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Effects of corneal biomechanical properties on rebound tonometry (Icare200) and applanation tonometry (Perkins) readings in patients with primary congenital glaucoma

Laura Morales-Fernandez[1,2] MD, PhD; Federico Saenz-Frances[1] MD,PhD; Pilar Pérez-García[1] MD; Julian Garcia-Feijoo[1,3,4], MD, PhD; Sofia Garcia-Saenz[1], MD, PhD; Rosario Gómez de Liaño[1,3,4] MD, PhD; Jose M. Martinez-de-la-Casa[1,4], MD, PhD.

1 Ophthalmology Unit, Hospital Clinico San Carlos, Dept. of Ophthalmology and ORL, Faculty of Medicine, University Complutense (Madrid), Institute "Investigacion Sanitaria del Hospital Clinico San Carlos" (IdISSC), Madrid, Spain

2 Hospital Universitario Quiron Pozuelo, Madrid, Spain

3 Institute "Investigaciones Oftalmologicas Ramon Castroviejo", University Complutense, Madrid, Spain

4 Cooperative Research Network on Age-Related Ocular Disease, and Visual and Life Quality, Instituto de Salud Carlos III, Madrid, Spain

Corresponding author: Laura Morales-Fernandez
Profesor Martin Lago SN, Madrid. Tel: 666-31-44-03
E-mail: lauramoralesfernandez@gmail.com
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Abstract

Objective: To assess the influence of corneal biomechanics on intraocular pressure (IOP) measurements made with the Icare200 (IC200) rebound tonometer and the Perkins hand-held applanation tonometer in patients with primary congenital glaucoma (PCG).

Materials and methods: 40 PCG patients and 40 healthy controls, age and gender-matched, were recruited. IOP was measured with the Ocular Response Analyzer (IOPc, IOPg), Icare200 and Perkins. The variables age, IOP, corneal hysteresis (CH), corneal resistance factor (CRF), central corneal thickness (CCT), best corrected visual acuity, spherical equivalent, medications and glaucoma surgeries were recorded for each subject. Uni and multivariate analysis were used to detect effects of variables on IOP measurements.

Results: Mean CCT was 545.65±71.88 μm in PCG vs. 558.78±27.58 μm in controls ($p=0.284$). CH and CRF were significantly lower in PCG group than in control group: mean CH 8.11±1.69 mmHg vs. 11.15±1.63 mmHg ($p<0.001$), and mean CRF 9.27 ± 2.35 mmHg vs. 10.71 ± 1.75 mmHg ($p=0.002$). Mean differences between IOP IC200-Perkins were 0.79 ± 0.53 mmHg in PCG vs. 0.80 ± 0.23 mmHg in controls ($p<0.001$) and mean differences IC200-IOPc were -0.89 ± 5.15 mmHg in PCG ($p<0.001$) vs. 1.60 ± 3.03 mmHg in controls (all $p<0.009$). Through multivariate analysis, CRF showed positive association and CH negative association with IOP measured with Perkins or IC200 in both subject groups. No association was detected for CCT, age or gender.

Conclusion: CH and CRF were identified as the main factors interfering with IOP measurements made with both tonometers in patients with PCG and healthy controls.

Key words: Primary congenital glaucoma, tonometry, biomechanical corneal properties, hysteresis, rebound tonometer, Icare200, Ocular Response Analyzer, Perkins applanation tonometer, central corneal thickness.

Introduction

Elevated intraocular pressure (IOP) is considered as the unique modifiable risk factor in glaucoma but its reliable measurement is not always easy in young glaucoma patients. Primary congenital glaucoma (PCG) is usually diagnosed within the first days or months of life and causes structural alterations in the eye due to the rapid increase in IOP. The changes of PCG affecting the cornea are due to a greater elasticity of this tissue and are manifested by Haab's striae, an increased corneal diameter, edema and leukoma (1).

