Ethnic variability of the vasculature of the optic disc in normal and in glaucomatous eyes

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PURPOSE. *Although disc pallor is associated with glaucoma, the structural macro-anatomy of the disc vasculature has received little attention, and possible ethnic differences have not been considered. Accordingly we studied the distribution of blood vessels crossing the rim of the optic disc.*
METHODS. *Thirty normal controls, and 50 glaucomatous cases with a unilaterally impaired visual field were studied. The sample populations included white Caucasian, and African and Afro/Caribbean volunteers. A b/w photographic method of imaging the blood vessels crossing the rim of the optic disc was used, the illuminant being green. The prints used in the analysis had been masked during their exposure to enhance contrast. The numbers of vessels, grouped into large (~60nm), medium (~30nm), and small (~10nm) lumina, were counted, the disc images being divided radially into eight equal sectors.*
RESULTS. *While the large vessels crossed the rim mainly along the vertical, the small ones did so mainly in the horizontal. The distribution of the medium vessels was unpolarized but their crossings predominated on the nasal side. The vessel patterns differed significantly between the two ethnic groups as regards both number and distribution along the rim of the disc, the smaller vessels being more numerous in Caucasian eyes (p~0.02). Rim crossings by vessels were smaller in glaucomatous eyes in both ethnic groups. In normal eyes there was a statistically significant age-related decline in the number of small vessels after the age of 20 years.*
CONCLUSIONS. *There exists a statistically significant inverse relation between the number of capillaries crossing the disc rim and the vertical cup/disc ratio. Caucasian rims show the larger number of capillaries crossing. (Eur J Ophthalmol 2004; 14: 501-507)*

KEY WORDS. *Disc vasculature, Ethnic differences, Vascular ageing, Vertical cup/disc ratio*

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INTRODUCTION

Although the colour of the optic disc is also used as an aid to the diagnosis of glaucoma (1), and a departure from the norm of the vasculature is likewise informative (2), remarkably little attention appears to have been paid to the discrete form of the vascular system of the living human optic disc. Kestenbaum (3) seems to be the only investigator to have concerned himself with the number of disc vessels; in point of fact, he had considered the problem in the 1948 edition of his book. However, his pioneering attempts at counting vessels were based on ophthalomoscopy as distinct from photography or other methods of recording the

Presentations of partial sections of the subject were made at the meeting of European Vision and Eye Research (EVER) in Alicante on the 12 October 2001, and at the Meeting of the Glaucoma Society of the International Congress of Ophthalmology, Chantilly 28-31 May 2003

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retinal image, and this led him inevitably to underestimate the number of existing vessels.

We were led to return to the question by a serendipitous observation of African and Afro-Caribbean discs: cursory, though repeated, observations suggested that their vasculature lacked the apparent symmetry of Caucasian discs. There was, therefore, a need to quantify this casual impression, if possible, and to relate it to patients and controls in both ethnic groups.

A preliminary study was designed to determine the optimal method of capturing the information. Neither colour photography nor the scanning electronic ophthalmoscope yielded as much detail or contrast as was to be achieved with traditional black-and-white photography, modified by the use of a really red-free filter (4). The word "really" needs stressing: current instruments are marketed as being provided with red-free filters, which, when tested, are found, lamentably, to fulfil this claim.

It was also necessary to try and classify the apparent widths of the blood vessels. Kestenbaum had counted all the visible vessels. It was, however, unlikely that the major vessels would undergo any variation either with disease or ethnicity. The vessel diameters were therefore divided into three groups that were relatively easy to identify visually (5): they were marked as large (L: ~60nm), medium (M: ~30nm) and small (S: ~10nm).

METHODS

Subjects

Permission to conduct the study having been obtained from the relevant Ethical Committees both at Moorfields Eye Hospital and University College London Hospital, each patient was informed of the nature of the study even if photography of the disc formed part of the clinical routine. The pupils of the eyes to be photographed were dilated with one drop of 1% tropicamide which is not known to have any effect on the disc vasculature.

Patients included in this study had previously been diagnosed as having primary open-angle glaucoma, based on the appearance of the optic disc, intra-ocular pressure over 21mmHg, and field loss in one eye only. Use was made of threshold perimetry of the central area. This was done with a Humphrey perimeter using Statpac 2 with the specific artificial intelligence system for testing the glaucoma hemifield. This allows a sensitive and specific determination of a glaucomatous field loss (6,7). The patients' eyes were identified as full field (FF) and glaucomatous with a reduced field (Gl) respectively.

