• Clinical Research •

Short-duration transient visual evoked potentials and color reflectivity discretization analysis in glaucoma patients and suspects

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Abstract

● AIM: To evaluate the use of short-duration transient visual evoked potentials (VEP) and color reflectivity discretization analysis (CORDA) in glaucomatous eyes, eyes suspected of having glaucoma, and healthy eyes.

● METHODS: The study included 136 eyes from 136 subjects: 49 eyes with glaucoma, 45 glaucoma suspect eyes, and 42 healthy eyes. Subjects underwent Humphrey visual field (VF) testing, VEP testing, as well as peripapillary retinal nerve fiber layer optical coherence tomography imaging studies with post-acquisition CORDA applied. Statistical analysis was performed using means and ranges, ANOVA, post-hoc comparisons using Turkey's adjustment, Fisher's Exact test, area under the curve, and Spearman correlation coefficients.

● RESULTS: Parameters from VEP and CORDA correlated significantly with VF mean deviation (MD) (P<0.05). In distinguishing glaucomatous eyes from controls, VEP demonstrated area under the curve (AUC) values of 0.64-0.75 for amplitude and 0.67-0.81 for latency. The CORDA HR1 parameter was highly discriminative for glaucomatous eyes *vs* controls (AUC=0.94).

● CONCLUSION: Significant correlations are found between MD and parameters of short-duration transient VEP and CORDA, diagnostic modalities which warrant further consideration in identifying glaucoma characteristics.

● KEYWORDS: short-duration transient visual evoked potentials; optical coherence tomography; color reflectivity discretization analysis; glaucoma

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INTRODUCTION

Glaucoma is the leading global cause of irreversible blindness[1]. Since early detection and treatment of glaucoma decreases the risk for vision loss and its attendant repercussions, there is a continuous need for identification of acceptable diagnostic tests that are safe, reliable, acceptable to patients, and easy to administer and interpret[2].

Visual evoked potentials (VEP) have been used as a method to assess the functional integrity of the pathway between photoreceptors and the occipital visual cortex. Any pathology along this pathway, such as demyelination, optic atrophy, or stroke, will cause a change in the recorded VEP signal. In patients with glaucoma, VEP could detect and monitor glaucomatous changes[3-6].

Conventional VEP techniques have been limited by issues including repeatability, protracted test duration, and subjective waveform analysis required for interpretation[7]. Technological advances are now allowing clinicians to record and analyze complex VEP signals in a more efficient manner. One such advance, termed short-duration transient visual evoked potentials (SD-tVEP), has been proposed as an objective, non-invasive means to evaluate the integrity of the visual pathway. Figure 1A demonstrates an example report generated by the short duration transient VEP system for a normal control subject. These modifications of VEP technology improve the subjective post-processing required for waveform assessment and demonstrate a capacity in discriminating healthy eyes from those with glaucomatous damage[8].

Color reflectivity discretization analysis (CORDA) is another novel method and software that was developed to evaluate various components of the retinal nerve fiber layer (RNFL) from spectral-domain optical coherence tomography (SD-OCT) images. In applying CORDA, each portion of the image is given a reflectance value, which correlates with a color that is superimposed on the final B-scan image as a colored

