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Wavefront Error Correction with Adaptive Optics in Diabetic Retinopathy

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Abstract

Purpose—To determine the effects of diabetic retinopathy (DR), increased foveal thickness (FT), and adaptive optics (AO) on wavefront aberrations and Shack-Hartmann (SH) image quality.

Methods—SH aberrometry and wavefront error correction were performed with a bench-top AO retinal imaging system in 10 healthy control and 19 DR subjects. Spectral domain optical coherence tomography (SDOCT) was performed and central FT was measured. Based on the FT data in the control group, subjects in the DR group were categorized into two subgroups with normal FT (DR-NFT) or increased FT (DR-IFT). SH image quality was assessed based on spot areas and high order (HO) root mean square (RMS) and total RMS were calculated.

Results—There was a significant effect of DR on HO and total RMS ($p = 0.01$), and RMS decreased significantly after AO ($p < 0.001$). SH spot area was significantly affected by DR ($p < 0.001$), but it did not change after AO ($p = 0.6$). HO RMS, total RMS, and SH spot area were higher in DR subjects both before and after AO correction. In DR subgroups, HO and total RMS decreased significantly after AO ($p < 0.001$), while the effect of increased FT on HO and total RMS was not significant ($p = 0.7$). There were no significant effects of increased FT and AO on SH spot area ($p = 0.9$).

Conclusions—DR subjects had higher wavefront aberrations and less compact SH spots, likely attributable to pathological changes in the ocular optics. Wavefront aberrations were significantly reduced by AO, though AO performance was suboptimal in DR subjects as compared to control subjects.

Keywords

diabetic retinopathy; Shack-Hartmann; wavefront error; adaptive optics; foveal thickness

Diabetic retinopathy (DR) is one of the leading causes of blindness in working-age adults in industrialized countries.¹ Central vision loss in DR is primarily due to retinal vascular abnormalities, which can lead to macular edema with increased foveal thickness (FT).^{2, 3} Additionally, vision of diabetic subjects can be adversely affected by disease-related

changes in the optics of the eye, including cataracts,⁴ and alterations in the crystalline lens caused by increased and variable blood glucose levels that affect refractive error.^{5–8} Furthermore, ocular high order (HO) wavefront aberrations and light scatter have also been shown to be increased in diabetic subjects, which may further contribute to visual impairment.^{5, 9–11}

Recently, improved visualization of retinal microvascular abnormalities in diabetic subjects has been demonstrated by adaptive optics (AO) retinal imaging technology.^{12–14} AO relies on Shack-Hartmann (SH) aberrometry for measurement and correction of HO wavefront aberrations to improve retinal image resolution. However, wavefront aberration measurements and SH image quality in DR subjects may be affected by pathological changes in the optical properties of the eye and retinal structure. The purpose of the current study was to determine the effects of DR, increased FT, and AO on wavefront aberrations and SH image quality.

PATIENTS AND METHODS

Subjects

This prospective research study was approved by an Institutional Review Board at the University of Illinois at Chicago. Prior to enrollment in the study, the protocol was explained to the subjects, and informed consent was obtained according to the tenets of the Declaration of Helsinki. Data were obtained in 10 healthy control subjects and 19 DR subjects diagnosed with non-proliferative diabetic retinopathy (NPDR) (N = 7) and proliferative diabetic retinopathy (PDR) (N = 12). Control subjects did not have a history of ocular disease or diabetes. Subjects did not have significant media opacities that precluded acquisition and analysis of SH images. Spherical and cylindrical refractive errors were measured by subjective refraction or SH aberrometry, and visual acuity (VA) was recorded during the clinical exam of DR subjects. Prior to SH imaging, pupils were dilated with one drop 2.5% phenylephrine hydrochloride.

Imaging

Spectral domain optical coherence tomography (SDOCT) was performed with the use of a commercial instrument (Spectralis, Heidelberg Engineering). In each subject, 19 horizontal raster B scans were obtained over a $20^\circ \times 15^\circ$ retinal area centered on the fovea. FT was manually measured using the instrument's software at the center of the SDOCT B scan image traversing the fovea. Thickness measurements were performed at the center of the fovea to coincide with the location of the incident laser for wavefront aberration measurement.

