5-1988

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Abstract

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Degree Type

Thesis

Degree Name

Master of Science in Vision Science

Committee Chair

Subject Categories

Optometry

RETINAL NERVE FIBER LAYER PHOTOGRAPHY AS AN INDICATOR OF NERVE FIBER INTEGRITY IN OCULAR HYPERTENSIVE SUBJECTS

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A thesis submitted to the faculty of the
College of Optometry
Pacific University
Forest Grove, Oregon
for the degree of
Doctor of Optometry
May, 1988

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ABSTRACT

To evaluate the usefulness of retinal nerve fiber layer (RNFL) photography in the estimation of nerve fiber layer integrity in ocular hypertensive subjects; we examined 89 eyes of normal (59 eyes, 48 subjects), ocular hypertensive (26 eyes, 14 subjects), and glaucomatous (4 eyes, 2 subjects) patients. Preliminary studies included verification of the Statpac program for the Humphrey field analyzer, and establishing the method of nerve fiber layer photography sensative enough to detect nerve fiber layer loss. The main study matched 14 ocular hypertensive subjects with 14 normal subjects. The subjects were matched by age (+/- 5 years), sex, and race. It was found that 2 of the 59 total normal eyes (3.4%) [2 of the 48 normal subjects (4.2%)]; 2 of the 15 matched normal eyes (13.3%) [2 of 14 matched normal subjects (14.3%)]; and 2 of 24 assessable ocular hypertensive eyes (8.3%) [2 of 13 subjects (15.4%)] showed suspected focal nerve fiber layer defects. None of our normal or ocular hypertensive subjects showed diffuse nerve fiber layer loss. We feel that retinal nerve fiber layer photography is a valuable indicator of nerve fiber layer integrity.

INTRODUCTION

Recent research has indicated that in the early stages of glaucoma, up to fifty percent (50%) of the optic nerve fibers, either diffusely or in a given area, may be damaged before standard visual field tests detect the classical glaucomatous defects.[1] Retinal nerve fiber layer (RNFL) abnormalities may occur as early as five years prior to the development of visual field defects.[2] Retinal nerve fiber layer photography may be a more sensitive indicator of subtle nerve fiber loss in ocular hypertensive (OHTN) patients or glaucoma suspects.

Preliminary studies involved: 1) the establishment of the method of nerve fiber layer photography to detect fiber loss and 2) verification of the newly designed StatPac program for the Humphrey Visual Field Analyzer (Allergan-Humphrey, Instrument Division, 3081 Teagarden, San Leandro, CA 94577). Statpac is a sophisticated statistical program which allows the clinician to compare a current patient's visual field with an age-matched population of normal visual fields. This program also has the ability to statistically analyze a given patient's visual field over time. Thus, when combined with clinical wisdom, the statpac program performs five functions:[3]

1) Statpac single test analysis assigns a point probability level to each tested threshold value within a patient's visual field by comparing it with an aged-matched normal population. The assigned probability level is the percentage of the normal population which would show such a value (eg. a 1% p-value would be seen in 1 out of 100 normal fields for that age). The probability levels are made on a "point-by-point" basis and can be read like an isopter plot.

2) Statpac adjusts the analysis of the raw test results for any changes in the height of the measured hill of vision (e.g.,a systematic overall depression produced by a cataract) to reveal hidden patterns of visual field defect.

3) Statpac provides reliability messages (fixation losses, false positive errors and false negative errors) so that proper weights can be given to the validity of the results.

4) Statpac provides global indices (with probability values when applicable) so that the clinician can analyze the entire visual field and hill of vision (in contrast to numbers 1 and 2 above).

5) Statpac analyzes results from a series of tests to assess and plot visual field changes over time.

The StatPac program would thus aid in the discrimination of probable true visual field loss from artifactual or expected age-related loss.

