Assessing the Relationship between Central Corneal Thickness and Retinal Nerve Fiber Layer Thickness in Healthy Subjects

Authors: Tarkan Mumcuoglu,[1*] Kelly A Townsend,[1*] Gadi Wollstein,[1] Hiroshi Ishikawa,[1] Richard A Bilonick,[1] Kyung Rim Sung,[1] Larry Kagemann,[1] Joel S Schuman,[1] on behalf of the Advanced Imaging in Glaucoma Study Group[2]

Affiliations: [1]UPMC Eye Center, Ophthalmology and Visual Science Research Center, Eye and Ear Institute, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]www.AIGstudy.net

Corresponding Author: Joel S Schuman, MD, UPMC Eye Center, Department of Ophthalmology, University of Pittsburgh School of Medicine, 203 Lothrop Street, Eye and Ear Institute, Suite 816, Pittsburgh, PA 15213, Tel: 412-647-2205, E-mail: schumanjs@upmc.edu

* The first two authors had equal part in preparation of the manuscript.

Introduction

Within the last several years, large glaucoma and ocular hypertension multi-center studies such as the ocular hypertension study (OHTS) and European glaucoma prevention study (EGPS) have been established to determine significant risk factors and predictors for development of open-angle glaucoma.[1, 2] One of the risk factors that has been shown to be a powerful predictor of glaucomatous development is central corneal thickness (CCT).[1, 2] The mechanism for this relationship has been hypothesized to be related to the connection between corneal thickness and the overall inherent structural and elastic properties of the eye, which may determine its vulnerability to glaucoma. However, the fundamental physical reason for this relationship is still not fully known.

However, while this relationship has been examined in glaucomatous eyes and eyes with ocular hypertension,[1, 3-10] there is little information on the relationship between retinal nerve fiber layer thickness (RNFL) and CCT in healthy subjects. Evaluating this relationship in healthy eyes will eliminate the inevitable confounder when evaluating glaucomatous eyes, due to the inherent effect of the disease on the RNFL that cannot be discerned from the fundamental relationship between CCT and RNFL thickness.

The purpose of this study was to evaluate the relationship between CCT and RNFL thickness in healthy subjects. Several imaging modalities are currently available for evaluating the RNFL thickness: scanning laser polarimetry (SLP), confocal scanning laser ophthalmoscopy (CSLO), and optical coherence tomography (OCT). Because each method uses light in different ways, therefore employing different properties to determine RNFL thickness, we chose to use all three modalities to evaluate RNFL thickness.

Methods

The participants in the study were prospectively enrolled at four clinical sites, as part of the Advanced Imaging in Glaucoma Study (AIGS), a prospective longitudinal study. AIGS was designed to develop and evaluate glaucoma diagnosis using advanced ocular imaging technology. Full details on the study and the manual of procedure can be found at www.AIGStudy.net.

Testing

All subjects received a comprehensive ocular examination, including medical history, best-corrected visual acuity, manifest refraction, intraocular pressure measurement by Goldman applanation, gonioscopy, slit-lamp examination, pachymetry, axial length measurement, central corneal thickness measurement, visual field (VF) testing and imaging with SLP (GDx-VCC; Carl Zeiss Meditec, Dublin, CA), CSLO (HRT II; Heidelberg Engineering, Heidelberg, Germany) and OCT (Stratus OCT; Carl Zeiss Meditec, Dublin, CA). Subjects underwent pupillary dilation after VF testing, prior to imaging, with 1% tropicamide and 2.5% phenylephrine. Both eyes were used for the study if were qualified according to the criteria listed below.

Inclusion criteria were no history of ocular pathology, trauma or surgery other than uncomplicated cataract surgery at least a year prior to enrollment, best corrected visual acuity greater than or equal to 20/40, spherical equivalent between -7.0 and +3.0 diopters with cylinder power < 3 diopters, central corneal thickness greater than 500 μm, IOP less than 21 mmHg, open anterior chamber angle and normal appearing optic nerve head (ONH) and RNFL. Normal appearing ONH was defined as intact neuroretinal rim without splinter hemorrhage, notches, localized pallor, or asymmetry of the cupping > 0.2 between the eyes, accounting for the disc size.