SH imaging was performed before and after correction of wavefront aberrations with a modified bench-top AO retinal imaging system previously described.¹⁵ A chin and forehead rest mounted on a xyz translation stage was used to align the pupil along the optical axis of the system. Alignment was monitored using a charge couple device (CCD) camera integrated into the AO system and real-time display of the pupil. A Badal optometer, incorporated into the AO system, was used to minimize the subject's spherical error, while

cylindrical error was corrected with trial lens placed near the pupil plane. An internal target was presented to the subject during image acquisition to ensure foveal fixation. A collimated 780 nm laser diode (40 μ W) was projected normal to the eye and focused on the center of the fovea. A lenslet array sampled the wavefront and an SH image was generated on a CCD camera (Princeton Instruments, Trenton, NJ). Wavefront aberrations were corrected using a microelectromechanical system deformable mirror with a stroke of 6 μ m (Boston MicroMachines, Cambridge, MA). Closed loop AO control minimized root mean square (RMS) wavefront error. SH images were acquired before and after AO correction. Four repeated corrections were performed in one eye of each subject.

Image Analysis

SH images were analyzed to estimate the wavefront aberration function for a 5 mm pupil diameter with the sum of 36 Zernike polynomials, using a least squares fitting technique.¹⁶ RMS wavefront error was calculated from the Zernike coefficients for HO (3rd through 7th) and total (2nd through 7th) wavefront aberrations, before and after AO correction. SH image quality was assessed based on SH image spot areas. A local region around each SH spot was thresholded using Otsu's method,¹⁷ and the number of pixels on the binary image assigned to 1 in a spot region was counted and converted to area units. Otsu's method determined an ideal threshold that separated two classes of pixels (SH spot and background) in the local region around each spot such that the within class variances were minimized. The areas of all SH spots within the 5 mm pupil were averaged from each SH image. A mean SH spot area was derived by averaging areas from 4 repeated SH images, before and after AO correction.

Statistical Analysis

DR subjects were categorized as having either normal or increased FT based on the mean and standard deviation (SD) of FT in control subjects ($219 \pm 13 \mu$ m; $N = 10$). In 11 DR subjects (NPDR: $N = 4$, PDR: $N = 7$), FT was normal (within two SD of the mean, or $193 - 245 \mu$ m), and these subjects were classified as DR-NFT. Six DR subjects (NPDR: $N = 1$, PDR: $N = 5$) had increased FT (greater than two SD above the mean, or $> 245 \mu$ m) and were classified as DRIFT. Two DR subjects (NPDR) had FT less than two SD below the mean, or $< 193 \mu$ m. Data obtained in these 2 subjects were removed from further analysis, since only the effect of increased FT was investigated. Age, refractive error, FT, RMS and absolute Zernike coefficients (2nd, 3rd and 4th order) were compared between groups (control and DR) and subgroups (DR-NFT and DR-IFT) using unpaired t-test. A two-way analysis of variance (ANOVA) with repeated measures was conducted to determine the effects of disease (control and DR) and AO (before and after) on outcome measures of RMS (HO and total) and SH spot area. Similarly, a repeated measures two-way ANOVA was performed in DR subgroups to determine the effects of FT (normal and increased) and AO (before and after) on each outcome measure. Linear regression analysis was performed to determine the relationship between total RMS after AO and SH spot area before AO in DR group. Statistical analysis was performed using SPSS version 21 (SPSS Inc, Chicago, IL, USA) and statistical significance was accepted at $p = 0.05$.

RESULTS

Subjects' Demographics and Characteristics

Age, visual acuity, and refractive error averaged in each group and subgroup are summarized in Table 1. Mean age and spherical and cylindrical refractive errors were not significantly different between control and DR groups, or between DR-NFT and DR-IFT subgroups ($p > 0.1$). Visual acuities of subjects in DR-NFT and DR-IFT subgroups were not statistically different ($p = 0.3$). Mean FT in control ($219 \pm 13 \mu\text{m}$; $N = 10$) and DR ($232 \pm 44 \mu\text{m}$; $N = 19$) subjects were similar ($p = 0.4$). Mean FT in DR-IFT subjects ($285 \pm 20 \mu\text{m}$) was significantly greater than FT in DR-NFT subjects ($216 \pm 13 \mu\text{m}$) ($p < 0.001$).