To measure IOP, Goldman applanation tonometry (GAT) remains the gold standard also in children with glaucoma. However, there are some situations in which this measurement is not possible or the reading is not reliable even when using a hand-held device (2). The introduction of new IOP measuring devices allows for complementary pressure measurements to GAT such that more measurements can be obtained in the ophthalmology office (3,4,5).
Icare200 (IC200, Tiolat Oy, Helsinki, Finland) is the new version of Icare (figure 1). This instrument consists of a probe that makes minimal contact with the corneal surface to provide a pressure reading according to its deceleration as it bounces off the corneal surface (rebound phenomenon). The accuracy and reproducibility of measurements are improved over those of the previous models (Icare TA0I, Icare Pro, Icare 100) as a detector confirms the central corneal position of the probe.
In our preliminary study (6), systematic and proportional differences between IC200 and Perkins IOP readings were found. These differences were higher in high values of IOP, especially in PCG patients. However, no predictor factors were previously identified to explain the difference between both tonometers.

In early work, central corneal thickness (CCT) was identified as one of the main factors affecting IOP measurements made with both older Icare rebound tonometry models and applanation devices (4). However, recent studies have reported the effects of corneal biomechanical properties on rebound tonometry readings irrespective of CCT (5).

The Ocular Response Analyzer (ORA, Reichert Inc, Depew, NY) determines corneal biomechanical properties using an applied force-displacement relationship produced by an air-puff (7). Parameters used to characterize the biomechanical properties of the

cornea include corneal hysteresis (CH) and corneal resistance factor (CRF). CH is a measure of the cornea's ability to absorb and dissipate energy (5). CH is the difference measured in the ORA waveform between air pressures in 2 applanation events: force-in applanation (P1) — force-out applanation (P2). The corneal resistance factor (CRF) is a measure of corneal resistance calculated as P1-kP2, where k is a constant derived from empirical observation of the relationship between P1, P2, and CCT. (7) In addition, ORA provides 2 measurements of IOP, one that is equivalent to Goldmann applanation tonometry (IOPg) and a reading corrected for the biomechanical properties of the cornea (IOPc).

It has been reported that both CH and CRF affect IOP measurements obtained with Icare TA01 in healthy subjects and in patients with glaucoma (8).
While it is widely accepted that patients with PCG feature abnormalities in their corneal biomechanics (9), to date the impacts of these variables on IOP measurements made using the IC200 have not been analyzed.
The present study was thus designed to determine whether differences in corneal biomechanical properties and CCT existing between patients with PCG and healthy subjects could affect IOP measurements made through rebound (IC200) and applanation (Perkins) tonometry.

Materials and methods

The participants of this cross-sectional study were 40 patients with PCG and 40 healthy subjects. Patients were recruited among those patients diagnosed with PCG at the Hospital Clínico San Carlos (Madrid, Spain). Healthy controls were recruited among the hospital staff and their relatives. The study protocol was in line with the principles of the declaration of Helsinki and received review board approval. Written informed consent was obtained from all participants aged 18 years or older. Those younger than this age gave their verbal consent to participate and their parents or guardians signed the informed consent form.
The PCG group was comprised of 40 eyes of 40 outpatients of the Primary Congenital Glaucoma Unit of our center. Inclusion criteria for participation were: PCG diagnosed according to its latest definition by the Childhood Glaucoma Research Network (1) (IOP >21 mmHg at diagnosis and clinical evidence of glaucoma such as an enlarged corneal diameter, Haab's striae and glaucomatous appearance of the optic nerve head), age older than four years, and capacity to cooperate in the clinic. Patients were excluded if

there was a secondary cause of glaucoma (eg, iridocorneal endothelial syndrome, Axenfeld-Rieger syndrome or Peter's anomaly), or if they had a systemic disease or situation that could impair cooperation with the tests, prior corneal surgery or alterations not related to glaucoma (eg, corneal transplant or trauma).
The control group was comprised of 40 eyes of 40 volunteers without clinical findings of glaucoma who were enrolled based on the findings of an ophthalmologic examination. Inclusion criteria were: subjects with similar age that those cases recruited in the glaucoma group, no signs/symptoms of glaucoma, best corrected visual acuity (BCVA) ≥ 0.9 (Snellen Scale), optic nerve cup-disc asymmetry ≤ 0.2, and a cup-disc ratio < 0.5 without focal neuroretinal rim loss, hemorrhage, or pallor.
In all participants, variables were recorded for only one eye. If both eyes fulfilled the inclusion criteria, the eye to be examined was randomly selected using a web tool (www.randomization.com).