All subjects had a reliable and normal Swedish interactive thresholding algorithm (SITA) standard 24-2 perimetry (Carl Zeiss Meditec, Dublin, CA). Reliable VFs had fewer than 30% fixation losses, false positive or false negative responses. A normal test was defined as one with mean deviation (MD) and pattern standard deviation (PSD) within 95% confidence limits of normal reference and glaucoma hemifield test (GHT) within normal limits.

CCT measurement was performed using ultrasound pachymetry (Pachette 2; DGH Technology, Exton, PA). CCT was measured as a mean value of multiple measurements automatically generated by the machine after obtaining adequate number of qualified measurements (up to 50 repetitive measurements). Axial length was measured using an ultrasonic A-scan device (IOL Master; Carl Zeiss Meditec, Dublin, CA). Five to six measurements were obtained for each eye and averaged.

RNFL imaging was performed using three devices: GDx-VCC, HRT II and Stratus OCT. For all devices, image quality was assessed both subjectively and by the standard quality parameter generated by the device.

The GDx (software version 5.5.1.5) uses the birefringence properties of the parallel placement of axons in the RNFL to determine its thickness. All subjects were scanned on the GDx using the variable corneal compensation method (VCC), the algorithm for which has been described elsewhere.[11-13] The images included in the study all had good focus, even illumination, well centered ONH, and quality score of 8 or better.

The HRT II (software version 1.4.1.0) uses confocal imaging to acquire a series of image planes of the ONH and surrounding peripapillary retina, and uses the planes to create a three-dimensional topographic map. Inclusion criteria of acceptable image quality were good focus, even illumination, well centered ONH, and pixels standard deviation of 50 or less. RNFL measurements are measured along the contour line, and are the height from the contour line to a reference place 50μm below the retinal surface along the section of the contour line in the papillomacular bundle (350° to 356°).

The StratusOCT (software version 4.0) uses low-coherence interferometry to generate cross-sectional images of the retina with high axial resolution (8-10 microns). The fast RNFL protocol was used to acquire data along a 3.4 mm diameter circle around the ONH in the peripapillary retina. All included images had appropriate centration, even illumination, signal strength 7 or better, and no obvious segmentation algorithm failure.

Statistical Analysis

A linear mixed effect model was used to assess the relationship between RNFL thickness and CCT, accounting for clustering of eyes within subjects, scan quality score from each device, family history of glaucoma, ethnicity, axial length, IOP, MD, PSD, testing site, and the interactions between these parameters. A separate model was created for each imaging technique. Alpha significance level was set a priori to 0.05. The R Language and Environment for Statistical Computing (Version 2.5.1, 2007-06-27) was used for statistical computations and graphics.[14] The R package *nlme* (Version 3.1-83, 2007-06-13) was used for the linear mixed effects analysis.[15]

Results

Subject Characteristics

Two hundred and eighteen eyes of 109 healthy subjects were enrolled in the study (31 male, 78 female). Average age of the subjects was 56.7 ± 10.3 years. The race characteristics of the study population were 99 white, 8 African Americans, and 2 Asians. Mean refractive error was -0.81 ± 1.94 diopters and mean axial length was 23.77 ± 0.98 mm. Mean corneal thickness was 558.6 ± 33.8 μm (range: 499 - 658). Figure 1 displays the distribution of CCT across all subjects. Mean RNFL thickness measurements from each of the three imaging devices can be seen in Table with the slope of the correlation between these measurements and CCT.

Linear Mixed Model Statistical Analysis

For GDx, MD and PSD were significant covariates of mean RNFL thickness ($p=0.002$ and $p<0.0001$, respectively). All other covariates (scan quality, family history of glaucoma, ethnicity, axial length, IOP and testing site) did not show a statistically significant relationship. The slope for RNFL vs. CCT was positive (0.024) but not statistically significant ($p=0.17$). High variability between sites was found with a few sites exhibiting significant slopes, but combining to display the non-significant slightly positive slope (Figure 2).

For HRT II, none of the tested covariates showed statistical significance with the RNFL measurements. The slope for RNFL vs. CCT was slightly negative (-0.001) but not statistically significant ($p=0.27$). As with GDx, HRT II displayed high variability between sites, however, in this case, the slope was not significant at any of the sites (Figure 2).