Wavefront Aberrations and SH Spot Area

Mean 2nd, 3rd and 4th order RMS and absolute Zernike coefficients in each group and subgroup are listed in Table 2. Mean 2nd order RMS in control and DR groups did not differ significantly ($p = 0.3$), while it was higher in the DR-IFT subgroup than in the DR-NFT subgroup ($p = 0.04$). Mean 3rd order RMS was not significantly different between control and DR groups ($p = 0.3$), while 4th order RMS was significantly higher in the DR group ($p = 0.005$). All 3rd and 4th order Zernike coefficients were higher in the DR group as compared to control group, and the difference in Zernike coefficient Z_{42} reached statistical significance ($p = 0.03$). Between DR subgroups, both 3rd and 4th order RMS were similar ($p = 0.2$), and only Zernike coefficient Z_{31} was significantly different ($p = 0.006$).

Mean HO RMS, total RMS, and SH spot area in control and DR subjects before and after AO are shown in Figure 1. Before AO correction, mean HO and total RMS were $0.28 \pm 0.11 \mu\text{m}$ and $0.40 \pm 0.15 \mu\text{m}$ in the control group, respectively. In the DR group, mean HO and total RMS were $0.45 \pm 0.16 \mu\text{m}$ and $0.61 \pm 0.22 \mu\text{m}$, respectively. The mean SH spot areas in the control and DR group were $0.0092 \pm 0.0024 \text{ mm}^2$ and $0.018 \pm 0.0076 \text{ mm}^2$, respectively. SH spot area in the DR group was on average 96% larger than in the control group. There was a significant effect of DR ($p = 0.01$) on HO and total RMS, and these metrics decreased significantly after AO ($p < 0.001$). HO and total RMS before and after AO were higher in DR subjects as compared to control subjects. There was not a significant interaction between disease and AO ($p = 0.3$). SH spot area was significantly affected by DR ($p < 0.001$), but did not change significantly after AO ($p = 0.6$). The interaction effect was also not significant ($p = 0.4$). In DR group, there was a significant correlation between total RMS after AO and SH spot area before AO ($R = 0.61$, $p = 0.006$).

Mean HO RMS, total RMS, and SH spot area in DR subgroups before and after AO are shown in Figure 2. Before AO correction, mean HO and total RMS were $0.48 \pm 0.17 \mu\text{m}$ and $0.60 \pm 0.21 \mu\text{m}$ in the DR-NFT subgroup, respectively. In the DR-IFT subgroup, HO and total RMS were $0.44 \pm 0.10 \mu\text{m}$ and $0.69 \pm 0.21 \mu\text{m}$, respectively. The mean SH spot areas in the DR-NFT and DR-IFT subgroups were $0.0193 \pm 0.0088 \text{ mm}^2$ and $0.0166 \pm 0.0061 \text{ mm}^2$, respectively. In DR subgroups, HO and total RMS decreased significantly after AO ($p < 0.001$), while the effect of increased FT on HO and total RMS was not significant ($p = 0.7$). The interaction effects were also not significant ($p = 0.2$). There were no significant

effects of increased FT and AO on SH spot area ($p = 0.9$), nor was there a significant interaction effect ($p = 0.1$).

DISCUSSION

The clinical utility and performance of AO retinal imaging systems depend on SH image quality which may be degraded due to disease-related abnormalities in the optics of the eye and retinal tissue. In the current study, we demonstrated that wavefront aberrations and SH spot areas were greater in DR subjects as compared to control subjects, though they were similar between DR subjects with and without foveal thickening. Furthermore, wavefront aberrations were significantly reduced by AO in control and DR subjects, with and without foveal thickening. However, wavefront error after AO was higher in diabetic subjects as compared to control subjects.

