Twenty-four hours intraocular pressure in keratoconic eyes assessed by applanation tonometry and Tono-Pen AVIA

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Abstract

- **AIM:** To assess intraocular pressure (IOP) during the daily curve of intraocular pressure (DCPo) in keratoconic eyes and compare Goldmann applanation tonometer (GAT), without and with astigmatism correction (nGAT and cGAT) and Tono-Pen AVIA (TPA) assessment methods.

- **METHODS:** Thirty-nine keratoconic eyes of 24 patients were assessed. DCPo was evaluated with five IOP measurements; four were performed with a GAT (nGAT and cGAT), and a Tono-Pen AVIA (TPA) at various times throughout the day.

- **RESULTS:** Mean IOP DCPo values (mm Hg) were: nGAT, 9.9±2.6; cGAT, 11.3±2.6; TPA 12.3±3.1. Mean IOP DCPo differences (mm Hg) and Spearman’s correlation coefficients were as follows: cGAT-c-nGAT, 1.32±1.31, rs=0.879 (P<0.01); cGAT-TPA, -1.02±2.08, rs=0.723 (P<0.01); and nGAT-TPA, -2.35±2.23, rs=0.730 (P<0.01). Bland-Altman analysis for agreement between cGAT-TPA and nGAT-TPA mean IOP DCPo measurements revealed a mean difference of 1.02 (95%CI, 0.35-1.70) and 2.35 (95%CI, 1.62-3.07) mm Hg, respectively. Regression analysis yielded the following equation: TPA IOP=5.49+0.775×cGAT-0.015×ACD-0.299×corneal astigmatism, which allowed us to infer TPA IOP values from other parameters.

- **CONCLUSION:** In keratoconic eyes, IOP peaks of DCPo measurements are identified at 6 a.m., independent of the tonometer. The mean DCPo values are: TPA>cGAT>nGAT. IOP TPA measures are predictive of cGAT values, adjusted according to anterior chamber depth and corneal astigmatism.

**KEYWORDS:** intraocular pressure; keratoconus; tonometry

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INTRODUCTION

It is established that elevated intraocular pressure (IOP) is the main risk factor for the onset and progression of primary open angle glaucoma (POAG)[1-2]. Advanced Glaucoma Intervention Study (AGIS) demonstrated that long-term IOP changes are related to the progressive visual field deterioration in patients with low mean IOP measurements, but not in patients with high mean IOP measurements[3]. It is broadly accepted that IOP varies according to a 24-hour cycle. For some authors, the daily curve of intraocular pressure (DCPo) based on IOP measurements acquired by applanation tonometry at 6:00 a.m., in the dark, with the patient lying down in bed, is very important in establishing the diagnosis of glaucoma suspect and assessing IOP in glaucoma[4-7].

Keratoconus is an ectatic corneal disease, with non-inflammatory progressive thinning and anterior projection that results in an irregular conical shape[8-10]. It is an asymmetrical bilateral condition that appears at youth. Topographic (inferior steepening, inferior-superior asymmetry, and irregular astigmatism) and clinical (conical protrusion, corneal stromal thinning, Fleischer ring and Vogt striae) signals are commonly considered together for staging and diagnosing the disorder[11]. Emerging ocular imaging technologies, such as Pentacam (Oculus Inc, Wetzlar, Hesse, Germany), have yielded precious information with regards to corneal and anterior ocular segment assessment. Diagnosing keratoconus has been refined by corneal pachymetric arrangement, curvature (elevation) maps, corneal volume and anterior segment information, all of which have been generated using a variety of currently available equipment[11-18].
Proper IOP measurement is essential to the follow-up treatment and diagnosis of glaucoma. Goldmann applanation tonometer (GAT) is the gold standard method for IOP measurement. But, it might be interfered by variations in corneal thickness, structure, and curvature. Corneal alterations due to keratoconus may probably lead to inaccurate determining IOP in this status. Prior researchers have demonstrated that GAT tends to under evaluate IOP in keratoconic patients primarily because of differences in the cornea's biomechanical properties and characteristically reduced corneal thickness. And also, some studies showed evidence that the IOP values of the dynamic contour tonometry (DCT) and a corneal-compensated IOP value (IOPcc) obtained by the ocular response analyzer (ORA) are less influenced by central corneal thickness (CCT) than GAT measurement, and should be considered more suitable to evaluate IOP in keratoconus.