For OCT, there was a statistically significant relationship between overall RNFL thickness and ethnicity, axial length, and signal strength ($p=0.01$, $p<0.0001$, $p=0.02$, respectively). All other parameters did not show significant relationship. The overall slope for RNFL vs. CCT was positive (0.037) but also not statistically significant ($p=0.34$). OCT displayed similar variability between sites, with a few sites exhibiting small but significant slope (Figure 2).

Discussion

Central corneal thickness has been an area of much recent interest as a major risk factor for the development of glaucoma.[1, 3-10] It has been suggested that the relationship exists because corneal thickness is a surrogate indicator of the overall structure and biomechanical properties of the eye.[5] In this study, we used three commonly used ocular imaging devices to measure the RNFL thickness. Using all three methods, we did not detect any statistically significant relationship between CCT and RNFL thickness in healthy eyes.

There are limited data regarding CCT as it relates to RNFL thickness in normal controls included in other studies. Kaushik et al[10] included a normal subset for comparison to ocular hypertensives in their OCT study of 35 healthy eyes from 35 subjects. After stratifying their data between CCT≤555μm and CCT>555μm, they found no significant different in average, inferior average, or superior average RNFL thickness between the two CCT groups. The correlation between CCT and the three RNFL thickness parameters, cup/disk area ratio, cup area, rim area, and horizontally integrated rim width was all found to be non-significant in their normal subset, except for the overall average RNFL thickness, which had a Pearson's correlation coefficient of 0.482, resulting in p=0.003. This agrees with our finding of a positive relationship between CCT and RNFL as measured by OCT though the magnitude of the correlation was substantially lower and statistically insignificant in our study. Henderson et al[5] examined the relationship between CCT and RNFL thickness as measured by the GDx-VCC RNFL thickness parameters and Nerve Fiber Indicator (NFI) in ocular hypertensives and 48 healthy individuals. They found no significant correlation between NFI and CCT in their data set, similar to our mixed effects model, which displayed no significant relationship between CCT and GDx.

A possible source of the previously observed relationship stems from the measurement technique used for RNFL thickness measurement. Several studies looked at changes in RNFL measurement using GDx before and after surgeries such as excimer laser photorefractive keratectomy (PRK) and laser-assisted in situ keratectomy (LASIK) which both decrease corneal thickness, with several reporting a decrease in RNFL thickness measurements after surgery.[16-18] Initially with the GDx, fixed corneal compensation (FCC) was used to account for the cornea birefringent properties that affect the RNFL measurements. Roberts et al.[19] suggested that the changes in RNFL measurement with GDx-FCC after surgery were unlikely to be related to actual physical change to the ganglion cells, hypothesizing that the changes were artifacts due to the change in corneal birefringence after LASIK. In other studies, once VCC was applied, which accounts for the individual corneal properties, there was no difference in RNFL measurement before and after LASIK.[20-22] These studies raise awareness that the measurement methods must be considered when examining the relationship between CCT and RNFL thickness. Our study aimed to minimize this effect by measuring RNFL thickness with multiple modalities and comparing their results.

Some of the covariates evaluated in the study had a significant relationship to RNFL measurements. GDx RNFL thickness displayed a significant relationship with MD and PSD, which would be an expected result if the data set consisted of glaucomatous subjects. However, the reason for our finding in the presence of a narrow range of healthy eyes' MD and PSD is unclear. For OCT, there was a significant relationship between RNFL thickness and ethnicity, axial length, and signal strength. Differences in RNFL thickness have been previously observed between different ethnic groups.[23] However, our findings were the opposite of that study; RNFL was thinner in eyes of Caucasians than ones of African Americans. This relationship may be an artifact due the limited sample size of African-Americans in this study (n=8). There has been some evidence that axial length can affect RNFL measurement in OCT in myopic subjects, however, the range of axial length in the present study was more limited, probably due to our somewhat limited refraction inclusion criteria.[24] RNFL thickness measurements in OCT have also been shown to decrease with decreasing signal strength in OCT, in agreement with our findings.[25]

Overall, no statistically significant relationship was observed between CCT and RNFL thickness as measured by all three imaging modalities in healthy eyes. While there were a few selected slopes at specific sites that were significant, this was probably due to the small sample size from individual sites. The smaller sample sizes resulted in the slope being slightly positive at certain sites, for a specific device, while other sites were slightly negative, resulting in the overall slope that was not significant. This was true for all three devices, though which sites were positive and which were negative for which device varied. There was no consistent trend as to which way each site tended across devices, or which way each device tended over all sites.

