.8 ± 1.3	120.1 ± 2.7	117.1 ± 1.6	122.6 ± 2.9
Diastolic blood pressure (mm Hg)	73.3 ± 11.0	79.7 ± 2.1	72.1 ± 1.2	70.4 ± 2.2**a,b
Women, no. (%)	66 (53.2)	17 (68.0)	37 (50.0)	12 (48.0)
Obesity, no. (%)	27 (22.9)	5 (20.8)	19 (27.5)	3 (12.0)

Legend: Data are mean ± SE, or number (%). Age-adjusted means were calculated using general linear models with Bonferroni correction for multiple comparisons. The difference in the mean of the variables compared with the control group (a) and diabetes group (b) without retinopathy. *Abbreviations*: IGT - impaired glucose tolerance, LDL - low density lipoprotein, HDL - high density lipoprotein. *$p < 0.05$, **$p < 0.001$, for comparisons across all three groups.

measured velocities. We obtained peak systolic (PSV) and end-diastolic (EDV) velocity measurements in the ophthalmic artery, central retinal artery, posterior ciliary artery, and in the central retinal vein. We used these measurements to calculate vascular resistance (expressed by the resistivity index and pulsatile index) using the following formula:

Resistivity index = (PSV-EDV)/PSV [21] and
Pulsatile index = (PSV-EDV)/V_{mean}

where V_{mean} = 1/3 (PSV-EDV)+EDV [22], in all patients. The eye with the worse retinopathy was chosen for inclusion in study examinations. If both eyes had equal retinopathy, the right eye was assigned to the study.

Signals from the ophthalmic artery can be located in the medial section of an eyeball, superior to the optic nerve, just lateral to and abutting the visible hyporeflective stripe representing the nerve. The central retinal artery originates from the ophthalmic artery and can be found anterior to the optic nerve, around 7.5 mm behind the ocular bulb. The posterior ciliary arteries are also supplied with blood by the ophthalmic artery, and they divide into multiple branches to supply the pial arteries. These arteries have a diameter of around 0.2 mm and form the pial network which adheres to the optic sheath and contributes to the vascularization of the optic nerve [23]. Signals from short posterior ciliary arteries can be located in the lateral and medial sections of an eyeball. Although numerous branches of short posterior ciliary arteries were available for assessment, only the lateral branch was chosen for analysis in this study.

The following measurements were carried out in all participants: HbA1c (measured by spectrophotometer), fasting plasma glucose, triglyceride, cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) (calculated by the Friedewald Equation [24]), body mass index (BMI, kg/m^2), and systolic and diastolic blood pressure.

The study protocol was approved by the Institutional Review Board of Isfahan University of Medical Sciences, Iran. Patients were informed about the Doppler method, and gave informed consent.

Statistical analysis

On the basis of previous estimates (with standard deviation of 2.2) [10], we calculated that 25 patients per treatment group would be required to

Table 2. Age-adjusted means (SE) of parameters obtained from color Doppler imaging in the ophthalmic, central retinal, and posterior ciliary arteries, and in the central retinal vein in participants with and without diabetic retinopathy

Variable	Control	Diabetes and IGT (minimal retinopathy)	Diabetic retinopathy
Ophthalmic artery			
PSV (cm/sec)	30.5 ± 1.6	33.5 ± 0.9	32.8 ± 1.7
EDV (cm/sec)	7.7 ± 0.6	7.7 ± 0.3	6.7 ± 0.6
Resistivity index	0.76 ± 0.01	0.79 ± 0.01	0.82 ± 0.01^{**b}
Pulsatile index	1.72 ± 0.1	1.83 ± 0.06	2.1 ± 0.11^{*b}
Central retinal artery			
PSV (cm/sec)	11.5 ± 0.8	11.7 ± 0.5	11.8 ± 0.9
EDV (cm/sec)	3.9 ± 0.3	3.7 ± 0.2	3.6 ± 0.3
Resistivity index	0.70 ± 0.02	0.71 ± 0.01	0.74 ± 0.02
Pulsatile index	1.32 ± 0.06	1.31 ± 0.03	1.45 ± 0.06
Posterior ciliary artery			
PSV (cm/sec)	13.9 ± 0.8	11.1 ± 0.5	12.7 ± 0.9**
EDV (cm/sec)	5.0 ± 0.3	3.9 ± 0.3	4.2 ± 0.3^{*b}
Resistivity index	0.67 ± 0.01	0.67 ± 0.01	0.71 ± 0.01
Pulsatile index	1.19 ± 0.06	1.16 ± 0.04	1.29 ± 0.06
Central retinal vein			
PSV (cm/sec)	5.3 ± 0.3	5.5 ± 0.2	5.9 ± 0.3
EDV (cm/sec)	3.7 ± 0.2	3.8 ± 0.1	3.6 ± 0.2
Resistivity index	0.38 ± 0.02	0.36 ± 0.01	0.43 ± 0.02^{**b}
Pulsatile index	0.48 ± 0.03	0.44 ± 0.02	0.55 ± 0.04^{*b}