It is important to investigate how changes in IOP occur during DCPo in keratoconic patients, and when IOP peaks occur, based on 24-hour IOP measurements, including IOP measurements acquired by applanation tonometry at 6:00 a.m., in the dark, with the patient lying down in bed. And, it is possible to assess IOP in bed with GAT, that's why this gold standard method was chosen to perform DCPo. Finally, it is also critical to compare DCPo GAT values with those acquired using Tono-Pen AVIA (TPA; Reichert Inc, Depew, New York, USA) in order to derive the profiles of both tonometers in keratoconic eyes. To the best of our knowledge, this is the first study that DCPo was performed in keratoconic patients using GAT and TPA.

SUBJECTS AND METHODS

Ethical Approval Keratoconic patients attended at São Geraldo Eye Hospital were included. Written informed consent was acquired from all patients, and investigation that acceded to the tenets of the Declaration of Helsinki started after the approval of the protocol by Ethics Committee of Federal University of Minas Gerais. All participants underwent a detailed ophthalmologic examination.

Keratoconus Assessment Diagnosis of keratoconus was firm up using the subsequent criteria (one sign or a conjunction of signs): biomicroscopic signs: stromal thinning, conical protrusion, Fleischer ring, Vogt striae, and enlarged corneal nerves; an abnormal retinoscopy reflex; and Munson’s sign (V-shaped configuration of lower lid on down gaze). Diagnosis was recognized topographically with Oculus Pentacam (Oculus Inc, Wetzlar, Hesse, Germany) system (“Topographical Keratoconus Classification”; TKC). We excluded keratoconic eyes with acute corneal hydrops, corneal scarring, penetrating or lamellar keratoplasty, keratitis, intrastromal corneal rings, or corneal cross-linking procedure.

Ultrasound CCT was measured by DGH 5100e A-Scan/ Pachymeter (DGH Technology, Exton, Pennsylvania, USA).

Daily Curve of Intraocular Pressure Measurements Each subject underwent a DCPo which was comprised of 5 IOP measurements; 4 performed with GAT (Haag-Streit, Harlow, Essex, United Kingdom) and TPA at 9:00 and 11:00 a.m., 6:00 and 10:00 p.m. and in the morning of the next day at 6:00 a.m. with patient lying down in bed and in the dark and before they had become erect when applanation tonometry measurement was done with Perkins applanation tonometer (Haag-Streit, Harlow, Essex, United Kingdom) followed by TPA. Considering irregular corneal astigmatism in keratoconic eyes, the prism red line of the applanation tonometer (GAT or Perkins tonometer) was placed at prism degree mark corresponding to the flattest meridian (minus cylinder) to correct intraocular pressure measurement (cGAT) of each patient; also, measurement without that astigmatism correction was done (nGAT). All IOP measurements were performed by one glaucoma specialist (Cronemberger S) and registered by another (Veloso AW). The IOP was taken sequentially with nGAT, followed by cGAT and TPA with an interval of three minutes among them. GAT was performed after a drop of 0.5% proxymetacaine hydrochloride followed by a drop of 0.25% fluorescein sodium instillation. Using TPA, 10 IOP measurements were obtained with accuracy of at least 95%.

Statistics All statistical analyses were performed with software R version 4.0.3 (The R foundation) and SPSS Version 21 (IBM Corp., Armonk, NY, USA). Pearson correlation coefficients were calculated to report the power of linear relationship among IOP values. An r value of >0.5 revealed moderate significance. An evaluation of Bland-Altman correspondence was used to compare GAT and TPA values. An extent of accordance was determined as mean±2 standard deviation.