The study population consisted of a relatively wide range of CCT values (500-685 μm) and our findings of approximately zero slope were consistent across all modalities, which reinforces the validity of these findings. Because our data resulted in a nearly zero slope with all three devices, which measure RNFL thickness in very different ways, the zero slope finding is likely representing reality.

In conclusion, no significant relationship was observed between RNFL thickness and CCT in healthy eyes. Therefore the relationship observed previously in ocular hypertension and glaucoma subjects is likely coming to fruition as RNFL is lost due to the disease.

Acknowledgements/Disclosure

a. *Funding/Support:* Supported in part by NIH grants R01-EY013178, R01-EY013516, P30-EY008098 (Bethesda, MD), The Eye and Ear Foundation (Pittsburgh) and an unrestricted grant from Research to Prevent Blindness, Inc (New York, NY).
b. *Financial Disclosures:* Dr. Schuman receives royalties for intellectual property licensed by Massachusetts Institute of Technology to Carl Zeiss Meditec. Dr. Wollstein received research funding from Carl Zeiss Meditec and Optovue.
c. *Contributions of Authors:* Design of the study (TM, GW, JSS); Collection, management, analysis, and interpretation of the data (TM, KAT, GW, HI, RAB, KRS, LK); Preparation of the manuscript (TM, KAT, GW); Review of the manuscript (GW, HI, KRS, LK, JSS)
d. The participants in the study were prospectively enrolled at four clinical sites, as part of the Advanced Imaging in Glaucoma Study (AIGS), a prospective longitudinal study. AIGS was designed to develop and evaluate glaucoma diagnosis using advanced ocular imaging technology. Full details on the study and the manual of procedure can be found at www.AIGStudy.net. The study followed the principles of the Declaration of Helsinki and Health Insurance Portability and Accountability Act regulations and received full Institutional Review Board and ethics committee approval by the University of Pittsburgh, University of Miami and the University of Southern California, with informed consent obtained by all participants. ClinicalTrials.gov identifier: NCT00286637.
e. *Other Acknowledgements:* None.

Figure Legends

Figure 1: Histogram of central corneal thickness of eyes in all healthy subjects.

Figure 2. Scatter plots of central corneal thickness and retinal nerve fiber layer thickness (RNFL) as measured by optical coherence tomography (OCT), Heidelberg retina tomography (HRT) and nerve fiber analyzer (GDx-VCC) for each study center. A green line joins points for the two eyes of each subject. Red line is the spline fit for the data, taking into account the correlation between the two eyes.