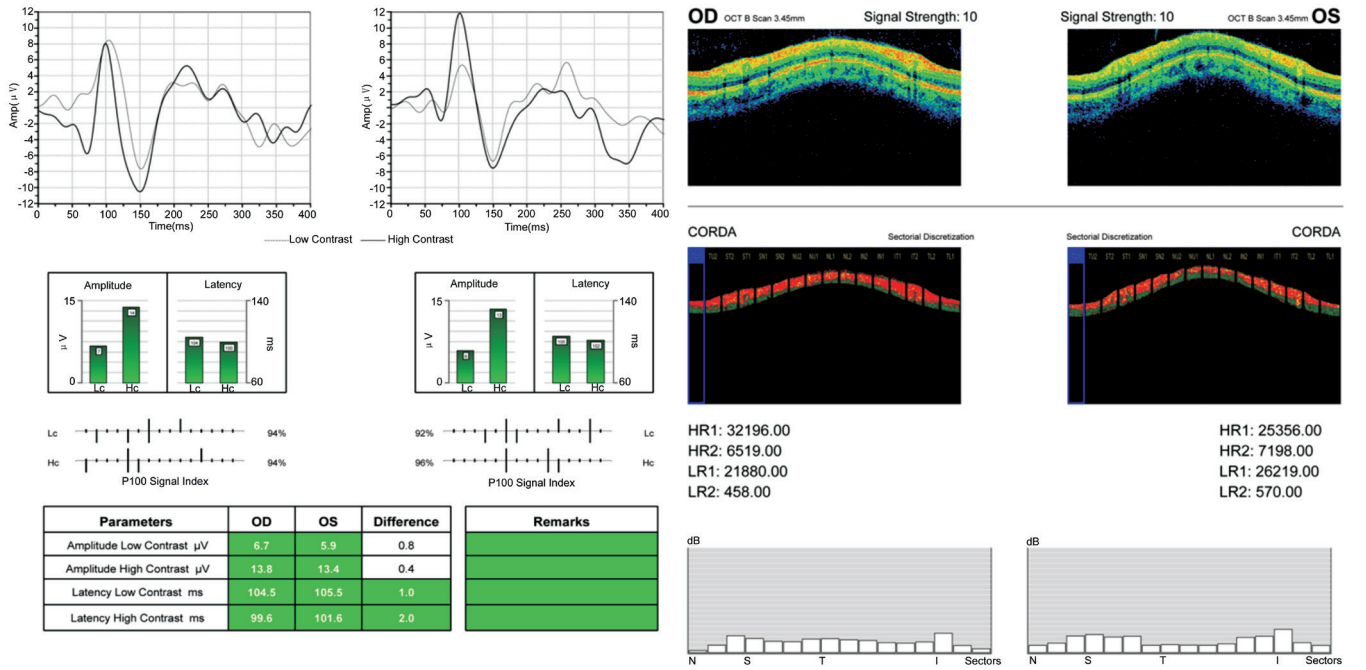

Figure 1 Examples of reports from SD-tVEP (left panel) and the CORDA (right panel) protocol in a normal control A: The output from the SD-tVEP protocol. This includes waveforms indicating the change in amplitude over time for each eye with respect to low contrast (gray) and high contrast (black) stimuli (top two graphs); B: The output from the CORDA protocol. This includes the traditional B-scan images for each eye (top two images) as well as sectorial discretization images (bottom two images) for each eye (left and right images). The CORDA parameters for high reflectivity (HR1 and HR2) and low reflectivity (LR1 and LR2) are presented for each eye.

pixel. The CORDA software quantizes the pixels according to predefined parameters; pixels are assigned values of either high reflectivity (HR1, HR2) or low reflectivity (LR1, LR2). The choice for reflectivity as a potentially beneficial property in RNFL analysis stems from prior studies suggesting that tissues with low reflectivity likely represent glial and vascular tissue, whereas highly reflective tissues likely represent neural elements[9-10]. By parsing out these properties within the RNFL, CORDA may provide a means to distinguish between these tissues and may improve the diagnostic utility of SD-OCT. Figure 1B demonstrates an example report generated by the CORDA system for a normal control subject.

The aim of this study was to assess correlations between visual field (VF), VEP, and CORDA and assess their ability to differentiate between glaucomatous eyes, eyes suspected of having glaucoma, and healthy eyes.

SUBJECTS AND METHODS

Subjects All participants in this study were recruited from the Glaucoma Service at Wills Eye Hospital. Informed consent was obtained from each participant, and all methods were approved by the Wills Eye Hospital Institutional Review Board and adhered to the Declaration of Helsinki for research involving human subjects.

All participants underwent ophthalmologic examination including assessment of medical and family history, best-corrected visual acuity testing, slit-lamp biomicroscopy of the anterior segment, gonioscopy, intraocular pressure measurement with Goldmann applanation tonometry, and fundus examination. VFs were assessed using the Humphrey 24-2 Swedish Interactive Threshold Algorithm (SITA) Standard perimeter (Zeiss Meditec, Dublin, CA, USA).

Patients with glaucoma, glaucoma suspects, and healthy controls were enrolled. The inclusion criteria for all participants consisted of best corrected visual acuity of 20/40 or better, a spherical refraction within ±5.0 D and cylinder correction within ±3.0 D, a normal slit-lamp anterior segment examination, and open angles on gonioscopy. Subjects were excluded if they met any of the following criteria: history of ocular surgery (with the exception of uncomplicated cataract and/or glaucoma surgery), media opacity affecting the ability to obtain good quality SD-OCT images, other diseases affecting the VF (*e.g.* demyelinating diseases, or diabetic retinopathy), use of medications known to affect VF sensitivity (*e.g.* vigabatrin, lamotrigine), or other ocular conditions related to secondary causes of increased intraocular pressure (*e.g.* iridocyclitis, trauma).