High order RMS wavefront errors in control subjects were in general agreement with measurements reported in previous studies.^{18–22} Wavefront aberrations were higher in DR subjects compared to control subjects, consistent with previous reports.^{9, 22} Increased high order aberrations in diabetic subjects may be due to disease-related changes in the crystalline lens and cornea, though retinal surface irregularities have also been implicated as a source for increased SH image blur in patients with retinal disorders.²² Ideally, SH wavefront sensors detect aberrations based on light returned from a single reflecting retinal layer. However, in the presence of retinal pathologies, light scattering from multiple intra-retinal interfaces and irregular retinal surfaces may blur the SH image that is analyzed to measure the wavefront error. The finding of similar RMS and SH spot area between DR subgroups suggests negligible contribution of foveal thickening to SH image quality and consequent wavefront aberration measurements. Future studies are needed to evaluate the effect of more extensive retinal pathologies on SH image quality and the resulting wavefront error measurements.

In the current study, wavefront aberrations were significantly reduced by AO in DR subjects. Previous studies have shown improved visualization of retinal microvasculature by AO retinal imaging in diabetic subjects without retinopathy or with NPDR, indicating wavefront error correction for high resolution imaging is feasible in diabetic subjects with mild retinopathy.^{12–14} In the current study, wavefront error was reduced in DR subjects with increased foveal thickness, though macular edema has been previously shown to hinder high resolution imaging of underlying retinal layers.²³ Our finding of higher RMS wavefront error after AO in DR subjects as compared to control subjects is indicative of suboptimal AO performance. This is likely attributable to physiological and technical factors, such as the initial level of wavefront error, SH image quality, fixation stability, and stroke of the deformable mirror.

In DR subjects, SH spots were less compact as compared to control subjects. SH spot area can be increased by elevated wavefront aberrations and light scatter due to ocular optics.^{5, 10, 11} SH spot area did not change significantly with AO correction in both control and DR subjects, contrary to our expectation of decreased spot size with reduction of aberrations. This finding suggests that either the corrected level of aberrations minimally

affected the spot size or light scatter (not correctable by AO) was the predominant factor in the observed SH spot area. Furthermore, the increased SH spot area in DR subjects may have influenced centroid detection and wavefront error correction. In DR subjects, wavefront aberrations after AO were still higher than in control subjects and linearly correlated with SH spot area before AO.

In conclusion, DR subjects had higher wavefront aberrations and less compact SH spots, likely attributable to pathological changes in the ocular optics. Wavefront aberrations were significantly reduced by AO, though AO performance was suboptimal in DR subjects as compared to control subjects.

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References

1. Congdon N, O'Colmain B, Klaver CC, Klein R, Munoz B, Friedman DS, Kempen J, Taylor HR, Mitchell P. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol*. 2004; 122:477–85. [PubMed: 15078664]
2. Holm K, Larsson J, Lovestam-Adrian M. In diabetic retinopathy, foveal thickness of 300 μm seems to correlate with functionally significant loss of vision. *Doc Ophthalmol*. 2007; 114:117–24. [PubMed: 17242898]
3. Gardner TW, Larsen M, Girach A, Zhi X. Diabetic macular oedema and visual loss: relationship to location, severity and duration. *Acta Ophthalmol*. 2009; 87:709–13. [PubMed: 19817721]
4. Stefek M, Karasu C. Eye lens in aging and diabetes: effect of quercetin. *Rejuvenation Res*. 2011; 14:525–34. [PubMed: 21978083]
5. Kato S, Shiokawa A, Fukushima H, Numaga J, Kitano S, Hori S, Kaiya T, Oshika T. Glycemic control and lens transparency in patients with type 1 diabetes mellitus. *Am J Ophthalmol*. 2001; 131:301–4. [PubMed: 11239860]
6. Wiemer NG, Dubbelman M, Ringens PJ, Polak BC. Measuring the refractive properties of the diabetic eye during blurred vision and hyperglycaemia using aberrometry and Scheimpflug imaging. *Acta Ophthalmol*. 2009; 87:176–82. [PubMed: 18547279]
7. Furushima M, Imaizumi M, Nakatsuka K. Changes in refraction caused by induction of acute hyperglycemia in healthy volunteers. *Jpn J Ophthalmol*. 1999; 43:398–403. [PubMed: 10580662]
8. Duke-Elder WS. Changes in Refraction in Diabetes Mellitus. *Br J Ophthalmol*. 1925; 9:167–87. [PubMed: 18168468]
9. Shahidi M, Blair NP, Mori M, Zelkha R. Optical section retinal imaging and wavefront sensing in diabetes. *Optom Vis Sci*. 2004; 81:778–84. [PubMed: 15557852]
10. Mori F, Ishiko S, Abiko T, Kitaya N, Kato Y, Kanno H, Yoshida A. Changes in corneal and lens autofluorescence and blood glucose levels in diabetics: parameters of blood glucose control. *Curr Eye Res*. 1997; 16:534–8. [PubMed: 9192161]
11. Di Benedetto A, Aragona P, Romano G, Romeo G, Di Cesare E, Spinella R, Ferreri G, Cucinotta D. Age and metabolic control influence lens opacity in type I, insulin-dependent diabetic patients. *J Diabetes Complications*. 1999; 13:159–62. [PubMed: 10509876]
12. Tam J, Dhamdhare KP, Tiruveedhula P, Lujan BJ, Johnson RN, Bearse MA Jr, Adams AJ, Roorda A. Subclinical capillary changes in non-proliferative diabetic retinopathy. *Optom Vis Sci*. 2012; 89:E692–703. [PubMed: 22525131]