All participants were subjected to a comprehensive ophthalmologic examination including best-corrected visual acuity (BCVA) using the Snellen decimal scale, refractive state expressed as spherical equivalent (SE), slit lamp biomicroscopy, CCT measurement, dilated fundoscopy examination and IOP measurements. CCT was measured by ultrasound pachymetry (Dicon P55; Paradigm Medical Industries Inc., Salt Lake City, UT) and expressed as the mean of 5 consecutive measurements obtained through an automated procedure. In all participants, medical history was reviewed and age and the eye selected for inclusion were noted.

Several patient data were compiled from clinical records: age at diagnosis, last BCVA, corneal state (corneal transparency, edema and leukoma), cup-to-disk ratio, glaucoma surgeries and treatments up until the time of the study.

All IOP measurements were made by the same examiner (LMF) in a single session, using topical fluorescein and topical anesthesia in sitting position.

The order of the instruments used was, first, Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA), second, rebound tonometer IC200 (Icare 200, Tiolat Oy, Helsinki, Finland) and third, Goldmann handheld applanation tonometer (Perkins; Clement-Clarke, Columbus, OH), to avoid corneal deformation after

applanation. All measurements were made consecutively between 9 am and 11 am to minimize the effects of diurnal variations.

Three pressure measurements were made with Perkins and IC200 tonometer and the mean value was recorded.

Multiple readings were taken using RT200 and only those with good reliability were recorded (green light). IC200 rebound tonometer offers an indicator of quality, obtained after six consecutive measurements (good quality of measurements offers a green lighter, yellow lighter if variation is borderline or a message of "repeat" if variation is bad).

Using ORA, three pressure measurements were made and the reading with the best waveform score was used in the data analysis. Only IOP measurements with a waveform score of >5 were accepted. The ORA was used to obtain measurements of CH, CRF, corneal-compensated IOP (IOPc) and Goldmann-correlated IOP (IOPg).

Statistical analysis

The Bland-Altman method was used to graphically depict agreement between the Perkins and IC200 IOP measurements and Intraclass correlation coefficient (ICC) with interval confidence 95% (IC95%) were calculated.

To compare the IOP readings made with the two tonometers between the glaucoma patients and healthy subjects, a t-test for paired samples was used. The impacts of age, gender, CCT, CRF and CH on the readings of both tonometers were assessed through univariate and multivariate linear regression models.

Results

In Table 1 we provide demographic data for the two study groups. There were no significant differences between the patient and healthy control groups in terms of age, gender, eye selected, and mean CCT. As expected, statistical differences were found between groups in terms of BCVA, SE and cup to disc ratio. All IOP readings made with both tonometers were higher in the PCG group. The corneal biomechanical variables CH and CRF were significantly lower in the PCG group.

The clinical characteristics of the glaucoma patients were: median age was 12 (9-19) years, mean age at diagnosis was 27.27 (23.57) months, mean BCVA was 0.51 (0.38), mean cup-to-disk ratio was 0.55 (0.30) and 85% of PCG patients were using some

hypotensive medications (mean number of medications used 1.70 (1.43). 5 patients were using 1 hypotensive medication, 7 patients were using 2, 11 patients were using 3 drops and 4 patients were using 4 medications. 12 patients were using topical prostaglandines drops. All required some glaucoma surgery (mean number of glaucoma surgeries per eye was 2.30 (1.92)). Of these glaucoma surgeries, in 24/40 eyes surgery was goniotomy with a mean of 1.15 (1.13) (range 1-3) goniotomies per eye; in 24/40 it was trabeculectomy, and in 7/40 surgery involved the implant of an Ahmed valve.

In Table 2 we provide pairwise differences in IOP measurements between the different devices used for each subject group. Significant differences between patients and controls in IOP readings were observed for differences between IC200-IOPc, Perkins-IOPc and IOPg-IOPc.