Eyes were categorized as glaucomatous if they had characteristic glaucomatous optic neuropathy (local narrowing, notching, or absence of the neuroretinal rim in the absence of disc pallor elsewhere) with corresponding VF defects. Eyes were classified as glaucoma suspect if optic discs appeared glaucomatous (*e.g.* increased cup to disc area ratio (>0.7), rim thinning, or RNFL defects) or if intraocular pressure was ≥21 mm Hg, with a normal VF result in both eyes. Healthy eyes were characterized

by intraocular pressure ≤21 mm Hg, normal optic disc appearance, and normal RNFL and VF results in both eyes.

Visual Evoked Potentials The VEP method used in this study was a modified extension of the Diopsys Enfant NOVA system (Diopsys Enfant Amp 100, Diopsys Inc., Pine Brook, NJ, USA). The stimulus was presented on an Acer V173 17-inch LCD monitor running at 75 frames per second. Output over time was verified using a luminance meter Mavo-Spot 2 USB (Gossen, GmbH, Nürnberg, Germany). Gold cup electrodes (10 mm) with commercially available skin preparation and eletroencephalogram (EEG) paste were used for recording the short duration transient VEP. Synchronized single-channel VEP recordings were produced, generating time series of 512 data points per analysis window. The room luminance was maintained at scotopic conditions (<0.3 candelas per square meter). Pre-adaptation was not required for the VEP recordings. Each phase reversal was set at 500ms and was monitored for effects due to blinking or eye movement. A technique for artifact rejection was used during VEP data recording; if half or more of the phase reversals were rejected, then the entire run was rejected. The maximum run time for a single test was limited to 20s. Each complete VEP protocol was comprised of multiple test instances, with a complete protocol presenting the stimulus for a maximum of 1min and 46s.

In all cases, the stimulus was viewed through non-dilated pupils with optimal refractive correction in place. The viewing distance was set to 1 m, yielding a total display-viewing angle of 15.5 degrees. The square black/white checkerboard pattern reversal stimulus had a height and width of 27 cm with a red circular ring used as a fixation target. The diameter of this target was approximately 1.5 cm with a ring thickness of 1.5 mm. The target ring was centered on the stimulus. The check size was 29.0min of arc. Two types of contrast patterns were used in the study, based on earlier studies suggesting that differential contrast stimulation activates distinct populations of cells within the visual pathway, which may show different rates of glaucomatous damage[11]. The two patterns used represented high contrast (HC, Michelson contrast of 85%) and low contrast (LC, Michelson contrast of 15%).

During each recording session, the contrast polarity of each stimulus check was temporally modulated at a reversal frequency of 1 Hz (2 pattern reversals equates to 1 reversal cycle); therefore, each reversal occurred at 2 Hz or twice per second. This stimulus was a pattern reversal stimulus with a duty cycle of 50%. The 15% and 85% contrast stimuli were presented for a period of 20s while the untested eye remained covered. The study eye was randomly selected for all patients.

In preparation for VEP recording, the skin at each electrode site was scrubbed with Nuprep (D.O. Weaver & Co, Aurora, CO, USA) using a cotton gauze pad. Electrodes were fixed in position with a small gauze pad applied with Ten20 conductive paste (D.O. Weaver&Co, Aurora, CO, USA). Electrode impedance was maintained below 10 kΩ and typically kept below 5 kΩ. The gain of the EEG analog amplifier/filter module in the Enfant system was set at 10 000 with band-pass filter frequency range of 0.5 to 100 Hz. The EEG signal was sampled at 1024 Hz using the Enfant system's analog-to-digital (A/D) converter, with the EEG filter gain of 10 000 representing the only gain in the entire data acquisition path, including the (A/D) 12-bit convertor. For this study, the A/D converter was programmed to operate across a voltage range of -1.25 V to +1.25 V with a resolution of 610 mV/quantum.

Output parameters from the VEP system included amplitude and latency measures for each of the contrast stimuli. The amplitude parameter (measured in microvolts) reflected the strength of the signal being transmitted through the visual pathway, essentially an indication of neural structural integrity, including axons. The latency parameter (measured in milliseconds) was a measure of the length of time for signal transmission along the primary visual pathway. Damage due to ischemia, compression, and toxic exposure can decrease VEP amplitude, whereas optic nerve demyelination can result in increased VEP latency[12]. Thus, the combination of these two VEP parameters may be helpful in assessing the overall health of the visual pathway.