Legend: Data are mean ± SE. Age-adjusted means were calculated using general linear models with Bonferroni correction for multiple comparisons. The difference in the mean of the variables compared with the control group (a) and diabetes group without retinopathy (b). The indices are calculated as follows: resistivity index = (PSV-EDV)/PSV, pulsatile index = (PSV-EDV)/V_{mean}, with V_{mean} = 1/3 (PSV-EDV)+EDV. *Abbreviations*: IGT - impaired glucose tolerance, EDV - end-diastolic velocity, PSV - peak systolic velocity, V_{mean} - mean time velocity. *p < 0.05, **p < 0.001, for comparisons across all three groups.

achieve the power of 80 percent required to detect a mean difference in blood flow velocity of 1.0 cm/s between the diabetic retinopathy and the control group, with a two-sided alpha of 0.05. Statistical methods applied included Student's t-test, one-way analysis of variance (ANOVA), Chi-square test, and the general linear model. Comparisons between PSV, EDV, and the resistivity and pulsatile indices were calculated by ANOVA followed by Tukey-Kramer post-hoc comparison. Non-normally distributed data were analyzed using the Kruskal-Wallis analysis of variance. Correlation between variables was detected using the Pearson's coefficient. Chi-square analysis was used where indicated. Age-adjusted means were calculated and compared using general linear models with Bonferroni corrections for multiple comparisons. Adjustments for age and HbA1c were examined in separate models. Further adjustments for age, gender, HbA1c, fasting plasma glucose, blood pressure, BMI, cholesterol, HDL, LDL, and triglycerides were analyzed to compare diabetes with and without retinopathy. The analyses were undertaken using SPSS version 18 for Windows (SPSS Inc., Chicago, IL, USA). All tests for statistical significance were two-tailed, and performed assuming a type I error probability of <0.05.

Results

Characteristics of study participants

The characteristics of the study participants are shown in Table 1. In age-adjusted comparisons of variables, age, fasting plasma glucose, and HbA1c, were more likely to increase, whereas LDL

Table 3. Pearson correlation coefficients between HbA1c, duration of diabetes, and hemodynamic parameters

Variable	HbA1c	Duration of diabetes
Ophthalmic artery		
PSV	0.076	0.131
EDV	- 0.080	- 0.186
Resistivity index	0.175	0.290*
Pulsatile index	0.149	0.261*
Central retinal artery		
PSV	- 0.103	- 0.137
EDV	- 0.201	- 0.224
Resistivity index	0.247**	0.327**
Pulsatile index	0.266**	0.322**
Posterior ciliary artery		
PSV	- 0.022	0.161
EDV	- 0.105	- 0.133
Resistivity index	0.167	0.365**
Pulsatile index	0.156	0.387**
Central retinal vein		
PSV	0.033	0.197
EDV	- 0.100	- 0.190
Resistivity index	0.201*	0.543***
Pulsatile index	0.157	0.582***

Legend: *p < 0.05, ** p < 0.01, *** p < 0.001.

and diastolic blood pressure tended to decrease across the three subject groups. The mean (standard error (SE)) age of patients was 52.2 (0.72) years. Of these participants, 53.2% (n = 66) were women. In diabetic patients, 65.1% were on oral agents, followed by diet (21.1%), and insulin (13.8%). Sulphonylurea derivatives were the most commonly used first-line treatments (51.5%), followed by metformin (36.5%), and combination treatments (12.0%), for those treated with oral agent.