The control group included only subjects free from any known ocular pathology, and were not being treated for any known systemic disease. Potential participants without clear media were excluded from the study.

The numbers and types of subjects were as follows:

	Patients	Controls
African and Afro-Caribbean	13 M, 5 F	7 M, 3 F
Caucasian white	14 M, 18 F	11 M, 9 F

Photographic procedure

Photography and processing were carried out by the two Hospitals' professional ophthalmic photographers. The principal factor requiring mention is the use of the gelatine filter: it was a green-pass Lee Filter equivalent to Rosco Supergel No. 389 with a peak (78%) transmission at 500nm and half this value at 480 and 540 nm. In other words, it was far superior to so-called green-pass filters provided with many present-day anterior segment cameras. The film used was Ilford FP4 Fine-grain (ASA125). Stock solution of ID11 developer was used with enhanced development time to optimize contrast. The magnification of the prints was ~15. Automatic printing was out of the question as this leads to the optimisation of the appearance of the retina with the usual over-exposure of the optic disc. To avoid this, the optic disc was partially occluded with an opaque fuzzy (i.e. unfocussed) disc-shaped mask so that the contact print might exhibit effectively equal exposures of the disc and the surrounding region (8). This maximized the detectability notably of the S vessels. The print paper was Kodak Kodalith Type 3, and processing was done automatically with an Ilfospeed 2 Automatic Processor.

Analysis of records

In order to analyze the records two transparent masks were drawn and photographed. They consisted of cir-

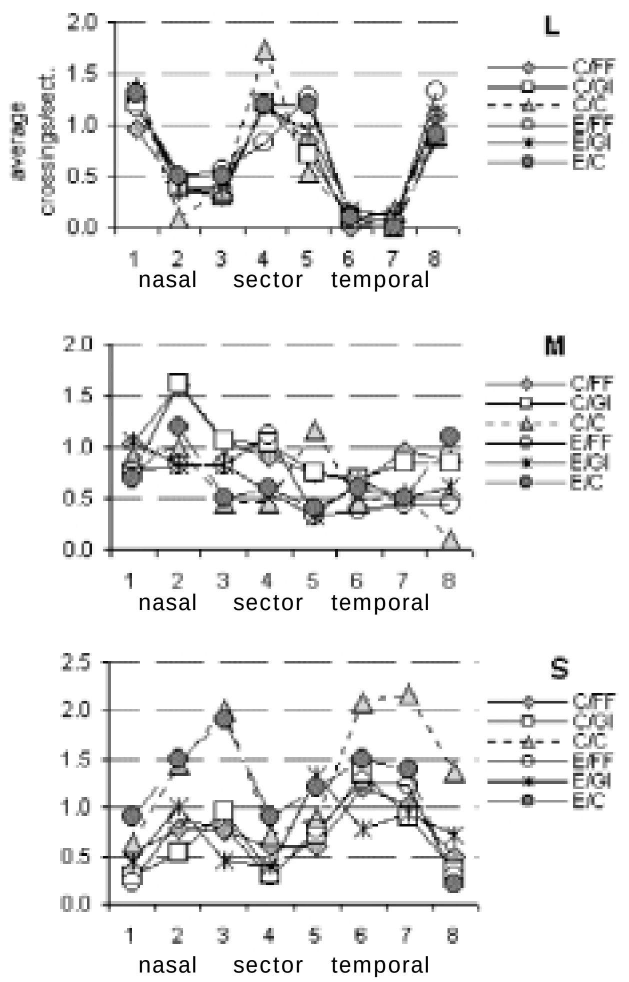

Fig. 1 - *The average number of crossings of the disc rim (vertical) as a function of the retinal sector (horizontal) centred upon the disc. C = Controls; N = normal field; Gl = Glaucomatous field. C: Caucasians; E: Africans/Afro-Caribbeans.*

cles, 5 cm in diameter, divided into 8 equal sectors, numbered clockwise and anticlockwise for right and left eyes respectively. Hence sectors 1-4 referred to the nasal side of the disc, and 5-8 to the macular or temporal side. The vessels crossing the rim of the disc were marked in each case accordingly as they belonged to class L, M, or S. The transparent masks were overlaid on each print in turn, and carefully centred on the disc prints, which were approximately 3cm in diameter. The number of vessels crossing the rim of the disc was counted for each sector, and noted. Counting of vessels was done by a very experienced observer (V.A.>6/5) who had seen neither the patients