Spectral-domain Optical Coherence Tomography Images The Cirrus HD-OCT system (Carl Zeiss Meditec, Inc., Dublin, CA, USA; Model 4000, software version 5.1.1.4) used for this study included a super luminescent diode emitting a wavelength of 840 nm. Details of this instrument have been described elsewhere[13-15]. In this study, the ONH cube 200×200 scanning protocol was used. This protocol consisted of raster scans containing 200×200×1024 samplings within 6×6 mm, resulting in 40 000 A-scans acquired in 1.48s. The resulting circular scan of 3.46 mm in diameter provided a B-scan image that was shown in a false color scale representing the multiple retinal strata. A certified operator completed the SD-OCT protocol in a single session.

Color Reflectivity Discretization Analysis The SD-OCT cross-sectional tomographs (B-scans) were exported as JPEG images and uploaded for further analysis to a separate workstation equipped with CORDA software (Diopsys Inc., Pine Brook, NJ, USA).

CORDA is an image analysis solution that was developed to evaluate the various components of the RNFL from SD-OCT images. The software quantifies the pixels within the image according to four discrete reflectivity parameters (HR1, HR2, LR1, and LR2) from which the relative occurrence of each parameter can be quantified. Areas designated with the HR parameters demonstrate high reflectivity (*e.g.* axons), whereas areas designated with the LR parameters demonstrate low reflectivity (*e.g.* glial or other supporting tissue).

The CORDA algorithm performs global and sectorial discretization of SD-OCT images and returns the information in a global RNFL pseudocolored image. In the sectorial analysis, the RNFL is divided into 16 sectors and the pixels in each sector are quantified. An average of superior and inferior sectors is then computed. An additional parameter, luminance, is calculated, which reflects overall average RNFL reflectivity.

Statistical Analysis Continuous variables (age, VF, SD-OCT, VEP and CORDA parameters) were summarized using means and ranges, and groups were compared with ANOVA followed by post-hoc comparisons using Tukey's adjustment for multiple comparisons. Comparisons among groups for discrete variables were analyzed using Fisher's exact test or non-paired *t*-tests.

The area under the curve (AUC) for the receiver operating characteristic (ROC) curve was estimated for each of the VF parameters as well as parameters from each calculation method (traditional SD-OCT, VEP, and CORDA) for differentiating eyes with glaucoma from normal controls, and for differentiating glaucoma suspect eyes from normal controls. Spearman correlation coefficients were calculated to assess the relationship between the VF parameters [mean deviation (MD) and pattern standard deviation (PSD)] and each of the parameters from the two novel methods (VEP and CORDA).

RESULTS

A total of 136 eyes were studied from the 136 subjects enrolled in the study. For each patient, the study eye was randomly selected. The average age of all subjects was 60.6y (range 24-86) and 58% were male. The 3 groups of study involved 49 glaucoma patients, 45 glaucoma suspects, and 42 healthy subjects. Demographic and clinical characteristics for the three groups are summarized in Table 1. Average RNFL thickness computed from SD-OCT was 71.2 microns for the glaucoma group, 87.5 microns for the glaucoma suspect group, and 95.1 microns for the normal control group (P<0.05 for all pairwise comparisons).

Correlations Between Glaucoma Testing Parameters In order to compare the methods evaluated in this study with existing standards, we first examined the correlation of each of the functional parameters with VF parameters (Table 2). Of the VEP parameters, latency at high contrast (LHC) and amplitude at low contrast (ALC) demonstrated the strongest correlations with MD (SCC -0.38, P<0.001 and SCC 0.34, P<0.001). The VEP parameters amplitude at high contrast (AHC) and latency at low contrast (LLC) were also significantly correlated with MD (SCC 0.30, P<0.001 and SCC -0.18, P=0.045, respectively). As seen in Figure 2, the CORDA parameters HR1 and HR2 were significantly correlated with MD (SCC 0.49, P<0.001 for HR1 and 0.50, P<0.001 for HR2). The correlations were less significant when broken down by patient groups however. This was true for HR1 (SCC 0.10, P=0.52 for normal patients, SCC 0.39, P<0.001 for glaucoma suspects, SCC 0.22, P=0.15 for glaucoma patients) and HR2 (SCC 0.11, P=0.48 for normal patients, SCC 0.42, P<0.002 for glaucoma suspects, SCC 0.18, P=0.23 for glaucoma patients).