References

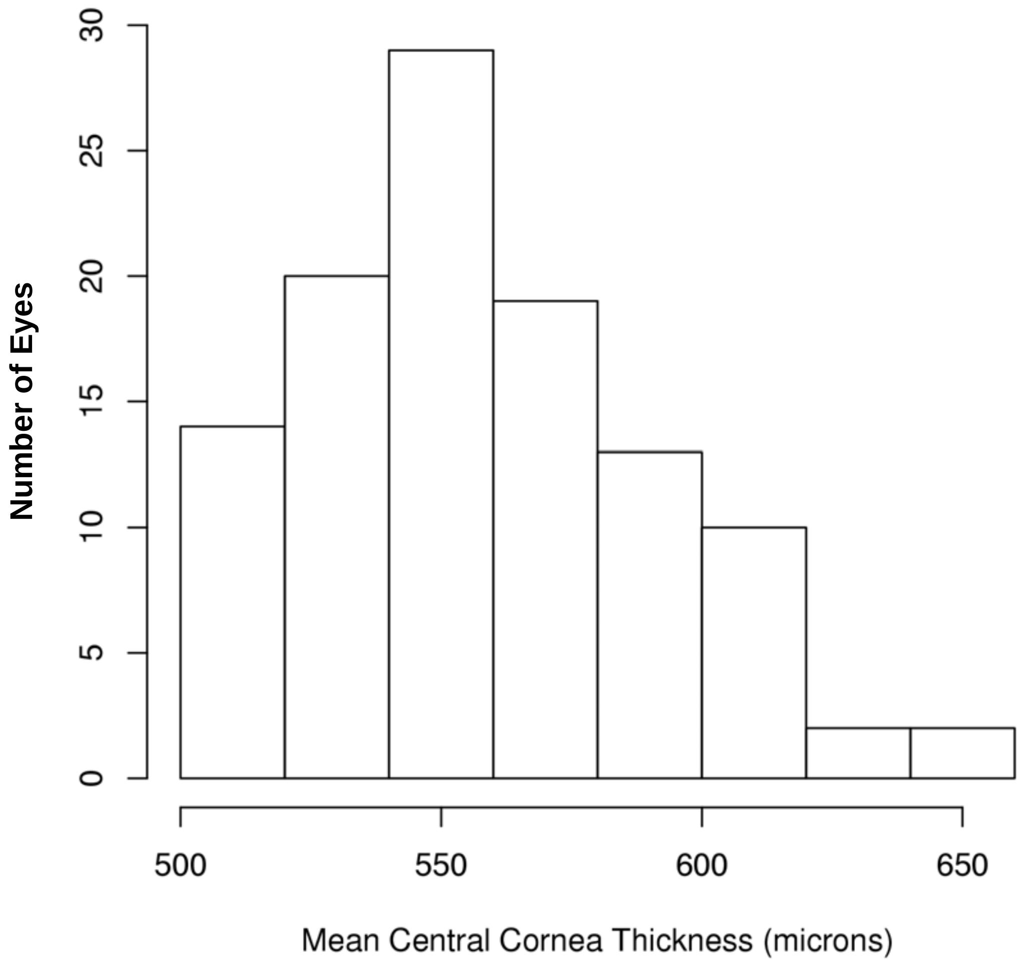
1. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. Arch Ophthalmol 2002;120:714-20.
2. Miglior S, Pfeiffer N, Torri V, et al. Predictive factors for open-angle glaucoma among patients with ocular hypertension in the European Glaucoma Prevention Study. Ophthalmology 2007;114:3-9.
3. Brusini P, Tosoni C, Parisi L, Rizzi L. Ocular hypertension and corneal thickness: a long-term prospective study. Results after two years. Eur J Ophthalmol 2005;15:550-5.
4. Choi HJ, Kim DM, Hwang SS. Relationship between central corneal thickness and localized retinal nerve fiber layer defect in normal-tension glaucoma. J Glaucoma 2006;15:120-3.
5. Henderson PA, Medeiros FA, Zangwill LM, Weinreb RN. Relationship between central corneal thickness and retinal nerve fiber layer thickness in ocular hypertensive patients. Ophthalmology 2005;112:251-6.
6. Iester M, Mermoud A. Retinal nerve fiber layer and physiological central corneal thickness. J Glaucoma 2001;10:158-62.
7. Jonas JB, Stroux A, Velten I, et al. Central corneal thickness correlated with glaucoma damage and rate of progression. Invest Ophthalmol Vis Sci 2005;46:1269-74.
8. Medeiros FA, Sample PA, Zangwill LM, et al. Corneal thickness as a risk factor for visual field loss in patients with preperimetric glaucomatous optic neuropathy. Am J Ophthalmol 2003;136:805-13.
9. Zeppieri M, Brusini P, Miglior S. Corneal thickness and functional damage in patients with ocular hypertension. Eur J Ophthalmol 2005;15:196-201.
10. Kaushik S, Gyatsho J, Jain R, et al. Correlation between retinal nerve fiber layer thickness and central corneal thickness in patients with ocular hypertension: an optical coherence tomography study. Am J Ophthalmol 2006;141:884-90.
11. Hoh ST, Ishikawa H, Greenfield DS, et al. Peripapillary nerve fiber layer thickness measurement reproducibility using scanning laser polarimetry. J Glaucoma 1998;7:12-5.
12. Zangwill L, Berry CA, Garden VS, Weinreb RN. Reproducibility of retardation measurements with the nerve fiber analyzer II. J Glaucoma 1997;6:384-9.
13. Zhou Q, Weinreb RN. Individualized compensation of anterior segment birefringence during scanning laser polarimetry. Invest Ophthalmol Vis Sci 2002;43:2221-8.
14. Team RDC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2007.
15. Pinheiro J, Bates D, DebRoy S, et al. nlme: Linear and Nonlinear Mixed Effects Models., 3.1-83. ed, 2007.
16. Gurses-Ozden R, Pons ME, Barbieri C, et al. Scanning laser polarimetry measurements after laser-assisted in situ keratomileusis. Am J Ophthalmol 2000;129:461-4.

