INTRODUCTION

Diurnal variations occur in many ocular parameters amongst which diurnal fluctuation of intraocular pressure (IOP) has been well studied.\textsuperscript{[1-3]}  Introduction

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

and evolution of optical coherence tomography (OCT) has made the evaluation of diurnal retinal thickness changes possible. OCT is now commonly used for reliable in vivo measurements of retinal thickness both in research and clinical practice. The development of spectral domain OCT (SD-OCT) with higher speed and resolution has led to highly precise and reproducible images as compared to the previous generation of time domain OCT (TD-OCT).\[4\]

Several studies have demonstrated diurnal fluctuation in retinal thickness among normal individuals as well as diabetic patients using older generations of OCT (OCT1) and TD-OCT.\[5,8\] They reported higher retinal thickness in the morning which corresponds to lower vision in the early morning particularly in patients with diabetic macular edema (DME). However, there are few studies evaluating diurnal variations in retinal thickness using SD-OCT in health and disease.\[9-12\] These studies have not shown significant diurnal retinal thickness changes using SD-OCT contradicting the results of TD-OCT.

Central corneal thickness (CCT) measurement is commonly performed as a part of glaucoma management for which ultrasonic pachymetry is currently the gold standard. Studies have shown diurnal variations in CCT with higher values in the early morning.\[13,14\]

Diurnal fluctuations in ocular parameters may affect interpretation of a single measurement. The present study was conducted to evaluate diurnal variations in ocular parameters including CCT, IOP, and macular and retinal nerve fiber layer (RNFL) thickness using SD-OCT, in normal and diabetic patients.

RESULTS

Baseline characteristics of the study groups which included 11 diabetic patients with DME and 11 healthy individuals are detailed in Table 1. The control group were younger than the diabetics (P = 0.035).

Table 1. Baseline characteristics of the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal (n=11)</th>
<th>Diabetic (n=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48±9.48 (37-71)</td>
<td>55±7.54 (45-65)</td>
<td>0.035</td>
</tr>
<tr>
<td>Female (%)</td>
<td>7 (63.6)</td>
<td>6 (54.5)</td>
<td>0.665</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>120±14</td>
<td>259±135</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BCVA (LogMAR)</td>
<td>0.1 (20/36)</td>
<td>0.7 (20/100)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

FBS, fasting blood sugar; BCVA, best corrected visual acuity; LogMAR, logarithm of the minimum angle of resolution; mg/dl, milligrams per deciliter

On the day of examination, all patients underwent a series of measurements at 9 AM, 12 PM, 3 PM and 6 PM including blood pressure (BP), best corrected visual acuity (BCVA), IOP using a calibrated Golmann applanation tonometer (GAT BQ900, Haag-Streit, Konitz, Switzerland), CCT using an ultrasonic pachymeter (Pachymeter SP 3000, Tomey, Nagoya, Japan), and macular and peripapillary RNFL thickness using Topcon 3D OCT-1000 (Topcon Corp, Tokyo, Japan). OCT scans were performed using a raster scan pattern (512 × 128 axial A-scans) in a 6 × 6 mm² area centered on the fovea or optic disc. Each measurement was repeated twice and the mean of the two readings for IOP, CCT, total macular volume (TMV), average macular thickness (AMT), and average RNFL thickness were used for further analysis. BCVA and fasting blood sugar were measured only once in the morning. We did not measure BCVA during the day because dilating the pupil and multiple measurements could affect vision.

Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA). To describe data, we used mean ± standard deviation (SD) and 95% confidence intervals (CIs). To compare values, absolute changes in any parameter as well as relative changes were calculated. To evaluate the reproducibility of the measurements, intraclass correlations (ICCs) were calculated. Mann-Whitney U test was used for comparing quantitative variables between the two groups. P-values less than 0.05 were considered as statistically significant.
Diurnal Variations in Retinal and Corneal Thickness; Sharifipour et al

decreased gradually during the course of the day to its lowest value at 6 PM. Diabetic patients had significantly higher CCT as compared to normal controls at all time points, and had the highest CCT at 6 PM and the lowest at 3 PM [Figure 1]. Diabetics had significantly lower RNFL thickness as compared to normal individuals. Both groups showed a non-significant decrease during the course of the day. The control group had significantly higher RNFL thickness at all time points.