Additionally, we examined correlations between VEP and CORDA and found that all 4 VEP parameters were significantly correlated with the CORDA parameter HR1. These results demonstrate the strongest correlation between

Table 1 Demographic and clinical characteristics of the study patients by group $\bar{x} \pm s$, (n)

Variables	Glaucoma (n=49)	Glaucoma suspect (n=45)	Normal controls (n=42)
Demographics			
Age (a)	68.2±11.5	56.2±15.7	56.4±14.7
Gender, *n* (%)			
Female	27 (55)	18 (40)	12 (29)
Male	22 (45)	27 (60)	30 (71)
Race, *n* (%)			
Caucasian	27 (55)	13 (29)	30 (72)
African-American	21 (43)	24 (53)	11 (26)
Hispanic	1 (2)	4 (9)	0 (0)
Asian	0 (0)	4 (9)	1 (2)
VF			
MD	-8.0±7.3 (48)[a,c]	-1.3±1.7 (45)	-1.0±1.0 (42)
PSD	5.7±3.9 (48)[a,c]	1.8±0.9 (45)	1.5±0.4 (42)
OCT			
Average RNFL	71.2±12.1 (49)[a,c]	87.5±13.4 (45)[a]	95.1±8.7 (42)
Rim area	0.9±0.3 (49)[a,c]	1.1±0.3 (45)[a]	1.3±0.2 (42)
Disc area	1.9±0.4 (49)	2.1±0.5 (45)[a]	1.8±0.3 (42)
Average C/D	0.8±0.3 (49)[a,c]	0.7±0.1 (45)[a]	0.5±0.1 (42)
Vertical C/D	0.7±0.1 (49)[a,c]	0.6±0.1 (45)[a]	0.4±0.1 (42)
Cup volume	0.5±0.4 (48)[a]	0.4±0.3 (45)[a]	0.1±0.1 (42)
SD-tVEP			
ALC	4.1±2.7 (45)[c]	7.1±3.9 (43)	6.7±3.3 (38)
AHC	6.6±4.5 (47)	11.2±14.1 (45)	9.1±5.6 (40)
LLC	130.4±18.4 (45)	123.1±14.5 (45)	122.8±17.6 (38)
LHC	124.1±14.7 (47)[a,c]	114.8±15.4 (45)	113.0±19.1 (40)
CORDA			
HR1	13396±4473 (49)[a,c]	17448±5773 (45)[a]	23035±4468 (42)
HR2	1967±1651 (49)[a,c]	3873±2568 (45)[a]	5709.1±1569 (42)
LR1	28361±4554 (49)	27920±4142 (45)	28668±2740 (42)
LR2	565±259 (49)[c]	860±374 (45)[a]	436±169 (42)
Luminance	38.3±3.8 (49)[a]	37.5±6.7 (45)[a]	43.5±3.8 (42)

VF: Visual field; MD: Mean deviation; PSD: Pattern standard deviation; OCT: Optical coherence tomography; RNFL: Retinal nerve fiber layer; C/D: Cup to disc ratio; SD-tVEP: Short duration transient visual evoked potentials; ALC: Amplitude at low contrast; AHC: Amplitude at high contrast; LLC: Latency at low contrast; LHC: Latency at high contrast; CORDA: Color reflectivity discretization analysis; HR1: High reflectivity parameter 1; HR2: High reflectivity parameter 2; LR1: Low reflectivity parameter 1; LR2: Low reflectivity parameter 2. [a]P<0.05 for Tukey-adjusted pairwise ANOVA comparisons *vs* normal controls; [c]P<0.05 for Tukey-adjusted pairwise ANOVA comparisons *vs* glaucoma suspects.

