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Relation between grade of diabetic retinopathy and perilimbal capillary density: digital fluorescein angiographic study

Zależność pomiędzy stopniem retinopatii cukrzycowej a gęstością kapilarów okołorąbkowych: badanie za pomocą cyfrowej angiografii

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Summary: Purpose: To evaluate the relation between the capillary drop out in perilimbal area and the stage of diabetic retinopathy using the new approach of digital fluorescein angiography and digital image analysis technology.
Material and methods: Anterior and posterior segment fluorescein angiography were performed in 100 diabetic participants (43 males and 57 females, mean age ± SD was 60 ± 10.9 years) and 81 healthy persons as control group (41 males and 40 females, mean age ± SD was 60.8 ± 16.7 years). The loss in perilimbal capillary was estimated objectively by measuring the perilimbal intercapillary area (PIA).
Results: A significant loss in the perilimbal capillary density was observed in all stages of diabetic retinopathy ($P<0.05$). 31.7±18% increase in perilimbal intercapillary area in average due to diabetes comparing to the control group, was observed.
Conclusions: The perilimbal capillary area drops and ischemic changes associated with diabetic retinopathies showed strong correspondence.

Słowa kluczowe: okołorąbkowe pole kapilarów, fluoresceina, cyfrowy obraz, retinopatia cukrzycowa.
Key words: perilimbal intercapillary area, surface area, fluorescein, digital image, diabetic retinopathy.

Introduction

Diabetes is a multiorgan disease which is known to affect the capillary density on the conjunctiva and perilimbal area (1-2). Different techniques like capillarscopy (3), conjunctival vessels morphometry (4), computer – assisted intravital microscopy (5) and automated analysis of red free images (6,7) have been employed to quantitatively study the microcirculation of the conjunctiva and perilimbic area in diabetic patients.

The new advancement in fluorescein angiography equipments allows one to quantify the perilimbal microcirculation. Furthermore, digital image format of vascular networks allow image manipulation, resulting in better visualization and objective analysis using computer-aided techniques. These computer softwares analyze digital images and convert positions and measurements in an image after calibration from pixel to real world dimensional measurements such as millimeters, microns, feet, miles, etc.

The aims of this study were to describe a new simple technique in assessing the capillary density in perilimbal area in digital fluorescin angiography images and to correlate the changes with grades of diabetic retinopathy.

Patients and methods

Participant selection:

A case control study was carried out at the Departments of Ophthalmology, Almowasat Clinic (Tripoli, Libya) and First Eye Hospital (Lublin, Poland).

100 participants with diabetes mellitus were recruited from diabetic patients referred for fluorescein angiography. 81 participants in control group were recruited from non diabetic patients referred for fluorescin angiography. Table I.

Diabetic retinopathy was graded clinically as no retinopathy, mild non-proliferative, severe non-proliferative or proliferative diabetic retinopathy in accordance with accepted criteria (8).

Exclusion criteria were contact lens wearing, active anterior segment disease, history of ocular surgery or long term use of ocular topical medication. The study was performed from July 2005 through March 2006.

Technique of angiography:

5 ml of 10% fluorescein were injected in the anticubital vein of the participants.

Two kinds of digital fundus camera were used for anterior segment photography; Kowa RC– XV3 (Almowasat clinic, Tripoli

	Number	Mean age (±SD)	Gender (Male/Female)
Control	81	60.8(±16.7)	41/40
Diabetic	100	60(±10.9)	43/57

Table I. Mean and standard deviation of age and male/female ratio of diabetics and control group (SD = Standard deviation).

– Libya), and Heidelberg Retina Angiogram 2 (First eye hospital, Lublin – Poland). 5 – 6 digital photographs were taken during the first two minutes. The photographs were concentrated on the lower quadrants of the bulbar conjunctiva. Posterior segment photographs were taken during the same session.

Technique of measuring the perilimbal intercapillary surface area (PIA):

The UTHSCSA Image Tool program (developed at the University Of Texas Health Science Center at San Antonio, Texas and available from the Internet from ftp://maxrad6.uthscsa.edu) was used to measure the Perilimbal Intercapillary Area (PIA). PIA provides capillary density estimation in the perilimbal network. The intercapillary areas are highlighted with the cursor in the digital image. Fifty randomly selected areas surrounded by capillaries are marked. The area described by the cursor is measured with the image tool program to obtain its surface area in μm^2. (Figure 1, A and B).