Subjects were obtained from the Ocular Disease and Special Testing Clinic at Pacific University College of Optometry clinic located in Forest Grove, Oregon and the Portland Community College Clinic. Other subjects were refered in from a local optometrist. First, visual field analyses were run on subjects without known previous field defects to determine whether StatPac verified no loss of field. Visual field analyses were also run on subjects with previously determined field defects to determine whether StatPac appropriately assigned a high probability of decreased sensitivity to the fields (i.e., a low pobability of occurance in the normal population). Second, a small sample of both normal and known glaucoma patients exhibiting nerve damage and visual field loss were photographed to test the ability to detect nerve fiber layer dropout using the Spectrotech SE-40 fluorescein exciter filter and the Spectrotech BPB 53 red-free filter on the Topcon TRC-50VT (Topcon Instruments Corp. of America, 65 W. Century Rd., Paramus, N.J. 07652) camera in conjunction with black and white Kodak Technical Pan 2415® film (Eastman Kodak Co., Rochester, N.Y. 14650).

The main study compared a sample of fourteen (14) ocular hypertensive subjects, who by definition have intraocular pressures of greater than 21 mm of Hg without statistically significant visual field loss as determined by Humphrey 30-2 visual field and Statpac analysis, with fourteen (14) subjects possessing intraocular pressures of less than or equal to 21 mm Hg, physiological cup to disc ratio of less than or equal to 0.6, and no statistically significant visual field defects as determined by the Humphrey 30-2 visual field and StatPac analysis. On each subject, a series of retinal photographs was taken. Monochromatic photographs provided maximum nerve fiber layer visibility. Additionally, color and stereo photographs were taken for optimum optic nerve cupping evaluation. The subjects were matched by age (±5 years), race, and sex to determine what differences in the appearance of the nerve fiber layer exist between the two populations.

Photographs and visual fields taken became part of a patient's permanent clinical record and can be used to note future changes in nerve fiber pattern or visual fields over an extended period of time. Sensitive analysis of ocular health can result in the initiation of earlier treatment in cases exemplifying nerve fiber loss.

Clinical usage of monochromatic light to view fundus detail was first demonstrated by Volk in 1925 with red-free ophthalmoscopy.[4] The benefit of utilizing monochromatic light to enhance retinal detail in photography has become increasingly evident during the last 20 years.[4] Poor image quality has previously led to limited clinical acceptance of spectral reflectance nerve fiber layer photography, inspite of systematic studies as early as 1940.[4] The photographs obtained have been improved with the introduction of Kodak Technical Pan 2415® film in 1982. Technical Pan is a high contrast, fine grain film yielding an increased resolution of 400 lines per millimeter, resulting in high resolution of nerve fiber layer images.[5] In a study involving over 1400 eyes diagnosed as either glaucomatous, ocular hypertensive or normal; retinal nerve fiber layer photographs of good quality confirmed the diagnosis of glaucoma in approximatedly ninety percent (90%) of the cases, with only seven percent (7%) of the normal cases mistakenly thought to possess an abnormal nerve fiber layer pattern.[6] Delori and Gragoudas, in a 1976 study of monochromatic light and examination of the ocular fundus, concluded that standardized monochromatic photography and ophthalmoscopy would be indispensible for making clinical comparisons over time.[7] Currently, monochromatic spectral reflectance photography for nerve fiber layer comparisons is most actively used in major universities and other research facilities.

METHODS

The StatPac program of the Humphry Visual Field Analyzer was validated for use in this study by taking patients who had previously had a central 30-2 full threshold visual field performed. Visual fields chosen for this validation included both those with significant visual field loss and diagnosed corresponding disease and those with no known pathology and apparent normal visual field from files of the Ocular Disease and Special Testing Clinic in Forest Grove, Oregon. Analysis of the type and frequency of the probability symbol(s) assigned for each visual field (if any) was performed. The StatPac program of the Humphrey Visual Field Analyzer was found to be sensitive in separating apparently clinically normal patients from those patients with visual field loss (eg. glaucomatous visual fields). Results are summarized in Table 1.

A sample of fourteen (14) ocular hypertensives, 4 males and 11 females between the ages of 17 and 62, exhibiting intraocular pressures greater than 21 mm Hg measured by Goldmann tonometry were matched by age (±5 years, Table 2), race, sex and cup to disc ratio to subjects with intraocular pressures less than or equal to 21 mm Hg and normal Humphrey 30-2 visual fields.