Good-excellent agreement was observed between all IOP measurements obtained with different tonometers in healthy group (see table 3) (all ICC>0.602). In PCG group, best ICC were obtained for IOP IC200-IOP Perkins=0.737, IC95%=0.492-0.882 (p<0.001) and ICC IOPg- IOPc=0.750, IC95%=0.580-0.857 (p<0.001%). The rest of values for ICC, were <0.561 in PCG group (see table 3).

The Bland-Altman plots in Figures 2a and 3a illustrate agreement between the IOP measurements made with IC200 and Perkins in both the patients and healthy controls. In the control group (figure 2a), the mean difference between the tonometers Perkins-IC200 was -0.80 mmHg (95%CI: -1.28--0.32) (p=0.016). The 95% lower limit of agreement was -3.81 mmHg (95% CI: -4.64- -2.98) whereas the 95% upper limit was 2.20 mmHg (95% CI: 1.37-3.02). As can be seen in the plot, 2 readings were below the limits of agreement (4.76% of the readings) and no reading was over the limit. In the PCG group (figure 3a), the mean difference between the tonometers Perkins-IC200 was -0.79 mmHg (95% CI: -1.87-0.28) (p=0.268). The 95% lower limit of agreement was -7.14 mmHg (95% CI: -9.02- -5.27) whereas the 95% upper limit was 5.55 mmHg (95% CI: 3.68-7.42). As can be seen in the plot, 1 reading was below and 1 was above the limits of agreement (2.70% of the readings respectively). Figures 2b,2c,3b and 3c depict the agreement between IOPc and IOP measurements obtained through Perkins and IC200 amongst healthy and PCG volunteers (see figures).

In the control group (figure 2d), the mean difference between the ORA IOP readings IOPc-IOPg was -0.14 mmHg (95% CI: -0.46-0.75) (p=0.899). The 95% lower limit of agreement was -3.69 mmHg (95% CI: -4.74- -2.63) whereas the 95% upper limit was 3.97 mmHg (95% CI: 2.92-5.03). As can be seen in the plot, 1 reading was below and 1

was above the limits of agreement (2.38% of the readings respectively). In the PCG group (figure 3d), the mean difference between the ORA IOP readings IOPc-IOPg was -2.53 mmHg (95% CI: -3.20- -1.87) (p=0.488). The 95% lower limit of agreement was -6.61 mmHg (95% CI: -7.76- -5.45) whereas the 95% upper limit was 1.53 mmHg (95% CI: 0.38-2.68). As can be seen in the plot, 1 reading was below the limits of agreement (2.50% of the readings respectively) and no reading was over the limit.
To assess the effects of age, gender, CCT, CRF and CH on the tonometer readings obtained in the patients and controls, we constructed univariate and multivariate linear regression models (see Table 4). IOP measurements obtained with Perkins and IC200 were significantly related to CCT and CRF in the univariate analysis. In the multivariate model, CRF and CH emerged as related to IOP measured with either Perkins or IC200 in both groups, whereas CCT had no significant impact on the measurements made with both devices.

Discussion

The addition of rebound tonometry and the new IC200 to the instruments used in routine clinical practice offers numerous benefits for the management and care of patients with PCG. However, this technological advance will only be of practical use provided there is good agreement with Goldmann applanation tonometry and we can identify any factors that could affect the validity of rebound tonometry measures (2).

In the present study, differences between IOP measurements through applanation and rebound tonometry (Perkins minus IC200) were -0.80 and -0.81 in the PCG and control groups respectively. However, despite the small mean difference between the reading of the two tonometers, the Bland-Altman plot indicates that the 95% limits of agreements are rather wide ranging from -3.81 to 2.20 in the control group and ranging from -7.14 and 5.55 mmHg in the PCG group. Indeed, good- excellent agreement was obtained for all IOP measurements using different tonometers in healthy group (all ICC>0.602), and in PCG group good agreement was detected for IOP measured by Perkins and IC200 (ICC=0.737).

This is consistent with results obtained in our preliminary study using IC200(6). Mean difference between IOP Ic200 and Perkins IOP readings was 1.26 mmHg, an upper limit of agreement of -8.06mmHg and a lower limit of agreement of 10.59mmHg.