Statistical Analysis

Mean values and standard deviations are given for all samples with normal distribution (Kolmogorov-Smirnov test). The Student's *t* test was used for unpaired (independent) samples with normal distribution. Findings with an error probability of <0.05 were considered to be statistically significant. The perilimbal intercapillary areas (PIA) were log transformed to give percentage differences. All the statistical calculations were performed using SPSS software version 14.0 for Windows (SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor Chicago, Illinois 60606, USA).

Results

Mean and standard deviation of PIA for control and diabetic participants and the percentage change in diabetic participants with different grade of retinopathy are given in table II.

Discussion

The present study adopted a new approach of using digital fluorescein angiography with digital image analysis technology.

This technology gives high magnification with excellent resolution enabling more precise study of conjunctival and limbal capillaries

Measurement of capillary density is a valuable diagnostic tool for differentiating capillary loss (9-12).

Our study describes a simple technique for quantitative assessment of capillary density in the perilimbal area by measuring the perilimbal intercapillary surface area (PIA) using image analysis software in digital fluorescin angiography images.

Measurement of intercapillary area in digital fluorescein images was first introduced by Arend and colleagues in 1991, for evaluating the perifoveal capillary network. It has been proven to be a valid and accurate tool in assessing the perifoveal capillary density in different ocular and systemic conditions (9).

Our results showed clearly the strong association between the capillary drop out in the perilimbal area and the grade of diabetic retinopathy.

The effect of diabetes mellitus on the microvascular system of the conjunctiva and limbus have been discussed in the ophthalmic literature mainly during the past few decades using different methods.

	Number	Mean(μm^2)	Standard Deviation	Percentage Change (%)		P Value*
				Mean	Standard Deviation	
Control	81	6546	529			
Mild Non-proliferative	37	10141	4452	14.9	19.3	< 0.0001
Severe non-proliferative	26	15377	2547	36.5	8.7	< 0.0001
Proliferative	37	18188	1863	44.3	4.5	< 0.0001
All Grades	100	14568	4712	31.7	18	< 0.0001

Table II. The Mean & SD of PIA for control group and diabetics and percentage changes in diabetic participants with differing grades of diabetic retinopathy.

* Unpaired Student's t test.

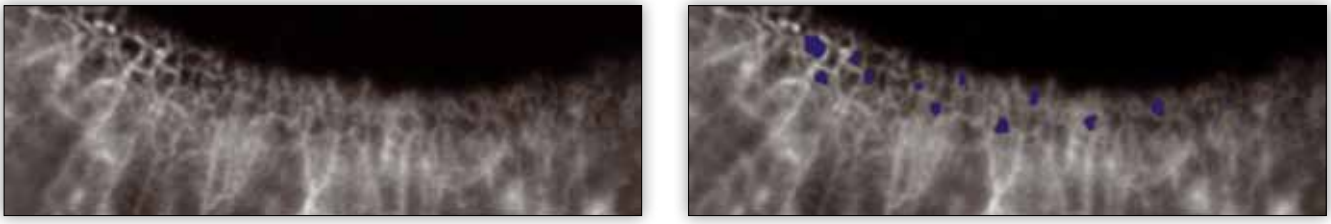

Fig. 1. (A) Normal fluorescein angiography image of the perilimbal area; (B) Perilimbal intercapillary areas are outlined by the cursor, the selected areas then easily measured by the image tool program, to obtain their surface areas in μm^2.

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For instance, Ioseliany using Quantitative capillarscopy noted that patients with diabetes mellitus with still no visible changes in the retina showed statistically significant reduction in the amount of functioning capillaries of the limbus (3).

Using the conjunctival morphometry, Worthen and colleagues, have noted that there is a decrease in the capillary vascularity by 25% among diabetic patients when compared to normal subjects (4).

Using red-free conjunctival images and an automated computer algorithm Owen CG and colleagues, have shown A strong positive association between the duration of diabetes and overall mean vessel width, resulting from changes in larger vessels (>80 μm in width). Conversely, the duration of diabetes showed a strong inverse association with vessel area that appeared to be driven by the trend observed in smaller vessels (<40 μm in width).

He also observed a 25% reduction in vessel density in those with type 1 diabetes and a 14% reduction in those with type 2 diabetes. The difference in the size of effect for type 1 and type 2 diabetes was accounted for by duration of diabetes, reflecting the longer duration of disease in those with type 1 diabetes compared with those with type 2 diabetes (median duration, 26 years and 7 years respectively). They also noted that grade of diabetic retinopathy showed less strong associations with the changes in conjunctival vessel indices (7).

Comparing with the present study, we found higher percentage of capillary loss due to diabetes (31.7%), and more strong association between the grade of diabetic retinopathy and perilimbal capillary loss.

We concluded the perilimbal capillary drop and ischemic changes associated with diabetic retinopathies showed strong correspondence.

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