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Distribution of normal superficial ocular vessels in digital images

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A B S T R A C T

Purpose: To investigate the distribution of different-sized vessels in the digital images of the ocular surface, an endeavor which may provide useful information for future studies.
Methods: This study included 295 healthy individuals. From each participant, four digital photographs of the superior and inferior conjunctivae of both eyes, with a fixed succession of photography (right upper, right lower, left upper, left lower), were taken with a slit lamp mounted camera. Photographs were then analyzed by a previously described algorithm for vessel detection in the digital images. The area (of the image) occupied by vessels (AOV) of different sizes was measured. Height, weight, fasting blood sugar (FBS) and hemoglobin levels were also measured and the relationship between these parameters and the AOV was investigated.
Results: These findings indicated a statistically significant difference in the distribution of the AOV among the four conjunctival areas. No significant correlations were noted between the AOV of each conjunctival area and the different demographic and biometric factors. Medium-sized vessels were the most abundant vessels in the photographs of the four investigated conjunctival areas. The AOV of the different sizes of vessels follows a normal distribution curve in the four areas of the conjunctiva. The distribution of the vessels in successive photographs changes in a specific manner, with the mean AOV becoming larger as the photos were taken from the right upper to the left lower area.
Conclusions: The AOV of vessel sizes has a normal distribution curve and medium-sized vessels occupy the largest area of the photograph.

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1. Introduction

The conjunctiva has attracted the attention of physicians since ancient times. As a tissue on which small vessels of the body are visible to the naked eye, it has served as a major site for the diagnosis of many diseases. In spite of the conjunctiva being such an important tissue for examination, little has been investigated regarding the quantification of its changes. This is due in part to the great variability in the distribution, shape and size of the conjunctival vessels and/or their brisk responsiveness to various stimuli which can cause instant changes in the vascular pattern. Previous attempts for studying conjunctival vessels have included the manual extraction of vascular data from their photographs

[1] or video captures [2]. In the automated world, computer algorithms can effectively serve this purpose. For example, Chen et al. [3] first used image processing algorithms to study conjunctival vessels. Owen and colleagues employed an algorithm for the detection of conjunctival vessels and the quantification of their changes in diabetic patients [4,5]. In another study, Fukushima and Tomita utilised a computer algorithm to study the changes induced by the histamine challenge of the conjunctiva in guinea pigs [6].

Previous studies have shown that the objective investigation of the conjunctival hyperemia is considerably more reliable and repeatable than subjective grading [4,7,8]. Therefore, attempts to employ computerized algorithms for the evaluation of conjunctival vessels are becoming more common during the past few years.

In the present study, a Radon transform-based algorithm for the detection of vessels in digital images [9] was used for the quantitative investigation of normal conjunctival vessels in a large population of normal individuals. This information may serve as

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baseline data for studying conjunctival vascular disorders and the response of conjunctiva to various stimuli.

2. Methods

2.1. Study population

Normal individuals between 30 and 60 years of age, from the same ethnic background, were selected for the current study. Cases were recruited either from individuals seeking corrective glasses or from healthy persons accompanying patients at the Eye Hospital outpatient clinics. After the experimental procedure had been explained, an informed consent was obtained from all participants. The study followed the tenets of the Declaration of Helsinki and was approved by the Research Ethics Committee. A detailed medical history was taken and those with any history of diabetes mellitus, hypertension, anemia, dysthyroid state, rheumatic disorders or allergy were excluded from the study. Photographs of conjunctivae were taken before any ocular examinations or the instillation of any drops. Complete ocular examinations were conducted afterwards and the data of those with any ocular or adnexal pathology, except for refractive errors, were excluded from the study. The blood pressure, height and weight, fasting blood sugar (FBS) and hemoglobin of all eligible participants were measured. In addition, excluded were cases of high blood pressure (systolic blood pressure (SBP) of more than 140 mmHg or diastolic blood pressure (DBP) of more than 95 mmHg measured after five minutes of sitting) or FBS of more than 126 mg/dl.

After an initial small pilot study of 15 individuals (unpublished data) and the age pyramid of the normal population in the local community, the sample size was calculated as 300 cases (power = 90%, α = 0.05).

