Vascular network changes in the retina during ageing in normal subjects: a computerized quantitative analysis

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Key words:
Fundus oculi; Retinal vascular network; Non-mydriatic retinography; Image analysis; Ageing.

Background. **Direct ophthalmoscopic examination of fundus oculi is widely used for the qualitative evaluation of target organ damage in several pathological conditions. At present, there are no reliable techniques available to quantify retinal vascular damage. The aim of this study was to develop a computerized technique for the quantitative analysis of fundus oculi.**

Methods. **We studied 68 non-smoking, normotensive normal subjects, with a visual acuity > 8/10. From each subject retinal images were taken using a non-mydriatic ophthalmoscope and digitized onto a personal computer. In each image the area of analysis was set to a circular selection, corresponding to 25% of the whole fundus oculi, concentric with the optic disc. From each selection arterial and venous area and mean arteriolar bifurcation angle were obtained. Measurements were taken in a blinded way by two operators and, by the same operator, twice at different times. Parameters were correlated with age and body surface area.**

Results. **Retinal arterial density (arteries 7.44 ± 1.25%; bifurcation angle 75 ± 16°) was significantly correlated with age, but not with body surface area. Intra- and interobserver coefficient of variation resulted 2.5 and 3.2%, respectively.**

Conclusions. **Computerized analysis of the fundus oculi with the evaluation of vascular density indexes represents a simple and reproducible technique that could be useful in identifying changes in retinal vascular network during ageing.**

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Introduction

Direct examination of fundus oculi is widely employed in several clinical conditions[1], such as arterial hypertension[2,3], diabetes mellitus[4,5] and atherosclerosis[6,7], for the qualitative evaluation of target organ damage. The retinal vascular tree is also known to be affected by ageing; a progressive reduction of arterial bifurcation angle, that is considered an index of vascular density, has been demonstrated[8,9], suggesting a retinal vascular rarefaction during ageing. Previous studies have demonstrated that retinal damage is characterized by progressive vascular alterations mostly involving the arterial component. Nowadays, thanks to early diagnosis and effective therapy, a smaller number of patients show macroscopical retinal vessel alterations, such as artero-venous crossings, exudates, hemorrhages and papillary edema[10]. Traditional qualitative techniques based on Keith-Wagener-Barker scale[11] often have a low reproducibility[12]. New techniques for the quantitative morphological evaluation of vascular retinal network have recently been proposed, showing a greater reproducibility than traditional ones, but requiring dedicated software and hardware[13,14] and fluorescein contrast[15]. At the moment there is not an easy and clinically useful technique, available for the quantitative determination of retinal vascular network. The aim of this study was: a) to set up a computerized technique for the quantitative analysis of retinal vascular density using a non-mydriatic retinograph in a population of healthy subjects; b) to evaluate morphological changes of retinal vascular network during ageing, by direct measurement of vessel area, that ensures a more independent approach.

Methods

Patient selection. Among all the subjects referred to our Department for a cardiovascu-

lar examination between January 1999 and July 1999, we enrolled 68 volunteer healthy subjects aged 8-78 years (32 males, 36 females; body surface area 1.68 ± 0.23 m^2), with normal arterial blood pressure (systolic blood pressure 118.7 ± 12.1 mmHg; diastolic blood pressure 75.8 ± 8.3 mmHg), non-diabetic, non-smokers, with visual acuity > 8/10 and without retinal abnormalities (grade 0 of fondus oculi according to Keith-Wagener-Barker scale).

Retinography. All subjects underwent a bilateral non-mydriatic retinography (Topcon TRC-NW3, Topcon Corporation, Tokyo, Japan). Images were captured using an analogic camera (Topcon MT-1), set in order to obtain reproducible images, on a color slide film (Kodacrome 100 ASA, Eastman Kodak Company, Rochester, NY, USA).