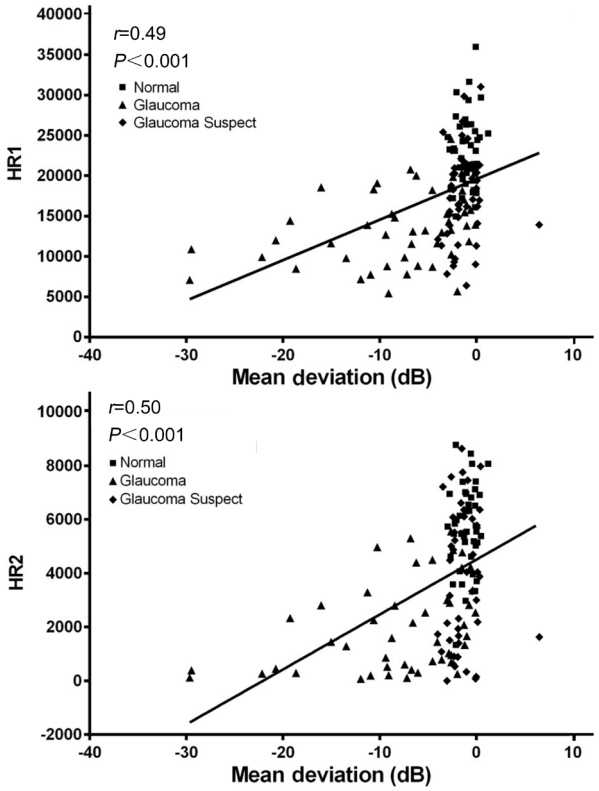

Figure 2 Correlation of MD and CORDA high reflectivity parameters (HR1 and HR2).

Table 2 Analysis of Spearman correlations between novel diagnostic clinical parameters and VF parameters MD and PSD for all subjects

Parameters	SCC (*P*)	
	MD	PSD
SD-tVEP		
ALC	0.34 (<0.001)	-0.29 (<0.001)
AHC	0.30 (<0.001)	-0.21 (0.018)
LLC	-0.18 (0.045)	0.26 (0.003)
LHC	-0.38 (<0.001)	0.31 (<0.001)
CORDA		
HR1	0.49 (<0.001)	-0.47 (<0.001)
HR2	0.50 (<0.001)	-0.48 (<0.001)
LR1	0.03 (0.720)	-0.12 (0.163)
LR2	-0.09 (0.289)	0.01 (0.888)

SD-tVEP: Short duration transient visual evoked potentials; ALC: Amplitude at low contrast; AHC: Amplitude at high contrast; LLC: Latency at low contrast; LHC: Latency at high contrast; CORDA: Color reflectivity discretization analysis; HR1: High reflectivity parameter 1; HR2: High reflectivity parameter 2; LR1: Low reflectivity parameter 1; LR2: Low reflectivity parameter 2.

the VEP parameter LHC and CORDA HR1 (SCC -0.47, *P*<0.001) with other significant correlations identified between LLC and HR1 (SCC -0.31, *P*<0.001), ALC and HR1 (SCC 0.28, *P*=0.001), and AHC and HR1 (0.19, *P*=0.026). Within CORDA analysis, we analyzed correlation between age and HR1 and HR2. There was a strong correlation with increasing age for HR1 (SCC -0.59, *P*<0.001) and HR2 (SCC -0.53, *P*<0.001), as seen in Figure 3.

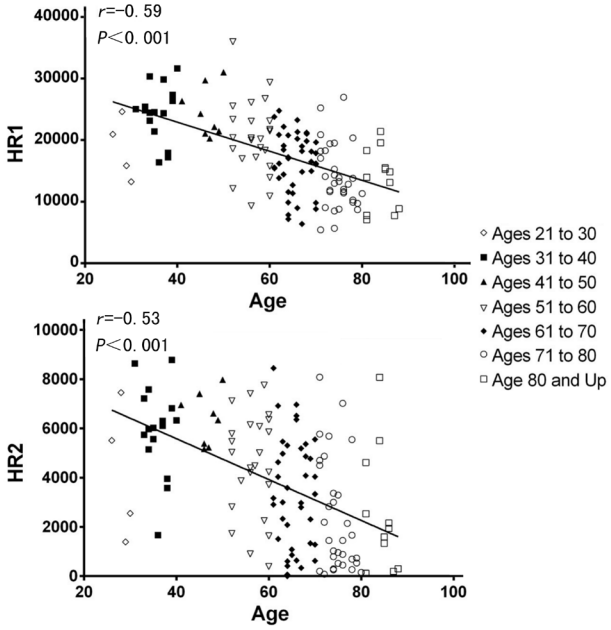

Figure 3 Age against CORDA high reflectivity parameters (HR1 and HR2).