2.2. Photography

The photos were taken by a trained photographer with an YZ5S digital slit lamp microscope (Suzhou 66 Vision-Tech Co., Ltd., Suzhou, Jiangsu, P.R. China). For all participants, a unique protocol was used for photography: with a 5 V input to the slit lamp and both red-free and diffuser filters in place, the illumination arm was set at an angle of 45° and a magnification of 25× was adopted. Illumination was provided by a halogen lamp and with a wide diffuse illumination of a slit lamp (8 mm circle). The photography of all measurements was performed under similar normal room illumination. Afterward, for taking images of the superior and inferior

conjunctiva, each participant was instructed to look completely up and down. Gentle pressure was applied to open the lids in order to ensure that they did not obstruct the conjunctiva during the photography. The sequence of the conjunctival photography was as follows: the superior and inferior conjunctiva of the right eye and then the respective areas of the left eye. Photographs without flash were taken as quickly as possible to avoid dry eye and irritation. This was followed by a one-minute break before photographing the left eye. With this protocol, four conjunctival photographs were taken for each case: right upper (RU), right lower (RL), left upper (LU), and left lower (LL).

2.3. Computer algorithm

The algorithm used (developed by Matlab; Mathworks, Inc., Natik, MA, USA) includes a local line detector based on the Radon transform for blood vessel segmentation [9]. Briefly, the green plane of the input image, as a gray scale image, is preprocessed and divided into overlapping windows. Subsequently, a circular mask is used for each window and the Radon transform is calculated over the masked window. The Radon transform is a matrix integral transform that summates the values of a matrix in different directions. The maximum value in the Radon space specifies the direction of the line. The profile of the Radon transform in this direction is used to detect the segment of the vessel (if present). Then, the extracted vessel segment is refined. Local vessel segments are eventually merged to make the final result. Since the Radon transform is based on integration, it is robust to noise when compared to other available algorithms (Fig. 1).

The output of the algorithm was the area occupied by vessels (AOV) of different sizes as a fraction of the total pixels of the image and this was provided as a percentage. The algorithm detected vessels from 1 to 27 pixels in diameter. Very small vessels (1–4 pixels in diameter) and very large vessels (21–27 pixels in diameter) were detected in only a few cases. Therefore, these pixels were excluded from further analysis and only vessels of 5–20 pixels in diameter (16 classes of pixels in diameter) were included in the present study. It should be noted that the photographs had been previously calibrated with photographs taken from a 0.5 mm scale ruler. This was performed by using the same settings of photography and finding a conversion factor of 18 μm for each pixel so that the vessels were comprised of vessels from 90 to 360 μm in diameter. The main outcome measure of the study was the distribution parameters of AOV (in percentages) in four conjunctival areas of the normal population aged 30–60 years.
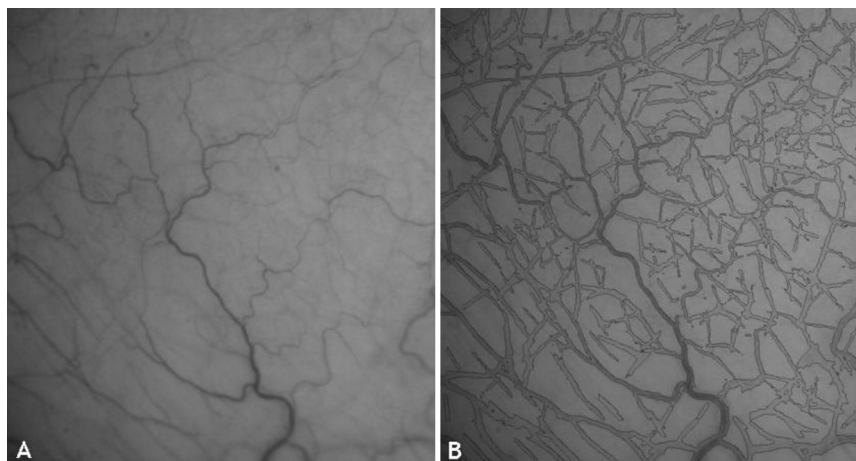

Fig. 1. Photograph of conjunctiva (A) and the result of segmentation of the vascular tree by the algorithm superimposed on it (B).