Image analysis. Images, one for each subject (right or left eye randomly chosen), were digitized by a scanner (Polaroid SprintScan 35/LE, Polaroid Corporation, Cambridge, MA, USA) in standard RGB mode with a 1200 pixel/inch resolution onto a personal computer (Power Macintosh® G3 av, 300 MHz, RAM 136 MB, HD 8 G, Apple Computer, Inc., Cupertino, CA, USA) and saved in Tagged Image File Format (TIFF). In each image the region of analysis (partial region of interest-ROI, average surface 206 073 ± 31 278 pixels), corresponding to the 25% of the whole fundus image (total ROI), was set in a circular area concentric with the optic disk. The algorithms of analysis were developed in our laboratory as a set of macros written in Pascal. The macros were executed with NIH Image, a previously described[16] integrated image processing software, distributed on a freeware basis by the National Institute of Health. Arteries and veins were recognized by visual judgment on the original picture; the visible vessels inside the optic disk were also included. From each image we obtained: a) the area corresponding to arteries and veins expressed as partial ROI percentage (%AA, %VV, respectively); b) the mean of arteriolar bifurcation angles (ω) resulting from three consecutive measurements along the superior temporal retinic artery. %AA, %VV and ω were taken as microvascular retinal density indexes and they were correlated with age and body surface area. All measurements were determined in a blinded way by two operators and by the same operator twice on two different occasions. In 15 randomly chosen subjects vascular density was measured both in partial and total ROI, in order to verify whether vascular network obtained from the partial ROI analysis is representative of fundus oculi vascularity (Fig. 1).

Statistical analysis. Data were analyzed by a statistical software (SPSS - Rel. 6.1.1), and expressed as mean ± SD. Associations between parameters were investigated using linear regression analysis. Reproducibility of the method was expressed as coefficient of variation. A p value of < 0.05 was considered statistically significant.

Results

Retinography was well tolerated by all subjects; the average examination time was 15 min/patient.

Quantitative data derived from fundus oculi analysis are reported in table I. Inside the partial ROI, %AA and %VV resulted 7.44 ± 1.25 and 7.55 ± 1.14%, respectively; ω was 75 ± 16°. %AA and ω showed a significant correlation with age (Fig. 2), but not with body surface area. No significant differences between sexes were found. We observed a progressive reduction of ar-

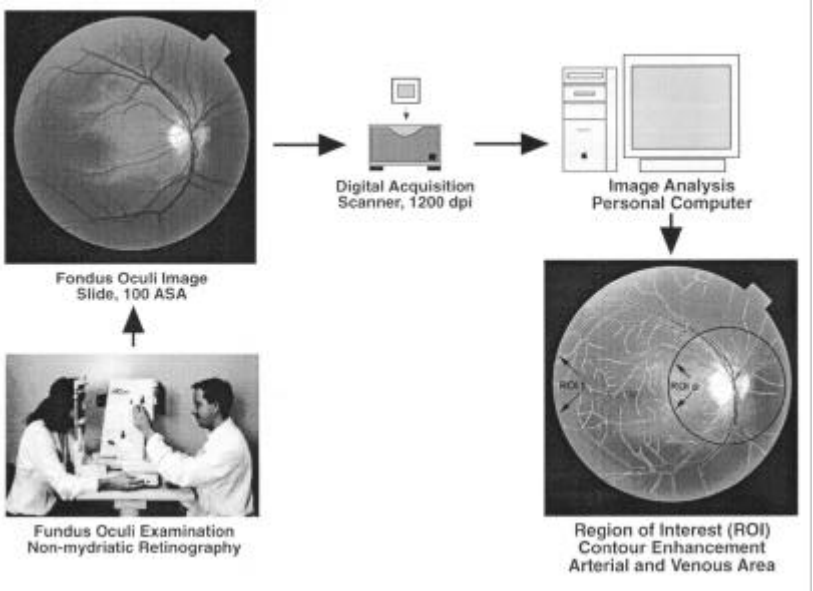

Figure 1. *Quantitative analysis of fundus oculi. The procedural steps from the retinography to the computerized analysis are reported clockwise. ROIp = partial region of interest; ROIt = total region of interest.*