17. Nevyas JY, Nevyas HJ, Nevyas-Wallace A. Change in retinal nerve fiber layer thickness after laser in situ keratomileusis. J Cataract Refract Surg 2002;28:2123-8.
18. Tsai YY, Lin JM. Effect of laser-assisted in situ keratomileusis on the retinal nerve fiber layer. Retina 2000;20:342-5.
19. Roberts TV, Lawless MA, Rogers CM, et al. The effect of laser-assisted in situ keratomileusis on retinal nerve fiber layer measurements obtained with scanning laser polarimetry. J Glaucoma 2002;11:173-6.
20. Halkiadakis I, Anglionto L, Ferensowicz M, et al. Assessment of nerve fiber layer thickness before and after laser in situ keratomileusis using scanning laser polarimetry with variable corneal compensation. J Cataract Refract Surg 2005;31:1035-41.
21. Toth M, Hollo G. Enhanced corneal compensation for scanning laser polarimetry on eyes with atypical polarisation pattern. Br J Ophthalmol 2005;89:1139-42.
22. Zangwill LM, Abunto T, Bowd C, et al. Scanning laser polarimetry retinal nerve fiber layer thickness measurements after LASIK. Ophthalmology 2005;112:200-7.
23. Poinoosawmy D, Fontana L, Wu JX, et al. Variation of nerve fibre layer thickness measurements with age and ethnicity by scanning laser polarimetry. Br J Ophthalmol 1997;81:350-4.
24. Leung CK, Mohamed S, Leung KS, et al. Retinal nerve fiber layer measurements in myopia: An optical coherence tomography study. Invest Ophthalmol Vis Sci 2006;47:5171-6.
25. Stein DM, Wollstein G, Ishikawa H, et al. Effect of corneal drying on optical coherence tomography. Ophthalmology 2006;113:985-91.

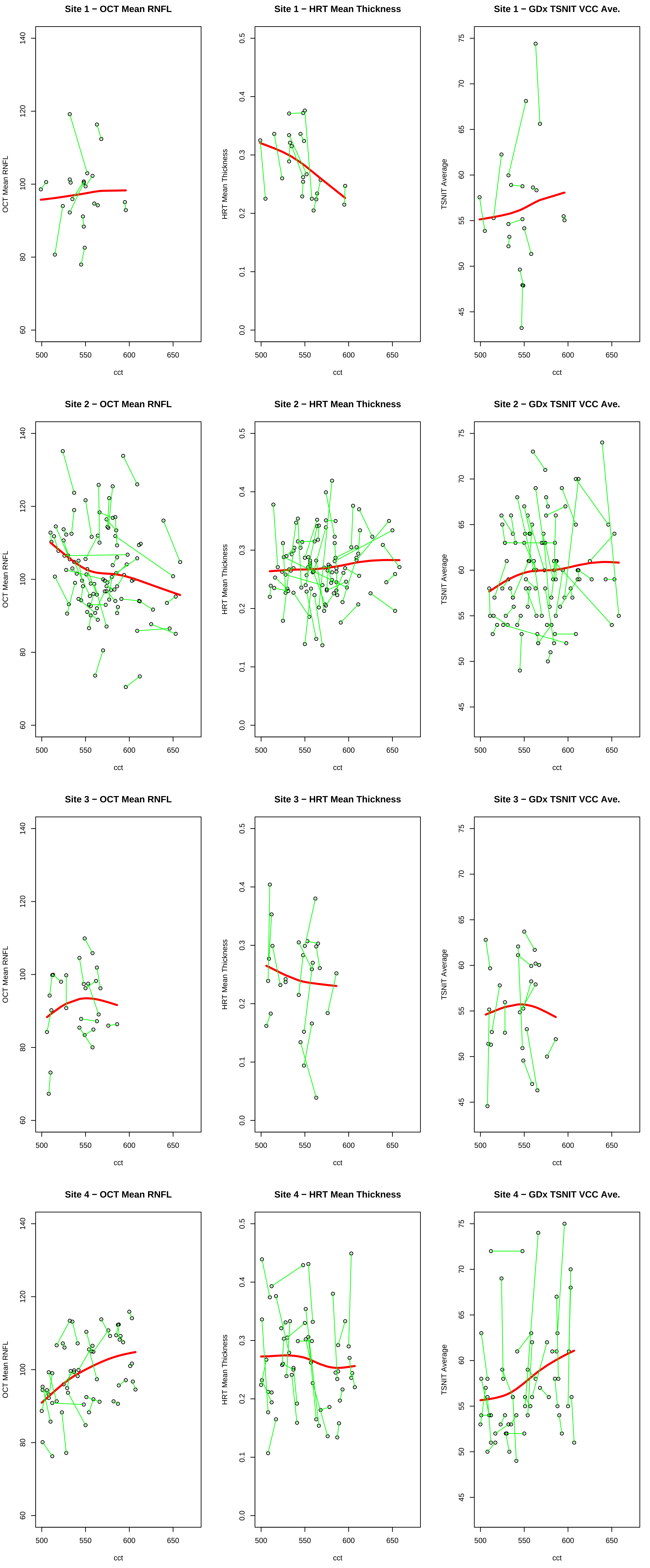
Table. Retinal nerve fiber layer (RNFL) thickness measured by three imaging devices and their relationship with central corneal thickness CCT.