Table 1
Baseline characteristics of the study population. Data are presented as mean ± standard deviation, with the range. BMI, body mass index; BP, blood pressure; FBS, fasting blood sugar.

	Total	Female	Male	*p*-Value
Age (year)	41.35 ± 6.99 (30–60)	41.02 ± 7.10 (30–60)	41.58 ± 6.55 (40–60)	0.020
Height (cm)	163.95 ± 10.36 (105–191)	159.67 ± 8.57 (120–187)	170.90 ± 9.19 (105–191)	0.000
Weight (kg)	70.73 ± 13.55 (19–122)	70.59 ± 13.84 (42–122)	70.94 ± 13.11 (19–109)	0.311
BMI (kg/m^2)	26.45 ± 5.37 (15.9–48.61)	27.80 ± 5.58 (16.79–48.61)	24.26 ± 4.18 (15.90–36.62)	0.270
Systolic BP (mmHg)	119.62 ± 10.54 (90–140)	118.68 ± 11.47 (90–140)	121.14 ± 8.67 (100–140)	0.016
Diastolic BP (mmHg)	69.15 ± 10.32 (40–95)	66.28 ± 10.59 (40–95)	73.29 ± 9.07 (50–90)	0.000
FBS (mg/dl)	78.67 ± 13.92 (57–118)	76.07 ± 13.32 (57–117)	83.82 ± 13.74 (58–118)	0.105
Hemoglobin (g/dl)	13.44 ± 1.19 (9.7–17)	13.08 ± 1.05 (9.7–16)	14.15 ± 1.15 (10.7–17)	0.000

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) and the significance level was set at *p*-values < 0.05. The Kolmogorov–Smirnov test was used to ascertain the normality of the data for each pixel in diameter (5–20 pixels in diameters) of all four investigated conjunctival areas.

Repeated-measures ANOVA was employed to determine whether there were differences in the measured AOV as a function of the conjunctival area. A Bonferroni correction with an adjusted p value was subsequently applied to all the possible multiple pairwise comparisons for the four investigated conjunctival areas.

The difference in AOV in each conjunctival area was investigated between the genders (*chi* square test). In addition, the relationship between the AOV of each conjunctival area was determined with other numerical demographic and biometric variables by using Pearson's correlation coefficient for variables with a normal distribution and Spearman's correlation coefficient for those without a normal distribution.

3. Results

Initially, 332 participants were enrolled in the current study. However, 37 cases were excluded from the study population due to one or more poor quality photographs or incomplete data. Of the remaining 295 subjects with complete demographic, laboratory and photographic data for analysis, 118 of these cases (40%) were male. The baseline characteristics of these 295 cases are summarized in Table 1.

The distribution of AOV in each of the four photographed conjunctival areas is presented in Table 2. As can be inferred from the data in Table 2, the mean AOV becomes larger as photos were taken from the right upper to the left lower area. The AOV of all vascular sizes in the four conjunctival areas of images had a normal distribution as determined by the Kolmogorov–Smirnov test; the medium-sized vessels had the largest AOV in the conjunctival photographs (Fig. 2). Repeated-measures ANOVA with one within-subject factor (conjunctival area) indicated that there was a statistically significant difference in the distribution of AOV among the four conjunctival areas (*p* < 0.001). In addition, multiple pair-wise comparisons (the Bonferroni correction with an adjusted p value) showed a statistically significant difference between all pairs of the conjunctival areas (*p* < 0.001), except between the

right-upper and right-lower areas (*p* = 0.34) and the left-upper and left lower areas (*p* = 0.20).

No statistically significant difference between genders was found in terms of AOV for the four conjunctival areas. The correlation of the AOV of each conjunctival area was analyzed in terms of the different demographic and biometric factors (age, height, weight, BMI, SBP, DBP, FBS, and hemoglobin). No statistically significant correlations were noted (either *p* > 0.05 or −0.2 < *r* < 0.2).