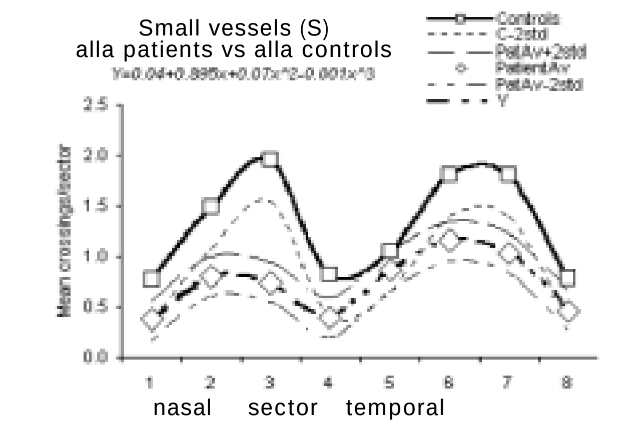

Fig. 2 - *Comparison of average S-counts per sector for controls and patients with confidence limits. Note that Y relates to the data represent by diamonds.*

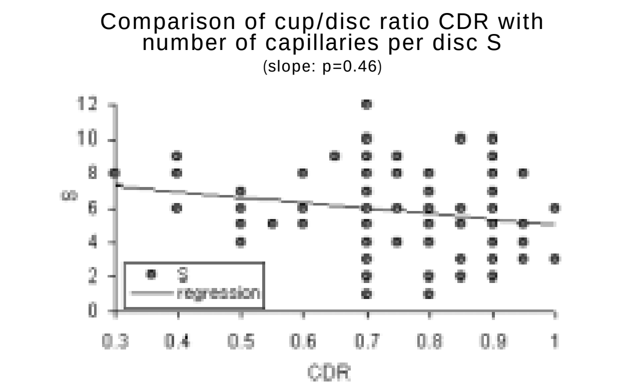

Fig. 3 - *The relation between S-counts per disc and age. The decline after the age of 20 years is significant (p = 0.02).*

Fig. 4 - *The relation between S-counts per disc and the cup/disc ratio CDR. The slope of the regression differs from zero (p = 0.033).*

nor the controls. The participants were identified by numbers, and logged as such on a spread-sheet where the data were entered for analysis. Estimates of the patients' field-loss, if any, and their cup-disc ratios were copied from existing notes in order to discover any potential relation between the photographic and the clinical data.
The data were subjected to a statistical analysis with the EXTEL package, and involved t-, F-, and Chi-tests, together with assessments of correlation coefficients; the statistical significance of the latter was read off Geigy Tables.

RESULTS

Counts of vessels

Figure 1 gives a global view of the averaged results for the Caucasians and the Africans and Afro-Caribbeans. The data for the large vessels (L) are at the top, those for the medium diameters (M) in the centre, and those for the smallest vessels (S) at the bottom. The symbols identify not only the ethnic group, i.e. Caucasian (C) and African/Afro-Caribbean (E), but also controls (C), and the eyes of patients (the eyes with full (FF) and those with impaired fields (Gl)). The abscissae give the average numbers of vessels per sector, numbers 1-4 lying on the nasal side, and 5-8 on the temporal (or macular) half. Note that the true vertical axis runs from half-way between 8 and 1 to halfway between 4 and 5. These radial distributions show the L vessels to leave the disc rim mainly at its top and bottom, irrespectively of ethnicity. The distribution of the S vessels is, broadly speaking, mainly orthogonal to that of L, whereas the M vessels are much less polarized. However, they predominate significantly on the nasal side ($p=0.03$). Table I shows the high negative correlations between L and S; the lower values for the Ethnics are partly attributable to the smaller number of patients. All the participants listed under Methods above were involved.
An analogous comparison of the two populations as regards similar vessels with type of eye as a parameter is shown in Table II, with similar limitations. In this case only participants over the age of 40 years were involved, with 11 Caucasians and 10 Ethnics.
The t-tests in Table III relate to comparisons between the sums of the means for all eight sectors, eye for eye, grouped according to type of vessel, with type of population and type of eye as subsets. As might be expected the situation regarding the large vessels (L) is similar in all cases. Again, all participants were involved. The control data for S for both populations were more numerous than for the patients, no matter whether the fields were normal or not. This is illustrated in Figure 2 which contains not only the averages but also plots for means ±2 x standard deviations for the patient data, and means –2 x std for the controls; there is a negligible, if any, overlap, and the two sets are found to differ, $p<0.0013$.
Table IV compares the nasal with the temporal (macular) sides. The test results for the total numbers of vessels are shown in the bottom three lines. Caucasians, but not Ethnics, show a significant correlation *r* between the two sides, thus bearing out the validity of the original observation which gave rise to this study. The F-test result compares the nasal sides of the two groups (columns 3 and 5), and also the temporal sides (columns 4 and 6), confirming that the distributions of vessels in the two halves of the disc differ significantly as between one group and the other. The result of the chi-test shows that the overall distribution of all the vessels differs greatly in the two ethnic groups.