13. Tam J, Dhamdhere KP, Tiruveedhula P, Manzanera S, Barez S, Bearse MA Jr, Adams AJ, Roorda A. Disruption of the retinal parafoveal capillary network in type 2 diabetes before the onset of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2011; 52:9257–66. [PubMed: 22039250]
14. Lombardo M, Parravano M, Serrao S, Ducoli P, Stirpe M, Lombardo G. Analysis of retinal capillaries in patients with type 1 diabetes and nonproliferative diabetic retinopathy using adaptive optics imaging. *Retina*. 2013; 33:1630–9. [PubMed: 23492950]
15. Wanek JM, Mori M, Shahidi M. Effect of aberrations and scatter on image resolution assessed by adaptive optics retinal section imaging. *J Opt Soc Am A Opt Image Sci Vis*. 2007; 24:1296–304. [PubMed: 17429475]
16. Liang J, Grimm B, Goelz S, Bille JF. Objective measurement of wave aberrations of the human eye with the use of a Hartmann-Shack wave-front sensor. *J Opt Soc Am (A)*. 1994; 11:1949–57.
17. Otsu N. A threshold selection method from gray-level histograms. *IEEE Trans Systems Man and Cybernet*. 1979; 9:62–9.
18. Liang J, Williams DR, Miller DT. Supernormal vision and high-resolution retinal imaging through adaptive optics. *J Opt Soc Am (A)*. 1997; 14:2884–92.
19. Thibos LN, Hong X, Bradley A, Cheng X. Statistical variation of aberration structure and image quality in a normal population of healthy eyes. *J Opt Soc Am (A)*. 2002; 19:2329–48.
20. Calver RI, Cox MJ, Elliott DB. Effect of aging on the monochromatic aberrations of the human eye. *J Opt Soc Am (A)*. 1999; 16:2069–78.
21. Salmon TO, van de Pol C. Normal-eye Zernike coefficients and root-mean-square wavefront errors. *J Cataract Refract Surg*. 2006; 32:2064–74. [PubMed: 17137985]
22. Bessho K, Bartsch DU, Gomez L, Cheng L, Koh HJ, Freeman WR. Ocular wavefront aberrations in patients with macular diseases. *Retina*. 2009; 29:1356–63. [PubMed: 19574950]
23. Lombardo M, Serrao S, Ducoli P, Lombardo G. Variations in image optical quality of the eye and the sampling limit of resolution of the cone mosaic with axial length in young adults. *J Cataract Refract Surg*. 2012; 38:1147–55. [PubMed: 22727285]

Wavefront aberrations were higher and Shack-Hartmann image quality was reduced in subjects with diabetic retinopathy with and without foveal thickening. Adaptive optics reduced wavefront error in subjects with diabetic retinopathy, though its performance was suboptimal as compared to control subjects.

Figure 1. Mean and standard deviation of high order root mean square (RMS) (A), total RMS (B), and SH spot area (C) in control and diabetic retinopathy (DR) subjects before and after AO. Asterisk denotes significant difference ($p < 0.05$).