Device	Parameter	Mean ± SD μm	Slope (CI)	P*
GDx-VCC	TSNIT Average	58.3 ± 6.0	0.024 (-0.010 - 0.059)	0.17
HRT II	Mean RNFL Thickness	265 ± 75	-0.001 (-0.003 - 0.001)	0.27
StratusOCT	Overall RNFL thickness	99.5 ± 11.4	0.037 (-0.039 - 0.112)	0.34

*P value for the statistical significance of the slope
SD – standard deviation, CI – confidence interval.



Number of Eyes
0
5
10
15
20
25
30
500
550
600
650
Mean Central Cornea Thickness (microns)


Site 1 − OCT Mean RNFL
Site 1 − HRT Mean Thickness
Site 1 − GDx TSNIT VCC Ave.
Site 2 − OCT Mean RNFL
Site 2 − HRT Mean Thickness
Site 2 − GDx TSNIT VCC Ave.
Site 3 − OCT Mean RNFL
Site 3 − HRT Mean Thickness
Site 3 − GDx TSNIT VCC Ave.
Site 4 − OCT Mean RNFL
Site 4 − HRT Mean Thickness
Site 4 − GDx TSNIT VCC Ave.
OCT Mean RNFL
60 80 100 120 140
HRT Mean Thickness
0.0 0.1 0.2 0.3 0.4 0.5
TSNIT Average
45 50 55 60 65 70 75
cct
500 550 600 650

Advance Imaging for Glaucoma Investigators:

University of Southern California Keck School of Medicine, Doheny Eye Institute, Los Angeles, CA: David Huang, MD, Rohit Varma, MD, MPH, Vikas Chopra, MD, Brian Francis, MD, Farnaz Memarzadeh, MD, Kenneth L. Lu, MD, Ou Tan, PhD, Srinivas R Sadda, MD.

University of Pittsburgh Medical Center: University of Pittsburgh School of Medicine, Pittsburgh, PA: Robert J Noecker, MD.

University of Miami, Bascom Palmer Eye Institute, Palm Beach, FL: David S. Greenfield, MD, Carolyn D. Quinn, MD, Mitra Sehi, PhD

Assessing the Relationship between Central Corneal Thickness and Retinal Nerve Fiber Layer Thickness in Healthy Subjects

Manuscript#: AJO-08-106

Central corneal thickness was suggested as an indicator of ocular structures including the retinal nerve fiber layer (RNFL). RNFL was measured in 218 healthy eyes with optical coherence tomography, scanning laser polarimetry and confocal scanning laser ophthalmoscopy. No statistically significant relationship was noted in healthy eyes between central corneal thickness and RNFL thicknesses as measured by any of the imaging devices.