4. Discussion

This study investigated the distribution of different-sized vessels in the digital images of the ocular surface. The main purpose of this work was to provide baseline quantitative data for the comparison of various conjunctival vascular conditions (e.g. conjunctival injection or reduced vascularity) with those of normal individuals and for studying the conjunctival reaction to various stimuli. As far as this study is concerned, no quantitative method for studying conjunctival vessels is clinically available. The results presented in this study may improve the understanding of the pathophysiology of conjunctival disorders and provide future practical clinical applications.

There have been many investigations conducted on the vessels of the bulbar conjunctiva, most using qualitative or semi-quantitative methods of study [6,10–12]. The purpose of most of these studies has been exploring the response of the conjunctiva to various stimuli or studying the effect of systemic diseases on the conjunctival vascular tree. For example, Fukushima and Tomita used an algorithm to study the hyperemia induced by the topical histamine challenge of the conjunctiva in guinea pigs [6]. In this study, the total pixels of the vessels were presented, but in the present study the effect of this challenge on vessels of different calibers was performed.

There are other ways of studying the physiology of conjunctival vessels. Some research has employed *in vivo* video recordings and analyzed the responses of the vascular tree to different stimuli [13] or diseases involving the microvasculature [14–17].

There are also many automated algorithms for blood vessel segmentation in digital images [3,4,14]. The current study's algorithm, which employs the Radon transform on overlapping windows of a picture, offers the advantage of being noise-resistant [9]. It has a high sensitivity and specificity when applied to fundus images and when compared with standard photographs [18]. However, in terms of conjunctival images, there are no standard manually extracted images of conjunctival vessels to report an ROC curve for the algorithm. The algorithm demonstrates a very accurate and acceptable performance when the results of the segmentation are subjectively compared with the initial photos (Fig. 1). Considering previous studies, Owen et al. [4] used an algorithm to detect conjunctival vessels. Their algorithm utilised a linear formula seemingly more prone to noise than a formula based on integration, which is very similar to what was used in the algorithm of the current study [9].

Table 2
Area occupied by vessels (AOV) in the 4 conjunctival areas of photographic images of normal subjects (*n* = 297). Data are presented as percentages of the image area.