Since the standard of care up until recently differentiated glaucomatous patients from ocular hypertensives by detectable visual field loss, each subject first had a central 30-2 full threshold visual field performed to ensure that they were indeed ocular hypertensives having no statistically significant visual field loss (p= 0.05). Fields showing points with an assigned probabilty value were rechecked for reliablity using a custom field. Best corrected visual acuities were measured monocularly prior to the test to establish a 20/30 minimum requirement. Trial lenses were put in place to correct all distance refractive error >3.00 D myopia, any hyperopia, and >0.75D astigmia (equivalent sphere was used for cylinder correction ≤ 0.75 DC). Near plus adds were put in place according to the table by age provided in the Humphrey Visual Field Analyzer operation manual.[8] A standard instruction set was provided by the help menu of the program.[8]

Prior to dilation, anterior chamber angles were graded using the Van Herick technique. Intraocular pressures were measured by Goldmann applanation tonometry using one drop of Fluress (0.25% sodium fluorescein and 0.4% benoxinate HCL) administered to each eye. The diagnostic pharmaceuticals instilled to obtain dilation were one drop each of 2.5% phenylepherine and 1.0% tropicamide. An assessment of each fundus was made using direct ophthalmoscopy, binocular indirect ophthalmoscopy, and/or Volk 90 diopter indirect ophthalmoscopy.

The Topcon TRC-50 VT retinal camera was the instrument used for the entire series of fundus photographs. Color stereo disc photographs were taken at a 35 degree field of view on Kodak Ektachrome ASA 100 film with no filter in place, and flash intensity set at 25 watt-seconds. These photographs were used for evaluation of optic cup to disk ratio and neuroretinal rim area. Monochromatic nerve fiber layer photographs were taken at 35 degree and 50 degree fields of view through both the Spectrotech BPB 53 red-free filter (100 nm band width, centered around 530 nm) and the Spectrotech SE-40 Exciter filter (35 nm band width, centered around 495 nm)[9]. Kodak Technical Pan 2415® film was used, with flash intensity set at 100 watt-seconds. Post procedural intraocular pressures were recorded before the subjects were released.

Due to negative underexposure, undiluted Kodak D-19 developer was used to process the Technical Pan film. Negatives were developed for 10 minutes at a temperature of 70 degrees Farenheight. Positive prints were produced from the negatives, with the total cost of development being approximately $20 per roll of film. Direct examination of the negatives can save both time and expense. Fulk and Van Veen[5] concluded that direct examination of negatives yielded equally as effective or better evaluation of the retinal nerve fiber layer.

Upon return from processing, the photographs were initially viewed with a 20 diopter lens against a diffuse white backlighted background. This was done to choose which prints were to be made into slides; usually 2-4 slides were made for each eye out of approximately 12 taken. Formal assessment of the nerve fiber layer slides was performed using the procedure of Fulk and Van Veen.[5] Each slide is taped to the headrest of a slit lamp biomicrosope, and a external light source is used to backlight the slides. The advantages of this method are: variable magnification, moveable light source, and binocularity. Viewing was done by two observers. The intra-observer consistency was checked by having more than one slide for each eye. Thus, after the masked viewing of all the normals was completed, all photographs for one eye were compiled and the observers impressions were compared for that one person to ensure that a consistency was obtained within photographic quality limits.

Assessment was performed within 2 disc diameters of the disc, since the RNFL begins to thin variably at this point.[10] This can give the RNFL the appearance of feathering or fanning out, which should not be confused with real nerve fiber loss. All slides were then put into one of four categories:

1) RNFL within normal limits.
2) RNFL with diffuse loss of fibers.
3) RNFL with local loss of fibers (rake-like, or slit defects).
4) Slide not useable.

Analysis began by first viewing all of the nerve fiber layer slides from 59 normal eyes, 132 slides in all. Slides were viewed in masked fashion, without knowing the identity of the subject. The normal variations of the nerve fiber layer among subjects with no apparent ocular pathology were studied.