We also compared average IOP and standard deviation of cGAT with normal upper limits (mean + two standard deviation of the DCPo and mean + two standard deviation of IOP) from normal subjects of the similar age range accessible in our Service. Multiple linear regression assessments were done using TPA IOP as a result with cGAT IOP and corneal parameters. P value of ≤0.01 was regarded statistically relevant. Five-fold cross-validation was used to find the model with the greatest generalization capacity. Linear mixed outcomes templates adjusting for nonindependence of right and left eye values were built and adjusted to the data.

RESULTS A total of 24 patients (48 eyes) with keratoconus were included in the study. Nine eyes of these patients were excluded from
Six had corneal transplantation and 3 had intrastromal corneal rings, leaving 39 eyes in final assessment. Table 1 summarizes clinical, ultrasound CCT and Oculus Pentacam features of patients.

DCPo are described in Table 2. IOP mean at 6:00 a.m. was higher than the other 4 DCPo measurements in all methods used of IOP assessment (cGAT, nGAT and TPA). Comparisons of mean DCPo values between cGAT-nGAT, cGAT-TPA, and nGAT-TPA are shown in Figures 1 and 2, and Table 3. We found that cGAT IOP DCPo mean was higher than nGAT (difference of 1.32±1.31; \( P < 0.01 \)) and lower than TPA (difference of -1.02±2.08; \( P = 0.004 \)), so DCPo was underestimated by nGAT and overestimated by TPA with a difference statistically significant. Moreover, nGAT was overestimated by TPA (-2.35±2.23; \( P < 0.01 \)) with statistically significant difference (Table 3).

A positive and statistically significant correlation was identified between cGAT-nGAT, cGAT-TPA and nGAT-TPA (Table 3).

Bland-Altman scenarios are displayed in Figure 3, describing mean difference (estimated bias) between cGAT-TPA and nGAT-TPA as well as the quantity of variance (±2 SD) around means. Mean difference between cGAT-TPA was 1.02 mm Hg and nGAT-TPA was 2.35 mm Hg, with most of values dropping within 2 SD of the mean.

Multiple linear regression models were built using TPA IOP as the dependent variable and covariates (cGAT, nGAT, Pentacam parameters: corneal astigmatism, maximum Ambrosio relational thickness (ART-Max), Belin/Ambrosio enhanced ectasia total deviation value (BAD-D), anterior chamber depth (ACD) as independent variables; since all of

### Table 1: Clinical, ultrasound CCT and Oculus Pentacam characteristics of the keratoconic patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23.5±7.7 (12-49)</td>
</tr>
<tr>
<td>Male/female, n (%)</td>
<td>7 (29.2) /17 (70.8)</td>
</tr>
<tr>
<td>White/mixed/black, n (%)</td>
<td>4 (16.6) /16 (66.8) /4 (16.6)</td>
</tr>
<tr>
<td>CCT (µm; n=39)</td>
<td>469±75.8</td>
</tr>
<tr>
<td>Keratometry average (D; n=39)</td>
<td>50.4±5.30</td>
</tr>
<tr>
<td>Keratometry 2 (D; n=39)</td>
<td>52.9±5.75</td>
</tr>
<tr>
<td>Keratometry maximum (D; n=39)</td>
<td>58.2±6.96</td>
</tr>
<tr>
<td>Corneal astigmatism</td>
<td>4.8±2.6</td>
</tr>
<tr>
<td>BAD_D</td>
<td>8.7±5.0</td>
</tr>
<tr>
<td>Pachymetry progression index</td>
<td>6.50±26.2</td>
</tr>
<tr>
<td>ARTmax</td>
<td>179.4±99.5</td>
</tr>
</tbody>
</table>

CCT: Central corneal thickness; BAD_D: Belin/Ambrosio enhanced ectasia total deviation value; ARTmax: Maximum Ambrosio relational thickness.
Figure 3 Bland-Altman assessment for accordance between Tono-Pen AVIA and GAT
A: Bland-Altman assessment for accordance between Tono-Pen AVIA and astigmatism corrected Goldmann applanation tonometer (cGAT) mean values of DCPo measurements demonstrating an average difference of 1.02 mm Hg, with the 95%CI of the limits of accordance between -3.05 and 5.09 mm Hg; B: Bland-Altman assessment for accordance between Tono-Pen AVIA and no astigmatism correction Goldmann applanation tonometer (nGAT) mean values of DCPo measurements demonstrating an average difference of 2.35 mm Hg, with the 95%CI of the limits of accordance between -2.02 and 6.72 mm Hg.