Comparison with clinical data

Visual fields

Percentage losses, appearing in field plots recorded at the time the vessels were photographed, were assessed for the two upper and two lower quadrants. Correlations were calculated as between those values and results for S vessels in neighbouring sectors, taken two at a time, to reduce the variability due to the relatively small numbers in each. The only statistically significant correlation ($r = -0.3918$) between the number of S vessels and field loss was found as between sector 4 and the lower temporal field (Figure 4; $p<0.05$). A study of much larger numbers would be needed to explain this observation.

The cup/disc ratio

Figure 4 shows the plot of counts of all the capillaries crossing a disc rim against the appropriate (ver-

TABLE I - CORRELATIONS BETWEEN VESSEL SIZES FOR THE THREE GROUPS OF EYE IN EACH OF THE TWO POPULATIONS.

	Caucasians			Ethnics		
Vessels	**Controls**	**FF**	**GI**	**Controls**	**FF**	**GI**
L/S	**-0.809**	**-0.884**	**-0.889**	-0.555	**-0.828**	-0.304
	P<0.03	P<0.01	P<0.01		P<0.05	
S/M	-0.394	-0.129	0.039	0.074	-0.488	-0.599
M/L	-0.394	-0.089	-0.210	-0.347	0.032	0.109

Significant values are here shown bold
FF refers to the eye with an unimpaired visual field,
GI to the eye with a reduced field

TABLE II - CORRELATIONS BETWEEN THE TWO POPULATIONS.

	Caucasians	Ethnics	
Vessels	**Controls**	**FF**	**GI**
L	0.7449	**0.8785**	**0.9818**
p	0.051	<0.01	<0.001
M	-0.0484	0.5442	0.3245
S	0.5507	**0.8979**	0.2468
p		<0.008	

Significant values are here shown bold
For key see Table I

TABLE III - P-VALUES FOR T-TESTS FOR THE SUMS FOR ALL EIGHT SECTORS.

L	C/C	C/FF	C/GI	E/C	E/FF	EGI
C/C		0.860	0.849	0.853	0.808	0.882
C/FF			0.987	0.683	0.634	0.709
C/GI				0.672	0.622	0.697
E/C					0.949	0.965
E/FF						0.913
E/GI						
M	C/C	C/FF	C/GI	E/C	E/FF	EGI
C/C		0.055	0.082	0.704	0.954	0.809
C/FF			0.855	0.075	**0.034**	**0.034**
C/GI				0.117	0.057	0.061
E/C					0.711	0.843
E/ff						0.931
E/GI						
S	C/C	C/FF	C/GI	E/C	E/FF	EGI
C/C		0.020	0.014	0.431	**0.021**	**0.023**
C/FF			0.654	0.058	0.845	0.963
C/GI				0.043	0.846	0.655
E/E					0.066	0.071
E/FF						0.829
E/GI						

Significant values bold: C = Controls; FF=Normal field;
GI= Glaucomatous field; C: Caucasians; E: Ethnics (see also Table I)

TABLE IV - COMPARISON OF THE TWO VERTICALLY DIVIDED HALVES OF THE DISC: THE FIGURES REPRESENT TOTAL COUNTS FOR GLAUCOMA PATIENTS AND CONTROLS.