Next, analysis was performed on all ocular hypertensive subjects (14 subjects, 26 eyes) and their matched normals (14 subjects, 15 eyes). Viewing was done in a masked fashion, without knowing subject identity or clinical findings. Masking of the disc was not included due to the similar C/D ratio findings between the ocular hypertensives and the normal subjects (table 3).

RESULTS

The normal variations and their frequencies which were noted among our subjects are listed in Graph 1. Two types of variation were noted among our sample of 59 normal eyes:

1) "Fanning" of the RNFL: a normal thinning of the dense arcuate fibers usually beginning approximately 1.5 to 2 disc diameters (DD) from the optic disc.[10] Graph 1 divides this category depending on the location at which this variation is first noted (eg: 1DD to 2DD, and beyond 2DD).

2) Color variations: these most likely are due to either photographic factors (angle, flash intensity, sheen, field of view, etc.) or retinal topographic factors. Most commonly, these areas are noted between two large retinal vessels as they course from the disc; possibly casting shadows on the retinal surface. Without exception, fibers can be noted within these areas (assessment is helped by noting the fibers crossing over small blood vessels[5]).

It was found that 2 eyes of the 59 total normals (3.4%), [2 of 48 subjects (4.2%)] showed areas of suspected nerve fiber dropout (0 diffuse and 2 focal). These two eyes (two subjects) were among our 14 matched normals. Thus for our 14 matched normal subjects (15 eyes), 2 showed areas of suspected focal nerve fiber dropout (14.3%)[2 of 15 matched normal eyes (13.3%)]. Etiologies of these suspected areas of nerve fiber defects are not known.

Of the 26 ocular hypertensive eyes, 2 (8.3%), [2 of 13 assessable subjects (15.4%)] showed areas of suspected focal nerve fiber atrophy. No cases of diffuse atrophy were shown in our sample. One subject's RNFL photographs (both eyes) were judged to be unassessable due to a very light reticulated fundus which did not permit enough contrast to view the RNFL. All photographic results are summarized in Table 4.

DISCUSSION

The normal variations we noted are supported by previous studies. Fulk and Van Veen[5] noted that apparent slit-like defects which occur especially in persons with dark fundi are in fact normal variations of a striated retina. Quigley, et al[10] stated that pseudodefects occur in the dense arcuate fibers as these fibers start their normal fanning out at approximately 2 disc diameters from the optic disc. In a later study, Sommer and Quigley, et al[6] suggested that "by ignoring focal 'defects' entirely the false-positive rate can be reduced by almost two thirds without appreciably impairing the ability to recognize glaucomatous nerve damage". This same study also stated that the fine focal changes within the nerve fiber layer most likely represent variations of normal nerve fiber layer with little diagnostic or predictive value.

The fact that we had two (3.4%) normal eyes who showed very suspicious areas of suspected focal nerve damage coincides with earlier studies. Quigley, et al[10] noted that 2 of their 67 normal eyes (3.0%) showed abnormal nerve fiber patterns. Airaksinen, et al[11] had a higher rate of abnormal retinal nerve fiber layers, 5 of 29 normal patients (17.24%). This higher figure could be due to including variations beyond 2 disc diameters, which we (and originally Quigley, et al[10]) included in our normal variations. We attempted to minimize the false-positive rate by analyzing our photographs within 2 disc diameters, and also by assessing all of our normal photographs first to factor out normal variations when assessing our ocular hypertensive subjects. Among our matched normals, the percentage of suspected nerve fiber layer abnormality was 2 eyes out of 15 (13.3%) [2 of 14 subjects, (14.3%)]. This percentage is inconclusive due to our small sample. However the mean age of our matched normals is 44.3 years, while the mean age for all other normals is 28.7 years; thus age could be a factor.

Of our 26 ocular hypertensive eyes (24 with assessable photographs), 2 (8.3%) showed suspected focal nerve fiber layer loss, while none showed diffuse loss of nerve fibers. In comparison, Sommer, et al[6] with two observers, showed 10 and 22 abnormal nerve fiber layers out of 430 and 442 eyes respectively (2.3% and 5.0%). Airaksinen, et al[11] in an earlier study showed 27 abnormal nerve fiber layer patterns out of 52 ocular hypertensive eyes (51.9%). Both these studies showed ocular hypertensives with diffuse atrophy, which was figured in to the above listed totals.