Table 2 IOP (cGAT, nGAT, and TPA) during DCPo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean DCPo</th>
<th>6:00 a.m.</th>
<th>9:00 a.m.</th>
<th>11:00 a.m.</th>
<th>6:00 p.m.</th>
<th>10:00 p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGAT</td>
<td>11.3±2.6</td>
<td>15.0±3.8</td>
<td>10.8±3.0</td>
<td>10.7±3.1</td>
<td>10.5±2.8</td>
<td>9.3±3.3</td>
</tr>
<tr>
<td>nGAT</td>
<td>9.9±2.6</td>
<td>12.5±3.1</td>
<td>10.4±3.1</td>
<td>9.3±3.2</td>
<td>9.4±3.0</td>
<td>8.0±3.1</td>
</tr>
<tr>
<td>TPA</td>
<td>12.3±3.1</td>
<td>14.3±3.3</td>
<td>12.6±3.4</td>
<td>12.7±4.2</td>
<td>11.6±3.4</td>
<td>10.3±3.3</td>
</tr>
</tbody>
</table>

Table 3 Paired t-test and Spearman’s correlation coefficient between mean IOP (cGAT, nGAT, and TPA) during DCPo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean difference</th>
<th>95%CI</th>
<th>P</th>
<th>r_s (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGAT-nGAT</td>
<td>1.32</td>
<td>0.90 to 1.75</td>
<td>&lt;0.01</td>
<td>0.879 (&lt;0.01)</td>
</tr>
<tr>
<td>cGAT-TPA</td>
<td>-1.02</td>
<td>-1.70 to -0.35</td>
<td>0.004</td>
<td>0.723 (&lt;0.01)</td>
</tr>
<tr>
<td>nGAT-TPA</td>
<td>-2.35</td>
<td>-3.07 to -1.62</td>
<td>&lt;0.01</td>
<td>0.730 (&lt;0.01)</td>
</tr>
</tbody>
</table>

IOP: Intraocular pressure; cGAT: Corrected astigmatism Goldmann applanation tonometry measurement; nGAT: Non-corrected astigmatism Goldmann applanation tonometry measurement; TPA: Tono-Pen AVIA; DCPo: Daily curve of IOP.

DISCUSSION
As far as we are aware, this is the first study to investigate changes in IOP during DCPo in keratoconic eyes, obtaining cGAT, nGAT and TPA IOP profiles, and also to assess correlations among these IOP measurements. Even though irregular corneas could interfere in IOP applanation measurements, we decided to use GAT, Perkins and TPA due to the fact that with them we were able to perform IOP 6:00 a.m. measurement with the patient lying down in bed and in the dark. Although the IOP values of DCT and IOPcc was used[30]. Using the K-fold averaging cross-validation model selection procedure yielded the following equation: TPA IOP=5.49+0.775×cGAT-0.015×ACD-0.299×corneal astigmatism with an average R^2 of 0.708 (95%CI 0.686 to 0.730), which means that these 3 covariates account for an average of 70.8% of the variance in TPA IOP.

With the generalized linear mixed model, TPA IOP was significantly related to ACD (P=0.027) and corneal astigmatism (P=0.007). Also, TPA IOP was significantly related to cGAT IOP (P<0.001) as for each increase in 1 mm Hg of cGAT IOP would mean an increase of 0.64 mm Hg in TPA IOP, with the other factors being fixed.