And similar results were reported for earlier Icare tonometer models. Despite good agreement between Icare TA01 and Perkins was described (2), the mean difference between the two devices being −0.79 ± 2.83 in PCG patients versus −0.52 ± 2.5 mmHg in healthy subjects (10), in both cases a wide range of limits of agreements has been observed. Indeed, similar results were reported between Icare PRO and Perkins (11).

In the present study, IC200 tended to overestimate pressures compared to the Perkins measurements in the two subject groups. Others have confirmed this overestimation on the part of ICare TA01 and Icare Pro, arguing that rebound tonometry leads to significantly higher IOP values (1.8 mm Hg) over GAT (4,12). However, the IC100 version (prior to IC200) seems to show different behavior. Thus, Molero et al. (13) found it significantly underestimated IOP versus Icare PRO and Perkins. Namakura et al. (14) also reported this underestimation of IOP by IC100 compared to ICare TA01 and Goldmann tonometry. This controversy observed in the literature could be explained by the Passing-Bablok regression equation recently reported: IC200-IOP= -4.63+1.28 Perkins-IOP (6). So, higher values of Perkins readings result on higher readings of IC200 IOP, however readings using IC200 could be lower, when Perkins IOP is lower than 16mmHg.

On the other hand, our data indicate significant differences in corneal biomechanical parameters between healthy subjects and individuals with PCG. Both CH and CRF were markedly lower in our glaucoma group. These results agree with that described by Perucho et al (15). While such differences can be attributed to the changes that take place in the cornea because of the disease itself (corneal edema and leukoma, Haab's striae, multiple prior surgeries and higher IOP among others) our study design precluded us from determining if such lowered values reflected a primary or secondary alteration.

While it has been described that patients with PCG will have a lower CCT (9), this difference was not observed here with similar values of 545.65 (71.88) μm vs. 558.78 (27.58) μm recorded in the PCG vs. control group respectively.

The positive correlation existing between CCT and IOP measured by Goldmann applanation tonometry has been widely described in the literature both in adult and

infantile glaucoma besides healthy individuals (2,4). This same effect of CCT has been described for rebound tonometry IOP readings (4,14).

Recently, the measurement of corneal biomechanical properties has evoked new concepts and controversial arguments. Some authors describe negative correlation between CH and GAT IOP, meaning that as CH increases, IOP measured in this manner tends to decrease (5,16).

In our univariate analysis, CCT was significantly associated with IC200 IOP and Perkins IOP measurements. However, through univariate analysis, while IOP measurements made using the two devices were found related to CCT, when CH and CRF were considered, the effect of CCT on IOP was abolished, leaving only CRF and CH as the main factors influencing IOP both in healthy individuals and glaucoma patients.

In both our subject groups, a lower CH was associated with a higher IOP determined with IC200 (Control: 1.17 mmHg higher IOP for each 1 mmHg CH reduction, P = 0.005, $R^2 = 0.44$; PCG: 1.58 mmHg higher IOP for each 1 mmHg CH reduction, P = 0.002, $R^2 = 0.53$) or Perkins (Control: 1.28 mmHg higher IOP for each 1 mmHg CH reduction, P = 0.001, $R^2 = 0.33$; PCG: 1.58 mmHg higher IOP for each 1 mmHg CH reduction, P = 0.004, $R^2 = 0.43$) (see Table 3).

In addition, in both the control and glaucoma groups, a positive effect of CRF was observed on IOP readings taken with either IC200 (Control: 1.29 mmHg higher IOP for each 1 mm Hg CRF increase, P = 0.002, $R^2 = 0.44$; PCG: 2.51 mmHg higher IOP, for each 1 mm Hg CRF increase, P = <0.001, $R^2 = 0.53$) or Perkins (Control: 1.33 mm Hg higher IOP for each 1 mmHg CRF increase , P = <0.001, $R^2 = 0.33$; PCG: 1.89mmHg higher IOP for each 1 mmHg CRF increase, P = <0.001, $R^2 = 0.53$).

These findings suggest that both CH and CRF similarly affect IC200 and Perkins IOP readings. No influence of age or gender were determined in our study.