Table 2. Measurements at four different hours on a single day in diabetics and normal subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal* Absolute change</th>
<th>Relative change (%)</th>
<th>Diabetic* Absolute change</th>
<th>Relative change (%)</th>
<th>P* (between the study groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL 9 am</td>
<td>100.2±5.6</td>
<td></td>
<td>95.4±28.3</td>
<td></td>
<td>0.045</td>
</tr>
<tr>
<td>RNFL 12 pm</td>
<td>99.7±5</td>
<td>−0.5±3.7</td>
<td>90.2±28.1</td>
<td>−5.2±6.5</td>
<td>0.002</td>
</tr>
<tr>
<td>RNFL 3 pm</td>
<td>99.6±6.7</td>
<td>−0.6±3.6</td>
<td>89.3±26.5</td>
<td>−6.1±6.8</td>
<td>0.001</td>
</tr>
<tr>
<td>RNFL 6 pm</td>
<td>94.9±12.3</td>
<td>−5.3±8.9</td>
<td>87.6±29.9</td>
<td>−7.8±10.7</td>
<td>0.005</td>
</tr>
<tr>
<td>TMV 9 am</td>
<td>7.5±0.4</td>
<td></td>
<td>9.4±1.5</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TMV 12 pm</td>
<td>7.4±0.5</td>
<td>−0.1±0.2</td>
<td>9.3±1.5</td>
<td>−0.1±0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TMV 3 pm</td>
<td>7.5±0.4</td>
<td>−0.1±0.1</td>
<td>9.2±1.4</td>
<td>−0.2±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TMV 6 pm</td>
<td>7.5±0.4</td>
<td>−0.1±0.1</td>
<td>9.3±1.3</td>
<td>−0.1±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CMT 9 am</td>
<td>214±28</td>
<td></td>
<td>291±105</td>
<td></td>
<td>0.044</td>
</tr>
<tr>
<td>CMT 12 pm</td>
<td>213±25</td>
<td>−1±22</td>
<td>280±122</td>
<td>−10±49</td>
<td>0.012</td>
</tr>
<tr>
<td>CMT 3 pm</td>
<td>205±28</td>
<td>−8±19</td>
<td>278±87</td>
<td>−12±60</td>
<td>0.033</td>
</tr>
<tr>
<td>CMT 6 pm</td>
<td>209±29</td>
<td>−4±9</td>
<td>264±81</td>
<td>−27±60</td>
<td>0.028</td>
</tr>
<tr>
<td>AMT 9 am</td>
<td>266±13</td>
<td></td>
<td>332±52</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AMT 12 pm</td>
<td>263±15</td>
<td>−3.3±5.9</td>
<td>324±58</td>
<td>−8.8±15</td>
<td>0.003</td>
</tr>
<tr>
<td>AMT 3 pm</td>
<td>264±13</td>
<td>−1.8±2</td>
<td>320±44</td>
<td>−12.7±25</td>
<td>0.001</td>
</tr>
<tr>
<td>AMT 6 pm</td>
<td>265±13</td>
<td>−1.1±2.5</td>
<td>333±45</td>
<td>0.9±18</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

, minimum value; , maximum value. *None of the values for each parameter were significantly different from the others. (ANOVA, all P>0.05), RNFL, retinal nerve fiber layer thickness (µm); TMV, total macular volume (mm³); CMT, central macular thickness (µm); AMT, average macular thickness (µm), †Based on Mann-Whitney U test

Figure 1. Diurnal variations in central corneal thickness (CCT) in controls and diabetics were not significant. The diabetic group had consistently higher CCT.

Figure 2. Diurnal variations in retinal nerve fiber layer (RNFL) thickness in control and diabetic groups. Both groups showed a non-significant decrease during the course of the day. The control group had significantly higher RNFL thickness at all time points.

DISCUSSION

The current study showed slight non-significant diurnal variations in retinal and corneal thickness both in diabetics and healthy individuals which seem to be clinically irrelevant.

The presence of diurnal IOP variations has been the subject of several studies in normal and glaucomatous
Diurnal Variations in Retinal and Corneal Thickness; Sharifipour et al

Figure 3. Diurnal variations in total macular volume (TMV) in the control and diabetic groups. While diabetics showed significantly higher values, the diurnal changes were not significant in either group.

Figure 4. Diurnal variations in central macular thickness (CMT) in the control and diabetic groups were not significant in either group. Diabetics demonstrated significantly higher CMT; both groups showed the highest value at 9 AM.

eyes. Most studies are in agreement that IOP is higher in the early morning.[1,2] IOP fluctuation in normal eyes usually does not exceed 6 mmHg; however, fluctuations more than 10 mmHg are suggestive of glaucoma.[3] In the present study, normal eyes had the highest IOP at 9 AM and the lowest at 12 PM; diabetics had their corresponding values at 6 PM and 12 PM, respectively. The range of IOP fluctuation was 2 mmHg in controls and 1.6 mmHg in diabetics which was comparable. We had a small sample size and did not measure IOP immediately upon awakening. Thus, we might have missed higher IOPs. Nevertheless, the normal group showed a pattern of IOP fluctuations comparable to previous reports.

The cornea is believed to have its greatest thickness on awakening with a rapid decrease in thickness in a few hours. However, in a study on corneal thickness over a 48-hour period, it was shown that corneal thickness is highly variable during any 24-hour period and does not follow a regular pattern of overnight swelling and exponential decay.[13] Another study measuring CCT and IOP in healthy individuals over a 24-hour period revealed the highest values on awakening at 7 AM which dropped rapidly to baseline by 9 AM.[14] Our study showed a similar pattern in normal individuals. However, diabetics had the highest CCT at 6 PM and the lowest at 12 PM and also significantly higher CCTs as compared to the control group at all time points.