Table 3 AUC from logistic regression of individual clinical parameters for distinguishing glaucoma patients versus normal controls (second column) and glaucoma suspects versus normal controls (third column)

Variables	AUC (95% CI)	
	Glaucoma *vs* normal	Glaucoma suspects *vs* normal
OCT		
Average RNFL	0.93 (0.88, 0.98)	0.66 (0.55, 0.78)
SD-tVEP		
ALC	0.75 (0.65, 0.86)	[1]0.52 (0.39, 0.65)
AHC	0.64 (0.53, 0.76)	[1]0.54 (0.42, 0.67)
LLC	0.67 (0.55, 0.79)	[1]0.55 (0.42, 0.67)
LHC	0.81 (0.71, 0.91)	[1]0.57 (0.45, 0.70)
CORDA		
HR1	0.94 (0.90, 0.98)	0.78 (0.68, 0.88)
HR2	0.94 (0.90, 0.99)	0.70 (0.59, 0.81)
LR1	[1]0.54 (0.42, 0.66)	[1]0.53 (0.41, 0.66)
LR2	0.65 (0.53, 0.76)	0.86 (0.77, 0.94)

OCT: Optical coherence tomography; RNFL: Retinal nerve fiber layer; SD-tVEP: Short duration transient visual evoked potentials; ALC: Amplitude at low contrast; AHC: Amplitude at high contrast; LLC: Latency at low contrast; LHC: Latency at high contrast; CORDA: Color reflectivity discretization analysis; HR1: High reflectivity parameter 1; HR2: High reflectivity parameter 2; LR1: Low reflectivity parameter 1; LR2: Low reflectivity parameter 2. [1]AUC not better than chance. Values presented represent area under the ROC curve (95% confidence interval).

Area Under the Receiver Operating Characteristic Curve

The area under the ROC curve was calculated for the methods evaluated in this study in two discriminatory tasks-glaucoma *vs* healthy eyes and glaucoma suspect *vs* healthy eyes (Table 3). Mean RNFL thickness calculated from traditional SD-OCT analysis had an AUC of 0.93 in distinguishing glaucomatous

eyes from healthy eyes. For the glaucoma versus control task, the VEP parameters had AUC values of 0.75 for low-contrast amplitude (ALC) and 0.81 for LHC. Both of the CORDA HR1 and HR2 parameters had an AUC of 0.94 in distinguishing glaucomatous eyes from healthy ones.

For the analysis distinguishing glaucoma suspects from healthy controls, the VEP parameters had AUC values of 0.55 for LLC and 0.57 for LHC. The CORDA parameter LR2 (AUC=0.86) showed superior performance to the traditional SD-OCT parameter of RNFL thickness (AUC=0.66).

DISCUSSION

The aim of this study was to investigate the utility of two novel diagnostic modalities in differentiating between glaucomatous eyes, glaucoma suspect eyes, and healthy eyes. Specifically, we evaluated parameters produced by SD-tVEP and CORDA post-acquisition analysis software, and examined their correlations with VF MD as a surrogate for disease severity. These results represent the first direct comparison of these modalities and their ability to discriminate between glaucomatous eyes, glaucoma suspect eyes, and normal control eyes.

VEP technology has shown a capacity in discriminating between healthy eyes and those suspect for glaucoma or those already with glaucomatous damage[5-6,16]. The VEP method offers an objective, non-invasive means to evaluate the integrity of the visual pathway. SD-tVEP is a modified VEP procedure that has been shortened and simplified while maintaining good repeatability at both low and high contrast[17]. In the present study, we demonstrate significant correlations between SD-tVEP amplitude parameters and glaucomatous damage as indicated by VF MD. These results are consistent with Prata *et al*[8], who demonstrated significant correlations between amplitude (ALC and AHC) and latency (LLC) SD-tVEP parameters in glaucomatous eyes. However, their study did not find significant correlations with LHC, a parameter that had the strongest correlation with VF MD in the current study (-0.38, $P<0.001$)[8]. The difference in patient populations could be a contributing factor to these discrepant results.

The CORDA software algorithm is based on measurements of RNFL reflectivity from SD-OCT B-Scan images. Dark colors (black and blue) represent regions of minimal optical reflectivity, whereas bright colors (red and white) represent regions of high reflectivity. This approach is believed to aid in the early detection of retinal structural defects, as signals attributed to lowly reflective tissues (*e.g.* supportive glial and vascular tissue) can be distinguished from those of highly reflective nervous tissue. Among the CORDA parameters tested in this study, the strongest correlations were found between VF MD and the high reflectivity CORDA parameters (HR1 and HR2).

Overall, the significant correlations identified between VEP, CORDA, and traditional VF parameters suggest that VEP and CORDA may be able to account for some of the functional findings typically identified in VF measurements while also providing new insight on structural changes, particularly with the CORDA high reflectivity parameters.