them expressed a P-value<0.05 in univariate analysis. In order to account for multicollinearity stepwise regression was used, which is a combination of forward and backward selection techniques. In stepwise regression, all variables are checked at each step to see if their significance has been reduced below a certain point. Variables with a P-value less than 0.05 are included in the model whereas if P-value goes above 0.1 the variable is removed. Seeking to find the model with the greatest generalization capacity, five-fold cross-validation was used[30].
values obtained by ORA are less influenced by CCT than GAT measurement, and should be considered more suitable to evaluate IOP in keratoconus, is technically impracticable to perform all measurements of the DCPo with these instruments. Iwaszkiewicz[35] assessed IOP diurnal fluctuations of 73 keratoconic eyes, however, he used a pneumotonometer, and IOP at 6:00 a.m. was not evaluated. In fact, the author took IOP measurements only from 7:00 a.m. to 10:00 p.m. He found the mean upper value of 19.24±2.84 mm Hg for right eye and 18.06±2.80 mm Hg for left eye. These values are higher than our mean IOP values, and means oscillation of IOP in a day equalized 7.00±2.41 mm Hg in the right eye and 6.00±2.38 mm Hg in the left eye, also higher than our findings. However, there are few reports using pneumotonometer, mainly in keratoconus, so it is difficult to know how accurate it would be in DCPo. As demonstrated by some studies, appropriate IOP assessment with its measurement taken with an applanation tonometer at 6:00 a.m. in bed and in the dark before the patient became erected is essential to detect IOP peaks[5,7]. As a matter of fact, our study is the first to investigate IOP keratoconus peak in this way, and we found the highest DCPo mean values at 6:00 a.m. independently of the way used to measure IOP (cGAT 15.0±3.8 mm Hg, nGAT 12.5±3.1 mm Hg, TPA 14.3±3.3 mm Hg). Normal and abnormal DCPo values have already been established in previous study[7]. The normal superior value for mean IOP and variability were 14.62 mm Hg and 2.28, respectively (age of patients from 15 to 25y), and 15.93 mm Hg and 2.28, respectively (age of patients from 26 to 35y), which implies that normal superior DCPo mean limits are 16.9 and 18.20 mm Hg for 15-25y and 26-35y respectively[9]. In the present study, all mean IOP values were under normal limits. Highest mean IOP DCPo value was observed with TPA (12.3±3.1 mm Hg), and lowest with nGAT (9.9±2.6 mm Hg) in keratoconic eyes. However, at 6:00 a.m. the highest IOP mean measurement was verified with cGAT (15.0±3.8 mm Hg). Therefore, in accordance with the normal superior DCPo IOP mean limits established[9], an IOP equal to or more than 17 mm Hg in keratoconic eyes would require glaucoma investigation with exams such as fundoscopy and optic coherence tomography. It is well established that, in corneas with an astigmatism greater than three diopter (3 D), GAT measurement is misleading[34]. The applanated area will not be circular, but elliptical. This mistake can be precluded by applanation at 43° to the axis of minus cylinder. To eliminate this error, we have performed IOP measurement by aligning the angle of minus cylinder with the prism with red mark on the prism holder. As far as we know, the present study is the first to compare IOP GAT measurements respectively with and without astigmatism correction (cGAT and nGAT) in keratoconus. We found cGAT higher than nGAT IOP DCPo mean values with a difference statistically significant, and also higher at 6:00 a.m. mean IOP measurement.