The reason our rate of abnormality among ocular hypertensive subjects is low could be due to a variety of reasons. First, our sample size is small, and of our 14 ocular hypertensive patients only 0-9% can be expected to progress to develop glaucoma.[12] Secondly, as previously mentioned, we attempted to keep our false positive rate low by assessing the nerve fiber within 2 disc diameters of the optic disc. Also, our sample had no one with diffuse atrophy. Sommer, et al[6] stated that diffuse atrophy is present in glaucoma patients with only minor field loss (<u>using Goldmann visual fields</u>). Since our present study used hypertensives without visual field loss as indicated by the more sensitive static threshold Humphrey Field Analyzer, further studies with larger sample sizes will be needed to determine a relationship between diffuse atrophy and Humphrey visual field (30-2) status. Another reason our sample may have lacked any subjects with diffuse atrophy is that our sample had no subjects with noteable loss of neuroretinal rim tissue. Airaksinen and Drance[13] have shown a correlation between loss of rim tissue and diffuse nerve fiber layer atrophy.

Photographically, our best results were with the Spectrotech SE-40 Exciter Filter, which enhanced the nerve fibers much more than the Spectrotech BPB 53 red-free filter in the majority of cases. We also felt that having both 35 degree and 50 degree field of views was advantageous. The 50 degree field allows assessment of nerve fibers as they course distally from the optic disc, while the 35 degree field helps to assess those fibers closest to the disc. With either field of view, best results are obtained when the optic disc is centered within the field. This allows for the best overall illumination. We found that the 100 watt-second flash intensity setting was most appropriate for both of the above mentioned filters for the majority of subjects. However; in very lightly pigmented, reticulated fundi the flash intensity may need to be decreased. At times a polaroid photograph is helpful in determining the appropriate flash intensity. Proper focus on the nerve fibers themselves is essential. One must be able to view the retinal nerve fiber layer in white light, or photography will be ineffective.

Of our 14 ocular hypertensive subjects, 8 had lightly pigmented fundi. Of these 8, another 3 also had a reticulated pattern (one of which was not assessable). We agree with many other earlier studies[2,4,5,6,10,14,15] that assessment is made more difficult in these lightly pigmented eyes. Assessment is also difficult or impossible in eyes with media opacities or poorly dilated pupils (<6mm).[5,6] The optimum ages for best results is 20 to 40 years. Patients younger than 20 exhibit a fairly large amount of sheen off of the internal limiting membrane, while patients over 40 have more likelyhood of having media opacities, and generally poorer nerve fiber layer visibility. However, we did have 1 subject younger than 20, and 22 subjects over 40 whose nerve fiber photographs were assessed without difficulty.

In summary, we feel that retinal nerve fiber layer photography is a valuable indicator of nerve fiber integrity. More studies are needed to establish a relationship between the sensitivity of the modern threshold perimeters such as the Humphrey Field analyzer, and nerve fiber layer atrophy in a large number of ocular hypertensive, glaucoma suspect, and glaucomatous eyes. We also feel that our particular photographic system needs more glaucoma patients with established visual field loss (and thus nerve fiber loss) to establish the sensitivity of our photographic system. Nerve fiber layer photography is a techique which currently is greatly under-used by eyecare professionals. It is hoped that as more practioners become familiar with the procedure, and knowledgeable in the assessment of photographs, ocular hypertensive and glaucoma suspect patients can be managed at a new standard of care.