		Caucasians		Ethnics	
		nasal	**temporal**	**nasal**	**temporal**
FF	L	92	64	55	50
	M	146	104	64	29
	S	85	107	39	64
GI	L	101	54	59	42
	M	154	100	59	41
	S	67	106	41	68
C	L	39	19	35	22
	M	31	25	30	26
	S	53	72	31	27
	r	0.681	p=0.05	0.004	n.s.
	F-test		0.5319		0.0139
	chi (C>4			7E-170	

FF refers to the eye with an unimpaired visual field, GI to the eye with a reduced field ; C: controls.

TABLEV - CORRELATIONS BETWEEN THE VERTICAL CUP/DISC RATIOS AND THE NUMBER OF SMALL DISC VESSELS PER DISK

Caucasians	**-0.2836**	p =	**0.044**
Afr/Afr-Car	-0.1196		n.s.
All	**-0.2183**	p =	**0.046**

tical) cup/disc ratio. The two quantities vary inversely with each other as set out in Table V. The data for the Caucasian patients show a significant negative correlation; that for the African/Afro-Caribbean patients is not statistically significant – perhaps on account of their smaller number – but the overall data show a statistically significant r.

DISCUSSION

The present study is concerned with an assessment of the number of different types of blood-vessel crossing the rim of the disc in two ethnic groups both in glaucomatous eyes and in controls. As such the object is anatomical rather than haemodynamic. Diffuse narrowing of the retinal vessels is found in glaucomatous and non-glaucomatous optic neuropathies.

In glaucoma the vessel diameter narrows with the decreasing area of the neuroretinal rim. A generalized reduction of the lumen is typical of optic nerve damage, but not characteristic of glaucoma; this suggests that the vessel reduction did not cause the glaucomatous optic nerve fibre loss, but may have resulted from a reduction in metabolic requirements following the loss of fibres (9).

Fluorescein angiography has shown an increased incidence of fluorescein filling defects in the optic nerve head in open-angle glaucoma as compared with normal eyes (10). Although such defects are also noticed in anterior ischaemic optic neuropathy, they are generally absent in nonglaucomatous optic atrophy (11). The extent and location of the filling defects tallied with predictions of their location based on focal visual field defects, and also with the severity of the glaucomatous field loss (10-13).

The filling defects are mostly located peripherally near the superior and inferior poles of the disc (10); and are more specific when located in the cup wall (14).

In the present study we have noted that both ethnic groups exhibit an inverse relation between the number of disc rim crossings of the major and the smallest vessels respectively. This is in keeping with the view that a high oxygen tension is not compatible with capillarogenesis (15). Both groups also exhibit a radial asymmetry in the crossings of the medium sized vessels M, the numbers tending to be larger on the nasal side (p =<0.03). Overall, the Caucasian discs show a greater symmetry than is observed for the others (Tab. IV).

Table III which compares all groups with one another, shows 5 significant difference in the section dealing with the capillaries. This would occur by chance with a probability of 0.026, and is, therefore, probably due to a real effect.

Only the S-values of the controls exhibit a correlation with age (Figure 3). The capillary vasculature appears to reach its optimum at maturity, and declines thereafter in both test groups. The rate of loss is similar to that observed for other capillaries. The number rises as adulthood is reached, and then drops with a normalized slope of -0.0053 p.a. This is not very different from the rate of loss of foveal capillaries (5). An age-related capillary loss may be a widespread phenomenon (1). On the other hand, the avascular zone of the retinal centre may be affected by prematurity (16): as compared with the norm, it is smaller in premature and low-birthweight babies, presumably because apoptosis of the capillaries occurs late in term. The disc capillaries do not appear to have been studied in this context so that a possible link between this condition and adult glaucoma has been left unexplored. It is, however, worth noting that a longitudinal study of ocular hypertension patients (17) has shown a link between the development of progressive glaucomatous atrophy and progressive changes in the vascular supply, associating increases in pallor area (with or without field defect) and delays in venous filling and also increases in the area with filling defects (4). The loss of capillaries in controls may be linked to the normal progressive age-related loss of optic nerve fibres (18).

The loss of capillaries relates to other clinical observations. It allows more white light to be reflected from the collagenous portion of the nerve head. Figure 4 is consistent with the observation that pallour increases with age. Also the maximum colour contrast generally coincides with the area of cupping. But in early glaucoma the area of pallour tends to be smaller than the area of cupping (18).

The number of capillaries varies inversely with the cup-disc ratio which makes it suitable as an additional criterion in the armament for diagnosing the risk of glaucoma, particularly when it comes to screening younger persons.

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