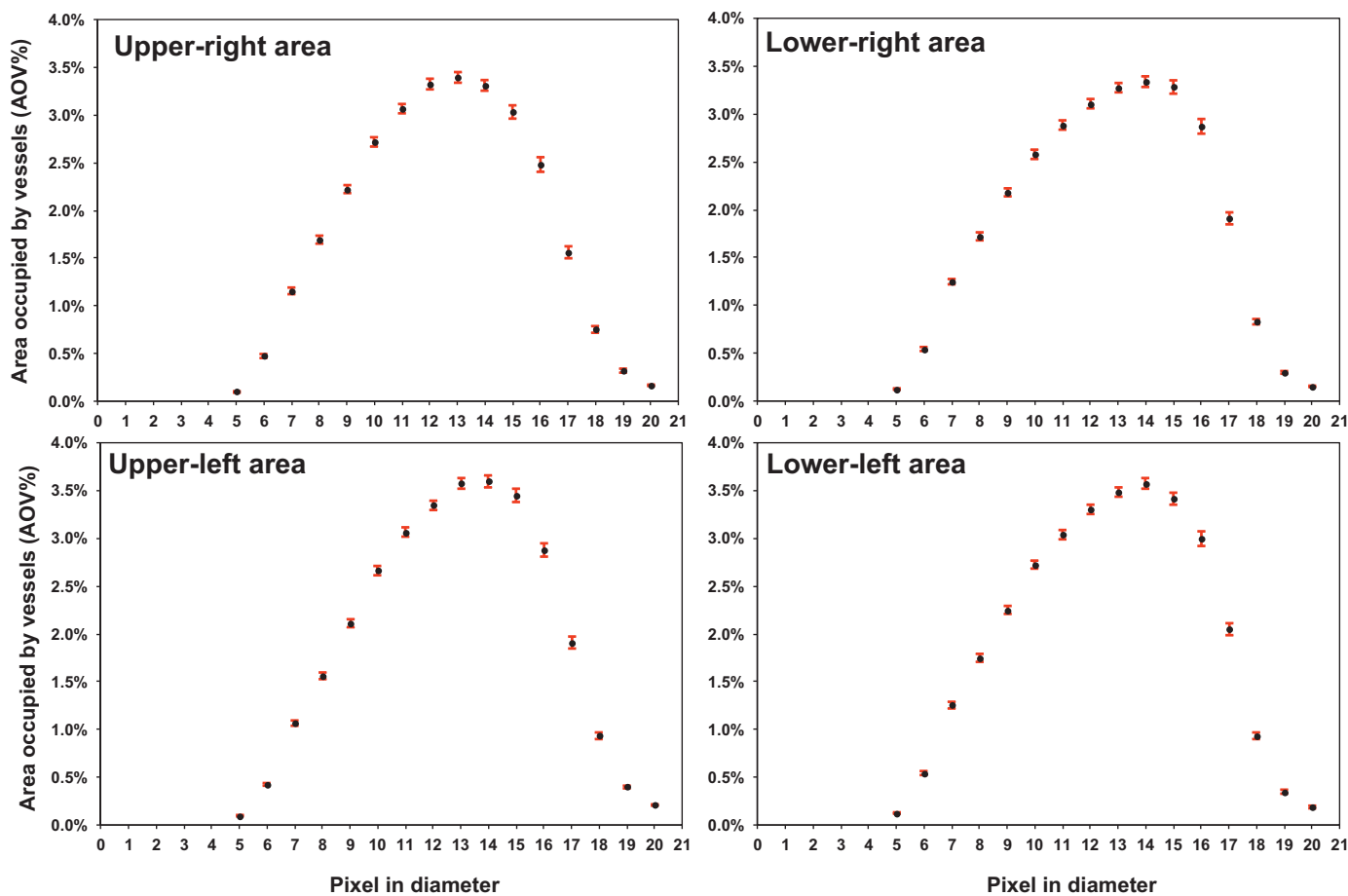| Image	Mean ± SD	Range	95% CI
Right-upper	29.58 ± 5.32	9.17–41.20	28.99–30.19
Right-lower	30.23 ± 5.73	6.61–40.34	29.55–30.86
Left-upper	31.19 ± 5.30	11.95–44.46	30.60–31.80
Left-lower	31.85 ± 4.86	13.34–45.53	31.29–32.39

Fig. 2. Area occupied by vessels of 5–20 pixels in diameter in four photographed conjunctival areas. Error bars indicate the standard error of the data.

The present study used AOV as the percentage of the image occupied by different-sized vessels instead of the actual area occupied by vessels. This enabled having a set of data that can be applied to photos taken by different cameras and with different magnifications. It was previously shown that this measurement technique correlates perfectly with the degree of the clinical grading of conjunctiva redness [19]. Nevertheless, a single parameter for total vessels was not used; instead, it was studied in different-sized vessels. The current study addressed the superior and inferior conjunctival areas and excluded the inter-palpebral conjunctiva due to the high variation of conjunctival tissue in the inter-palpebral area.

Refining the photography and image analysis methods of this study may be essential to provide more accurate and reproducible results along with producing a very sensitive tool for clinical studies. One way to reduce ocular irritation during the photography is to limit investigation to the inferior conjunctival fields, since this area of the conjunctiva may be photographed without holding the lids open, hence, minimizing eye irritation. To solve the problem of unfocused peripheral parts of the image, due to the convex surface of the globe, a modification can be the application of a mask, with a central predefined window over conjunctival images. This would exclude the peripheral unfocused parts from processing or taking advantage of de-blurring algorithms.

In conclusion, the present study described the normal distribution parameters of conjunctival vessels in a large population and found that medium-sized vessels have the largest AOV in conjunctival photographs. In addition, the mean AOV becomes larger as photos were taken from the right upper to the left lower area. This observed pattern may be due to an inherent difference between the four conjunctival areas or perhaps a mild irritation caused by

photography. Further studies are needed to randomize the photography sequence of the four conjunctival areas in order to rule out any bias from the method. The current study also found that the distribution of normal ocular vessels in healthy individuals is independent of age, gender, weight, height, BMI, hemoglobin and FBS.

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