Blood flow parameters

Table 2 shows the age-adjusted mean (SE) values for parameters obtained from color Doppler imaging measurements in the ophthalmic, central retinal, and posterior ciliary artery, and in the central retinal vein, in the following three groups:

1. Patients with diabetes and impaired glucose tolerance but without retinopathy
2. Patients with diabetes and with untreated retinopathy
3. Non-diabetic controls

There were no statistically significant differences between eyes with and without DR in PSV and EDV in the ophthalmic artery, central retinal artery, and central retinal vein. The mean resistivity (p = 0.009 after Bonferroni adjustment) and pulsatile (p = 0.029 after Bonferroni adjustment) indices in the ophthalmic artery were higher in eyes with DR than in healthy control eyes. The mean resistivity (p = 0.004 after Bonferroni adjustment) and pulsatile (p = 0.023 after Bonferroni adjustment) indices in the central retinal vein were also higher in eyes with DR than in diabetic and impaired glucose tolerance control eyes with minimal retinopathy.

However, PSV and EDV of the posterior ciliary arteries were significantly lower in patients with DR compared with healthy controls. We proceeded by stepwise inclusion of additional model adjustments. An initial comparison of the model adjusted for age alone with the model adjusted for age and HbA1c yielded no appreciable differences between eyes with and without DR in the resistivity and pulsatile indices in the ophthalmic artery and central retinal vein. After additional adjustment for age, HbA1c, and systolic blood pressure, the pulsatile index in the ophthalmic artery and central retinal vein was no longer significant. Finally, after further adjustment for age, gender, HbA1c, fasting plasma glucose, blood pressure, BMI, cholesterol, HDL, LDL, and triglycerides, only the resistivity index of the ophthalmic artery and central retinal vein remained significantly higher in patients with DR compared with healthy controls (p = 0.005 after Bonferroni adjustment).

Blood flow in relation to HbA1c and duration of diabetes

We evaluated the relationship between HbA1c, duration of diabetes, and hemodynamic parameters. The analysis showed significant positive correlations between HbA1c levels and the resistivity indices of the central retinal artery and the central retinal vein, as well as the resistivity and pulsatile indices of the central retinal artery (Table 3). HbA1c was negatively correlated with EDV of the central retinal artery. Duration of diabetes was positively correlated with the pulsatile and resistivity index of the ophthalmic artery, central retinal artery, posterior ciliary artery, and central

retinal vein. There was no correlation between HbA1c levels and duration of diabetes (Table 3).

Discussion

Compared with healthy controls, patients with DR had a significantly higher resistivity index in the ophthalmic artery and central retinal vein. Several studies have performed color Doppler imaging to assess orbital blood flow velocities in DR. These studies focused only on some of the orbital vessels and measured indices and velocities in specific diabetic or control groups. They were usually of limited sample size, and the results produced were inconsistent [3-12].

There is conflicting evidence as to whether blood flow velocity is increased, unchanged or decreased in DR. The inconsistency may be explained in part by different techniques and sites of measurement, differences in patient characteristics, definitions of DR, and there may be an element of chance. While differences in hemodynamic parameters over the course of the disease may partly account for the range of results seen in the literature, it is much more likely that the main component of variance in DR Doppler studies results from differences in instrumentation and technique.

Our findings are consistent with those of Grunwald *et al*. who found no differences in blood flow velocity in healthy eyes compared with the eyes of diabetic patients with or without retinopathy [8]. Patel *et al*., using laser Doppler velocitometry, showed an increase in blood flow velocity in patients with type 1 and type 2 diabetes with untreated retinopathy compared to non-diabetic controls and diabetics without retinopathy [3]. In contrast, studies using color Doppler imaging reported a reduced blood flow velocity in patients with diabetes which appeared to become further reduced as retinopathy progressed [6, 25]. Two other studies evaluated the blood flow velocities of the ophthalmic artery, central retinal artery, and posterior ciliary artery and found decreased PSV and EDV in the central retinal artery in patients with retinopathy [9, 11]. Similar results were reported by Mendivil *et al*., who analyzed 25 type 1 and type 2 diabetes patients with proliferative retinopathy and 30 non-diabetic controls [5]. In contrast to the results obtained in our study, these studies reported significant differences in blood flow velocities between healthy and DR eyes.