Previous studies on CORDA have demonstrated the utility of the technique in distinguishing between normal and glaucomatous eyes[18], but the aim of this study was to compare its utility to SD-tVEP. The AUC results for discriminating glaucomatous eyes from normal eyes revealed that CORDA parameters (particularly HR1) showed comparable accuracy to that of the traditional SD-OCT mean RNFL thickness (0.94 and 0.93, respectively). Of the VEP parameters, high contrast latency (LHC) and low contrast amplitude (ALC) demonstrated the highest AUC in differentiating between glaucoma patients and controls; however, AUC valuesfor the VEP parameters were lower than those of the CORDA and SD-OCT parameters. Overall, the VEP parameters demonstrated lower AUC values in identifying glaucoma suspect eyes.

In distinguishing glaucoma suspect eyes from healthy eyes, the CORDA HR1 (AUC=0.78) and LR2 (AUC=0.86) parameters demonstrated higher AUC compared to the traditional SD-OCT RNFL thickness (AUC=0.66). For comparison, Lisboa *et al*[19] used RTVue SD-OCT to evaluate AUCs of different parameters for differentiating glaucoma suspects from normal. The highest AUC they found was of mean RNFL (0.89), followed by ganglion cell complex average thickness (0.79) and vertical cup-to-disc ratio (0.74)[19]. Further study is needed in order to directly compare the new CORDA parameters and macular as well optic nerve head measurements obtained by traditional SD-OCT.

Prior studies have indicated that gender can affect the VEP results[20]. To account for this, gender comparisons were done using a non-paired *t*-test among the 4 parameters for SD-tVEP, as well as the 4 parameters for CORDA. Only the ALC (P=0.0029) of the VEP and the HR1 (P=0.0276) of the CORDA showed statistically significant differences between the genders. This could be accounted for by our smaller sample size, or our skewed gender distribution in all groups. The glaucoma group skewed slightly female, while the glaucoma suspect and normal groups skewed male as shown in Table 1. Future studies should aim to analyze a more equally distributed population.

This prospective study was limited by a relatively small sample size in proportion to the number of comparisons. The patients included in this study were evaluated at a single large academic eye hospital centrally located in a large urban setting. Future studies should use a larger sample size, consider other patient populations and use multiple locations so results can be applied more generally. Additionally, further analyses comparing CORDA and SD-tVEP to several advanced methods for improved glaucoma detection (*e.g.* the ganglion cell-inner

plexiform layer[21] or inter ocular comparison of VEP[22]) may be useful. Another important limitation in this study is the age difference between the groups of glaucomatous eyes (68±11.5y), eyes suspect for having glaucoma (56±15.7y), and healthy control eyes (56±14.7y). Age has been demonstrated as a contributing factor to RNFL thinning[23] as well as some gradual attenuation in VEP amplitude, and thus may contribute additional confounding to the differences observed between these groups. Figure 3 shows age plotted against HR1 and HR2, and both parameters show a trend of decreasing with increasing age group. Our study population fell primarily in the 71-80 age range, and future studies should incorporate analysis with more subjects different age groups to increase the reliability of our CORDA and VEP comparison. Moreover, another important limitation to consider relates to the CORDA methodology, which is based upon developing a reflectivity profile of the RNFL. Although the broad differentiation in components of RNFL based on relative reflectivity may be beneficial, the groupings may still be too discrete to account for subtle changes occurring as glaucoma begins to develop. This may be reflected in the wide range of HR1 and HR2 values observed in Figure 2 for patients with low MD (presumably accounting for the majority of healthy controls and glaucoma suspects). Future studies could incorporate alternate approaches to analyzing the CORDA data, such as dividing it into upper and lower field and analyzing these separately.

In conclusion, this study demonstrates evidence of the discriminatory ability of short duration transient VEP and CORDA methods, which may support their consideration as ancillary tests in differentiating glaucomatous eyes from normal controls. We found significant correlations between VEP parameters and the structural measurements from CORDA, though the diagnostic ability of VEP was less than CORDA. In addition, the results from the AUC analysis for glaucoma suspects suggest that the novel analytical approach using CORDA may demonstrate additional utility in identifying early glaucomatous optic nerve changes prior to the onset of VF deficits. Extension of this work into further investigation of the role of VEP and CORDA methods for evaluation of glaucoma progression is warranted.

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