Many studies have compared different tonometers for measuring IOP, such as GAT, tonopen, dynamic contour tonometer, ORA, and rebound tonometry, in keratoconic eyes[20-21,23-25]. However, our study is the first to compare GAT and TPA during the DCPo in keratoconic eyes. We have found a statistically significant difference between the mean IOP of cGAT-NGAT, cGAT-TPA, and nGAT-TPA. TPA presented the highest IOP mean DCPo values. Hypothetically, the highest IOP mean DCPo values found with TPA are due to the lower diameter of area and strength of applanation than those with cGAT. Therefore, GAT continues to be the gold-standard, even in keratoconic eyes, being more accurate to measure IOP with the cGAT than with TPA, especially in DCPo. In the present study, it was verified a mean IOP DCPo value of 12.3±3.1 (TPA), 11.3±2.6 (cGAT) and 9.9±2.6 (nGAT) mm Hg. On the contrary, some authors reported higher cGAT values (varying from 11.12 to 13.76 mm Hg, in different stages of the keratoconus) than those of tonopen IOP measurements (varying from 9.24 to 11.51 mm Hg) in keratoconic eyes, however, DCPo was not done[31]. These authors have measured IOP 3 times between 9:00 a.m. and 11:00 a.m., but they used Tono-Pen XL (Medtronic Solan, Jacksonville, FL, USA) which is different from our study[31], and also, they found that only DCT IOP and ORA IOPcc didn’t have association with CCT in keratoconus eyes. In another study, Tono-Pen XL IOP measurements were 3.6±10 mm Hg higher than GAT in keratoconic eyes, similarly to our research, and DCT IOP was 2.7±6 mm Hg higher than GAT, nevertheless, DCPo was not executed as well[20]. DCT and IOPcc were again found to be independent of CCT together with corneal hysteresis[29]. In the same way, in keratoconus patients after intrastromal corneal ring segments implantation, Tono-Pen XL IOP values were 0.8±3.07 mm Hg higher than GAT, and DCT IOP was 1.0±3.26 mm Hg higher than GAT, and DCP IOP measurement wasn’t affected by CCT[23]. In our study, TPA IOP mean DCPo values were 2.35±2.23 mm Hg higher than nGAT, and 1.0±2.08 mm Hg higher than cGAT values. The fact that DCT IOP measured is higher than GAT IOP in keratoconus was described in other study (DCT 14.8±3.07 mm Hg, GAT 13.1±2.9 mm Hg) that also verified CCT independence of the DCT IOP measurement[22]. ORA IOPcc value was found to be higher than GAT in keratoconus (ORA IOPcc 13.3±2.5 mm Hg, GAT 10.9±2 mm Hg), nevertheless, ORA reading seemed to be affected by corneal curvature[20]. Cronemberger et al[37] have reported isolated IOP measurements taken by GAT (10.50±2.22 mm Hg for right eye and
1.08±1.89 mm Hg for left eye) at 720 postoperative days in eyes who underwent laser-assisted in situ keratomileusis (LASIK). These values were a little higher than those mean IOP DCPo value (9.9±2.6 mm Hg) were found in keratoconic eyes, but, without a statistically significant difference (the two-tailed P=0.4741). These findings may be explained by differences in cornea’s biomechanical properties and the mean lower CCT in keratoconic eyes (469.0±75.8 µm) than in the eyes that underwent LASIK (492.7±20.4 µm). Based on our findings, the upper normal limit of GAT IOP (mean±2 SD) in keratoconus would be 16.5 mm Hg which is below the superior limit we found for normal eyes (18 mm Hg). Therefore, when using GAT for evaluating keratoconus, it is necessary to use correction of astigmatism (cGAT). Besides this, it is important to emphasize that an IOP≥17 mm Hg in one patient with keratoconus should be considered at least suspected of glaucoma depending on the findings of cup-to-disc ratio and retinal nerve fiber layer[7].

Our study has some limitations. IOP applanation measurement at 6:00 a.m. with the patient lying down in bed and in the dark was done using Perkins tonometer (in which it is possible to do astigmatism correction), however, both GAT and Perkins handheld are applanation tonometers and their possible to use DCT or ORA both considered more accurate in keratoconic eyes. Therefore, when using GAT for evaluating keratoconus, it is necessary to use correction of astigmatism (cGAT). Besides this, it is important to emphasize that an IOP≥17 mm Hg in one patient with keratoconus should be considered at least suspected of glaucoma depending on the findings of cup-to-disc ratio and retinal nerve fiber layer[7].

In conclusion, we demonstrated that, in keratoconic eyes, highest DCPo values are obtained at 6:00 a.m. measurement. So, our results suggest that an IOP peak in a keratoconic patient should be assessed at 6:00 a.m. in a supine position in bed and in darkness independent of the tonometer used. Furthermore, we found that TPA had higher mean DCPo values than GAT, and cGAT higher than nGAT with statistically significant difference. Also, a positive and statistically significant correlation was verified among TPA, cGAT, and nGAT mean IOP DCPo measurements. Besides, linear regression assessment yielded an equation that, when corneal astigmatism and anterior chamber depth are considered, may enable transformation of cGAT IOP into TPA IOP measurements. Forthcoming studies should target to authenticate this equation and establish other corneal elements, such as CCT.

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