Our finding of an increased resistivity index in the ophthalmic artery and central retinal vein is similar to that reported by MacKinnon *et al*. [11]. The finding that the resistivity and pulsatile indices of the central retinal artery, posterior ciliary artery, and ophthalmic artery in patients with and without retinopathy are significantly greater than those of healthy individuals is also in agreement with certain other studies [6, 25-27]. It has been suggested that an increased resistivity index in the ophthalmic artery in the DR group may be due to downstream vascular changes related to diabetes in both retinal and choroidal vasculature.

Evans *et al*. also used color Doppler imaging to investigate retrobulbar vascular reactivity in early DR [12]. While under conditions of isocapnic hyperoxia, diabetic patients exhibited a significantly lower resistivity index in both the ophthalmic and central retinal artery compared to controls. Hypoxia induced a higher EDV in the central retinal artery, and a lower PSV in the ophthalmic artery compared to the healthy group. The higher resistivity index of the ophthalmic artery found in the retinopathy group can be indicative of decreased retinal blood and decreased perfusion. It was shown that choroidal blood flow decreased with the increase in severity of DR because of increased vascular resistance and decreased perfusion pressure [28].

There are many different results relating to the association of blood flow velocities and DR in the literature. The interpretation of these data is complicated by the demographic variability in the patients, the methodological problems related to the outcome measures in these studies, and their subsequent analyses. In addition, statistical analyses did not always deal with the small number of patients and the small effect sizes. To elucidate the complex links of orbital blood flow velocity with DR is a challenge for future research.

The precise mechanism of action of orbital blood flow velocities in DR outcome appears to be a complex pathology. The variability in orbital blood flow velocities may be caused by chance. There is a need to assess the mechanism for the association, whether it is through change in retinal blood flow, change in perfusion pressure, change in intraluminal capillary pressure, poor glycemic control, chance, or a combination of these and other factors.

The strength of our study is based on the fact that none of the patients with type 2 diabetes were on treatment for or had systemic diseases. We excluded these patients to focus on the effect of diabetes on orbital blood flow in type 2 diabetic patients. An experimental study by Guthoff *et al*. has shown that an increase in intraorbital pressure leads to a decrease in central retinal artery flow [30]. Great care was thus taken to apply as little

pressure as possible to the patient's eye during color Doppler imaging examination.

There are also some critical issues to consider. Firstly, retinopathy was diagnosed by an expert ophthalmologist using an indirect ophthalmoscope with the patient's pupils diluted. Ophthalmoscopy may be less exact than fundus photography in the detection of DR. However, we classified the patients into two groups only, and with such a limited number of categories, discrepancies by misdiagnosis are largely excluded. Moss *et al*. [31], and Kinyoun *et al*. [32], found that ophthalmoscopy compared reasonably well with fundus photography, with 85.7% and 85.9% agreement respectively at any one examination. Secondly, the sample size of 123 patients is relatively small, even though examinations were thoroughly and carefully performed.

The data from most studies suggest an association between nephropathy, as manifested by microalbuminuria, and DR [29]. The association between nephropathy and retrobulbar hemodynamic alteration is not clear. The increase in resistivity index in DR may be an indicator of increased microalbumin in the urine and other vascular complications of diabetes. However, no information on renal function or nephropathy was recorded. Further investigation is needed to extend the study by these data.

In conclusion, our finding of an increased resistivity index in the ophthalmic artery and central retinal vein may indicate disturbances of retinal and choroidal circulation in patients with DR. These events may be among the early changes in DR. Further studies with larger groups of patients are needed to understand better the role of retrobulbar hemodynamics in the pathogenesis of DR.

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