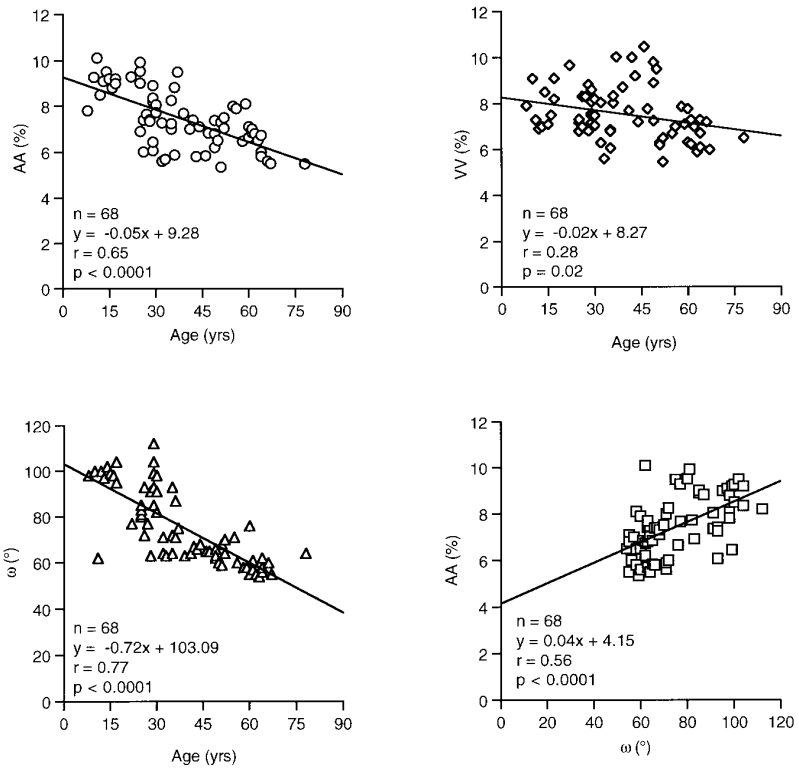

Figure 2. *Changes in retinal vascular network during ageing. AA = arterial area; VV = venous area; ω = arteriolar bifurcation angle.*

Table I. Quantitative analysis of retinal vascular network.

	Pixels	% partial ROI
Partial ROI	201 985 ± 33 805	100
OPA	22 383 ± 3790	11
AA	15 042 ± 3840	7.44 ± 1.25
VV	15 339 ± 3661	7.55 ± 1.14
AA + VV	30 380 ± 6978	14.9 ± 1.87
AA/VV	0.99 ± 0.17	0.99 ± 0.17
ω*	75 ± 16	

AA = arterial area; OPA = optic papilla area; ROI = region of interest; VV = venous area; ω = arteriolar bifurcation angle. * expressed in degrees.

terial area (0.05% per year) and arteriolar bifurcation angle with age.

Partial and total ROI vascular density were significantly correlated in both arterial and venous components (%AA: r = 0.78, y = 2.98 + 0.63 , p < 0.01; %VV: r = 0.69, y = 2.58 + 0.68 , p < 0.001).

Intra- and interobserver coefficient of variation resulted 2.5 and 3.2%, respectively.

Discussion

Fundus oculi provides a unique window to microcirculation useful for the clinical evaluation of target organ damage in systemic diseases like arterial hypertension and diabetes mellitus. Several studies have shown that quantitative retinal vascular alterations, not detectable by traditional examination, may be demonstrated by computerized analysis of optical fundus[13,15]. A recent study has reported a decrease in retinal arterovenous ratio associated with higher blood pressure values by measuring the cross-sectional diameters of the retinal vascular tree in a reduced region of analysis concentric with the optic disk[4]. In this study we observed a significant correlation between the extension of the retinal vascular network and age. The ageing process is associated with a retinal arterial reduction of 0.05% per year. This result is in agreement with arteriolar bifurcation angle reduction during ageing, as already observed by other authors[8,9].

The area under study, that was limited to a small part of fundus oculi in order to reduce the examination time, is however representative of total vascular density, as it includes the origin of the main retinal arteries[17,18]. Furthermore the selection of a limited ROI, concentric with the optic papilla, ensures a greater reproducibility of the region of analysis than other proposed methods.

In conclusion this technique, that requires a nonmydriatic retinograph and a certain skill to take the picture, presents the advantage of being suitable to any personal computer. Moreover it is simple to perform (mean time of execution 15 min/patient), does not need pupillar dilation or the use of fluorescein contrast, and has been proven to be reproducible and capable of iden-

tifying retinal vascular network alterations due to ageing.

Chronic pathological conditions like arterial hypertension, diabetes mellitus and atherosclerosis are known to affect arterial microcirculation; whether or not they could accelerate the process of rarefaction of retinal vessels due to age and modify the relation observed between arterial vascular density and ageing has not yet been demonstrated.

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