Our results are consistent with those of recent studies by Shin et al. (16) and Brown et al. (5), who found that CCT was significantly correlated with Icare pressure measurements in their univariate model, but only CRF and CH maintained a significant relationship with IOP in a multivariate analysis. Brown et al. (5) observed that a lower CH resulted in a higher IOP using Icare HOME (5.17 ± 2.50 mm Hg higher IOP for

each 10 mm Hg CH reduction) and GAT (7.23 ± 2.67 mm Hg higher IOP for each 10 mm Hg CH reduction), and thus proposed that GAT IOP could be more affected by CH than Icare HOME IOP. Shin et al (16) also concluded that IOP measurements may be underestimated in eyes with a higher CH and lower CRF, and may be overestimated in eyes with a lower CH and higher CRF. The implications of this for patient management could be excessive treatment or suboptimal treatment, revealing the importance of considering the biomechanical properties of the cornea along with CCT when interpreting pressure measurements (17,18). This becomes especially important for IOP measurements in PCG patients who show significant alterations in both CH and CRF.

The influence that corneal biomechanics has on the pressure measurements of these tonometers could explain the greater differences detected in our study in ORA-IOPc, as this IOP measure is compensated for corneal biomechanical properties compared to the remaining IOP measures (Perkins, IC200 and ORA-IOPg).

A markedly different behavior was observed between our two subject groups as IOPc pressures were much higher than remaining measures in the PCG patients, who showed altered corneal biomechanical variables. These high IOPc values are consistent with the notion that IC200 and Perkins readings are affected by corneal properties and not only by the actual IOP of the eye or corneal thickness (18). These findings can explain the poor correlation obtained in our study between IOPc-IOP IC200 and IOPc-IOP perkins (ICC<0.561). In line with this observation, similar poor correlation has been previously reported between ICare rebound tonometry readings and IOPc (18).

The main limitations of our study arise from the difficulty encountered to recruit patients as PCG is rare and there will always be selection bias given that our center is a reference center for this condition and most of our PCG patients show poor intraocular pressure control and severe PCG. This restricted recruitment explains the large age range which could interfere with results, although the variable age was included in our analysis. A further limitation of the present study was that we did not examine possible effects of potentially relevant factors, including axial length (19), corneal characteristics such as corneal curvature (9), corneal diameter (9) and corneal densitometry (20) or effects of glaucoma treatment (21,22), and surgeries (23) on the differences in IOP observed with different instruments.

In conclusion, we identified CH and CRF as the main factors interfering with IOP measurements made both through applanation and rebound tonometry. These findings highlight the importance of including these biomechanical variables in our clinical practice during the follow up of glaucoma in addition to CCT, and the benefit of IOPc measurements obtained by ORA in these cases. These variables can explain the lack of agreement between rebound tonometer IC200 in some cases. So, these measures should be considered in patients with PCG as they show both structural and biomechanical corneal alterations.