Normal physiological variations have been reported in retinal thickness along with most studies reporting greater macular thickness in the morning. Similarly, in patients with DME, a significant decrease has been reported in retinal thickness measurements by OCT over the course of the day.[5,7] Larsen et al evaluated overnight retinal thickness changes in patients with DME and normal individuals and observed no significant changes in normal individuals.[6] However, patients with DME showed significantly higher macular thickness and lower BCVA in the morning as compared to the evening. In these studies, OCT images were obtained using the first generation of OCT with lower resolution and reproducibility. In our study, however, we observed the lowest retinal thickness at 6 PM. In a study on DME and control eyes, Polito et al measured macular thickness by Stratus OCT at 4 time points on a single day.[7] No significant diurnal variation in retinal thickness was detected in control eyes and DME patients with CMT <300 μm. However, those with macular thickness ≥300 μm showed a significant decrease in thickness during the day. Moreover, it has been reported that foveal thickness decreases in both upright and recumbent positions.[12] In a report by Diabetic Retinopathy Clinical Research Network using Stratus OCT3 in 156 eyes with DME, a non-significant decrease in CMT was observed between 8 AM and 4 PM.[8]

Considering the continuous evolution of OCT technology from OCT1 to OCT3 and SD-OCT which has resulted in more reproducible and highly precise images, studies with older instruments should be interpreted cautiously. In one study comparing OCT1 and Stratus OCT3 in normal and DME eyes,[15] Stratus measurements were significantly higher than OCT1 readings, and artifacts were significantly more frequent with OCT1 and observed only in DME. Other studies using Stratus OCT3 showed a reproducibility of 10% in diabetic patients without macular edema[14] and 11% in subjects with DME.[17] It was concluded that changes more than 10-11% seem to be real and those less than these values are probably due to measurement errors.

Odell et al evaluated sources of error in macular thickness measurements by Cirrus SD-OCT and found that errant fixation has the greatest impact on the accuracy of OCT images specifically in macular diseases.[18] Fast
scans and differences in axial length may also affect the accuracy and reproducibility of OCT images. Thus, it is possible that some of the significant changes reported in older studies were actually due to measurement errors especially in patients with DME and low vision. This may cause fixation errors resulting in measurements not to be performed exactly in previously measured area. SD-OCT has shown higher reproducibility for both macular and RNFL thicknesses.

It has also been suggested that hypoglycemia may play a role in the lack of diurnal CMT decrease in patients with DME. In the present study, we were not able to evaluate such a correlation because only one single morning blood sugar measurement was obtained. Most studies investigating diurnal macular changes using SD-OCT have revealed non-significant changes in retinal thickness over the course of the day in normal eyes and patients with DME. Furthermore, SD-OCT machines have shown more reproducible thickness measurements as compared to TD-OCT. SD-OCT measurements have been reported to be significantly higher with more repeatability as compared to TD-OCT in DME patients.

Although a few studies have evaluated diurnal variations of retinal thickness in patients with DME, we observed a nearly comparable pattern in normal and DME eyes. All macular parameters except for AMT in diabetics were highest at 9 AM with a subsequent decrease during the day. In our study, the highest reproducibility of 3D-OCT 1000 was for CMT measurements and the lowest was for TMV.

RNFL measurements have been reported to be less consistent as compared to macular thickness measurements. Menke et al showed that reproducibility of RNFL measurements using Topcon 3D-OCT 1000 is 4% in normal individuals. Pierro et al reported good reproducibility (ICC, 0.8) for RNFL thickness measurements by Topcon OCT 2000.

In the current study, we observed a non-significant diurnal decrease in RNFL thickness in both groups with the highest RNFL thickness at 9 AM and the lowest at 6 PM. The control group had significantly higher RNFL thickness at all time points as compared to the diabetic group. Reproducibility of RNFL measurements using Topcon 3D-OCT 1000 was high (ICC, 0.9) and comparable to other studies. However, there are some drawbacks to our study; the sample size was small, normal individuals were younger than diabetic subjects and measurements were not taken upon awakening.

In summary, the results of this study are in agreement with other studies performed using SD-OCT machines in which the amount of diurnal changes in macular and RNFL thicknesses is non-significant and negligible in clinical practice. Thus, OCT images can be interpreted irrespective of the time of acquisition between 9 AM and 6 PM. Previous reports on significant diurnal changes in these parameters using older generations of OCT are likely the result of lower reproducibility and not real tissue changes.

**Financial Support and Sponsorship**

Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**REFERENCES**

Diurnal Variations in Retinal and Corneal Thickness; Sharifipour et al