Figure 2. Mean and standard deviation of high order RMS (A), total RMS (B), and SH spot area (C) in diabetic retinopathy subjects with normal (DR-NFT) and increased (DR-IFT) foveal thickness before and after AO. Asterisk denotes significant difference ($p < 0.05$).

Table 1

Comparison of age, visual acuity, and refractive error between control and diabetic retinopathy (DR) groups, and DR subgroups with normal (DR-NFT) and increased (DR-IFT) foveal thickness.

Variables	Groups			Subgroups		
	Control (N = 10)	DR (N = 19)	P value	DR-NFT (N = 11)	DR-IFT (N = 6)	P value
Age (years)	50 ± 15	53 ± 15	0.6	54 ± 14	44 ± 13	0.1
Visual Acuity (Log MAR)	-	0.30 ± 0.25	-	0.33 ± 0.28	0.19 ± 0.18	0.3
Spherical Refractive Error (D)	0.48 ± 1.32	-1.32 ± 2.87	0.4	1.66 ± 3.17	-1.29 ± 2.72	0.8
Cylindrical Refractive Error (D)	0.66 ± 0.44	0.94 ± 0.78	0.3	1.00 ± 0.92	0.67 ± 0.49	0.4

Table 2

Mean and standard deviation of 2nd, 3rd and 4th order root mean square (RMS) and absolute Zernike coefficients in control and diabetic retinopathy (DR) groups, and DR subgroups with normal (DR-NFT) and increased (DR-IFT) foveal thickness.

Zernike Coefficients	Groups			Subgroups		
	NL (N = 10)	DR (N = 19)	P value	DR-NFT (N = 11)	DR-IFT (N = 6)	P value
2 nd Order RMS (μm)	0.29 ± 0.14	0.38 ± 0.23	0.3	0.31 ± 0.19	0.55 ± 0.24	0.04
Z ₂ ⁻²	0.13 ± 0.10	0.23 ± 0.19	0.2	0.19 ± 0.18	0.33 ± 0.21	0.2
Z ₂ ⁰	0.12 ± 0.10	0.13 ± 0.08	0.6	0.12 ± 0.07	0.18 ± 0.10	0.2
Z ₂ ²	0.18 ± 0.14	0.23 ± 0.17	0.5	0.17 ± 0.11	0.35 ± 0.23	0.05
3 rd Order RMS (μm)	0.23 ± 0.11	0.27 ± 0.10	0.3	0.31 ± 0.11	0.24 ± 0.07	0.2
Z ₃ ⁻³	0.12 ± 0.08	0.14 ± 0.07	0.4	0.15 ± 0.08	0.13 ± 0.05	0.6
Z ₃ ⁻¹	0.13 ± 0.11	0.15 ± 0.10	0.7	0.16 ± 0.11	0.15 ± 0.07	0.7
Z ₃ ¹	0.07 ± 0.06	0.11 ± 0.09	0.2	0.14 ± 0.09	0.04 ± 0.02	0.006
Z ₃ ³	0.07 ± 0.05	0.09 ± 0.07	0.5	0.08 ± 0.06	0.09 ± 0.08	0.7
4 th Order RMS (μm)	0.11 ± 0.05	0.20 ± 0.11	0.005	0.18 ± 0.10	0.25 ± 0.14	0.3
Z ₄ ⁻⁴	0.03 ± 0.02	0.06 ± 0.05	0.3	0.07 ± 0.10	0.06 ± 0.05	0.8
Z ₄ ⁻²	0.02 ± 0.01	0.03 ± 0.02	0.3	0.02 ± 0.01	0.04 ± 0.03	0.1
Z ₄ ⁰	0.08 ± 0.06	0.14 ± 0.11	0.1	0.13 ± 0.08	0.18 ± 0.15	0.4
Z ₄ ²	0.02 ± 0.01	0.07 ± 0.06	0.03	0.06 ± 0.05	0.10 ± 0.07	0.2
Z ₄ ⁴	0.04 ± 0.03	0.04 ± 0.03	0.9	0.03 ± 0.02	0.06 ± 0.05	0.1