TABLE 1: STATPAC PROGRAM SENSITIVITY

TYPE OF STATPAC PROBABILITY SYMBOL (P-VALUE)
(Probability at which a given threshold value would be present)
(in the normal age-matched population)

NORMALS N=32 EYES	**5%**	**2%**	**1%**	**0.50%**	**TOTAL**
TOTAL # of p-values/field	36	5	1	0	42
MEAN # of p-values/field	1.125	0.156	0.0313	0	1.313
STANDARD DEVIATION	1.462	0.448	0.177	0	1.595
RANGE (min-max # of p-values/field)	0-5	0-2	0-1	N/A	0-5

ABNORMALS N=25 EYES	**5%**	**2%**	**1%**	**0.50%**	**TOTAL**
TOTAL # of p-values/field	136	62	43	59	300
MEAN # of p-values/field	5.64	2.48	1.72	2.36	12.00
STANDARD DEVIATION	4.707	2.917	2.319	4.949	11.913
RANGE (min-max # of p-values/field)	0-17	0-10	0-9	0-18	0-35

(SEE TEXT FOR FURTHER EXPLAINATION)

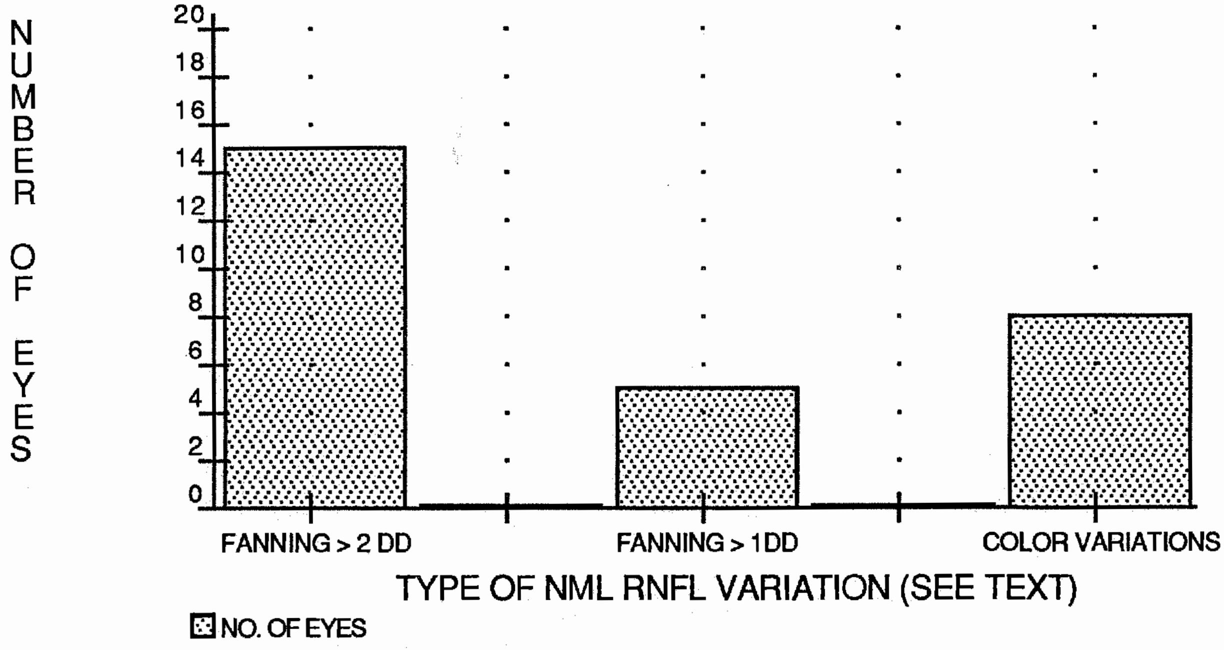
TABLE 2: AGES OF SUBJECTS AND MATCHED NORMALS

SUBJECT NUMBER	AGE	MATCHED NORMAL NUMBER	AGE
3	43	1	47
4	16	2	42
5	56	7	25
6	51	13	32
8	47	14	64
9	48	15	53
10	62	16	36
11	41	17	48
19	34	18	21
20	44	23	54
21	27	24	46
22	49	25	43
27	45	26	52
30	41	28	57
MEAN	43.14	**MEAN**	44.29

TABLE 3: CUP/DISC RATIOS: SUBJECTS AND MATCHED NORMALS

SUBJECT NUMBER	EYE	HOR C/D	VER C/D	MATCHED NORMAL #	EYE	HOR C/D	VER C/D
3	OD	0.15	0.15	1	OD	0.4	0.35
3	OS	0.35	0.3	1	OS	0.4	0.35
4	OD	0.2	0.2	2	OD	0.5	0.6
4	OS	0.2	0.2	2	OS	0.5	0.6
5	OD	0.4	0.4	7	OD	0.4	0.4
5	OS	0.3	0.4	7	OS	0.4	0.4
6	OD	0.45	0.4	13	OD	0.3	0.3
6	OS	0.35	0.35	13	OS	0.3	0.3
8	OD	0.35	0.4	14	OD	0.6	0.6
8	OS	0.35	0.4	14	OS	0.6	0.6
9	OD	0.65	0.75	15	OD	0.3	0.35
9	OS	0.4	0.4	15	OS	0.35	0.35
10	OD	0.25	0.25	16	OD	0.2	0.2
10	OS	0.25	0.25	16	OS	0.1	0.1
11	OD	0.4	0.4	17	OD	0.25	0.3
11	OS	0.5	0.5	17	OS	0.25	0.3
19	OD	0.4	0.4	18	OD	0.4	0.4
19	OS	0.3	0.3	18	OS	0.4	0.4
20	OD	0.4	0.4	23	OD	0.2	0.2
20	OS	0.35	0.35	23	OS	0.2	0.2
21	OD	0.2	0.2	24	OD	0.1	0.1
21	OS	0.1	0.1	24	OS	0.1	0.1
22	OD	0.65	0.65	25	OD	0.5	0.5
22	OS	0.6	0.65	25	OS	0.5	0.5
27	OD	0.4	0.45	26	OD	0.25	0.25
27	OS	0.3	0.3	26	OS	0.15	0.15
30	OD	0.15	0.15	28	OD	0.35	0.35
30	OS	0.15	0.15	28	OS	0.35	0.35
MEAN VALUES		**0.34**	**0.35**	**MEAN VALUES**		**0.33**	**0.34**
STANDARD DEVIATION		0.145	0.16	**STANDARD DEVIATION**		0.14	0.15
RANGE		0.10-0.65	0.10-0.75	**RANGE**		0.10-0.60	0.10-0.60

GRAPH 1: NORMAL VARIATIONS OF THE RNFL



1) "Fanning" of the RNFL: a normal thinning of the dense arcuate fibers usually beginning approximately 1.5 to 2 disc diameters (DD) from the optic disc.[10] Graph 1 divides this category depending on the location at which this variation is first noted (eg: 1DD to 2DD, and beyond 2DD).

2) Color variations: these most likely are due to either photographic factors (angle, flash intensity, sheen, field of view, etc.) or retinal topographic factors. Most commonly, these areas are noted between two large retinal vessels as they course from the disc; possibly casting shadows on the retinal surface. Without exception, fibers can be noted within these areas

TABLE 4: SUMMARY OF RESULTS

CATEGORY

	1 (RNFL WNL)	**2** (DIFFUSE LOSS)	**3** (FOCAL LOSS)	**4** (UNUSEABLE)
ALL NORMALS (N=59 EYES)	57 [96.6%]	0	2 [3.4%]	0
OHTN SUBJECTS (N=26 EYES)	22 [84.6%]	0	2 [8.3%]	2 [7.7%]
MATCHED NORMALS (N=15 EYES)	13 [86.7%]	0	2 [13.3%]	0

(NOTE: SEE TEXT FOR FULL EXPLAINATION OF SUBJECTS AND CATEGORIES)

ACKNOWLEDGEMENTS

The authors would like to thank the following individuals for their contribution to our study:

-Mark Evans and Pat Wallace of Oregon Health Science University Dept. of Ophthalmology-Photograpthy developed all of our nerve fiber layer photograpths, and gave us photographic advice.

-Greg Kautz, O.D. for referral of ocular hypertensive patients.

-Dori Carlson for putting up with our daily imposition on her work area.

-Shawna Dornan-Yount for helping us go through the files at the Portland Optometric Clinic.

This study was financed by the Pacific University College of Optometry research fund.

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