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LEGENDS

Figure 1. The IC200 rebound tonometer. Note the green light ring around the probe indicating its correct position and the screen displaying the intraocular pressure reading.
Figure 2. Bland-Altman plots showing agreement between IOP measurements made using the different tonometers amongst healthy volunteers. Grey square indicates upper and lower limits of agreement. intermittent lines represent mean difference value.
2a) Bland-Altman plot depicting the agreement between IOP measurements obtained through Perkins and iCare. Mean difference (Perkins-Icare): -0.81 mmHg (95% CI: -1.29- -0.33). Lower limit of agreement: -3.81 mmHg (95% CI: -4.64- -2.99). Upper limit of agreement: 2.2 mmHg (95% CI: 1.37-3.03).
2b) Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA (IOPg) and IC200 tonometer. Mean difference (IOPg-Icare): -1.46 mmHg (95% CI: -2.24- 0.69). Lower limit of agreement: -6.33 mmHg (95% CI: -7.66- -4.99). Upper limit was 3.40 mmHg (95% CI: 2.06-4.74).
2.c. Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA (IOPg) and Perkins. Mean difference (IOPg-Perkins): -0.66 mmHg (95% CI: -1.36- 0.04). Lower limit of agreement: -5.06 mmHg (95% CI: -6.27- -3.85). Upper limit: 3.74 mmHg (95% CI: 2.53-4.96).
2.d. Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA. Mean difference IOPc-IOPg: -0.14 mmHg (95% CI: -0.46-0.75). Lower limit of agreement: -3.69 mmHg (95% CI: -4.74- -2.63). Upper limit: 3.97 mmHg (95% CI: 2.92-5.03).
Figure 3. Bland-Altman plots showing agreement between IOP measurements made using the different tonometers in patients with primary congenital glaucoma. Grey square indicates upper and lower limits of agreement. intermittent lines represent mean difference value.
3a) Bland-Altman plot depicting the agreement between IOP measurements obtained through Perkins and IC200 tonometer: Mean difference (Perkins-Icare): -0.79 mmHg (95% CI: -1.87-0.28). Lower limit of agreement: -7.14 mmHg (95% CI: -9.02- -5.27). Upper limit of agreement: 5.55 mmHg (95% CI: 3.68-7.42).
3b. Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA (IOPg) and IC200 tonometer. Mean difference (IOPg-Icare): -0.66 mmHg (95% CI: -1.36- 0.04). Lower limit of agreement: -5.06 mmHg (95% CI: -6.27- -3.85). Upper limit: 3.74 mmHg (95% CI: 2.53-4.96).
3c. Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA (IOPg) and Perkins. Mean difference (IOPg-Perkins): -0.88 mmHg (95% CI: -2.57- 0.82). Lower limit of agreement: -11.26 mmHg (95% CI: -14.20- -8.33). Upper limit: 9.51 mmHg (95% CI: 6.58-12.45).
3.d) Bland-Altman plot depicting the agreement between IOP measurements obtained through ORA. Mean difference IOPc-IOPg: -2.53 mmHg (95% CI: -3.20- -1.87). Lower limit of agreement: -6.61 mmHg (95% CI: -7.76- -5.45). Upper limit: 2.53 mmHg (95% CI: 0.38-2.68).

Table 1. Demographic characteristics of the patients with primary congenital glaucoma (PCG) and healthy control subjects included in this study.

	PCG (n=40)	**Control (n=40)**	**P**
Age (years)	12 (9-19)[2] *(4-38)*	15 (10-21)[2] *(5-36)*	0.157**
Female/male	16/24	17/23	0.282[+]
Right/left eyes	20/20	20/20	0.829[+]
CCT (microns)	545.65 (71.88)[1] *(442-771)*	558.78 (27.58)[1] *(493-618)*	0.284*
BCVA	0.51 (0.38)[1] *(0.2-1.20)*	0.94 (0.02)[1] *(0.9-1)*	<0.001*
SE (D)	-1.75 (-5-0)[2] *(-15-4)*	1.12 (0.31-2.93)[2] *(-4.37-5.87)*	<0.001**
Cup to disk ratio	0.55 (0.30)[1] *(0.2-1)* n=36	0.2 (0.03)[1] *(0.0-0.3)* n=40	<0.001*
CH	8.11 (1.69)[1] *(5.3-12.2)*	11.15 (1.63)[1] *(11.5-16.2)*	<0.001*
CRF	9.27 (2.35)[1] *(4.8-13.1)*	10.71 (1.75)[1] *(6.8-14.9)*	0.002*
IOP Perkins (mmHg)	19.13 (5.64)[1] *(10-31.5)*	15.07 (2.05)[1] *(10-20)*	<0.001*
IOP IC200 (mmHg)	20.10 (6.37)[1] *(11.5-39.8)*	15.87 (2.52)[1] *(9.7-21.3)*	<0.001*
IOPg (mmHg)	18.26 (6.86)[1] *(7.0-36.7)*	14.41 (2.76)[1] *(8.8-22.4)*	0.001*
IOPc (mmHg)	20.80 (6.23)[1] *(9.9-39.3)*	14.27 (2.72)[1] *(8.5-21.8)*	<0.001*

PCG: primary congenital glaucoma; CCT: central corneal thickness; BCVA: Best corrected visual acuity; SE: Spheral Equivalent; D: diopters; CH: corneal

hysteresis; CRF: corneal resistance factor; IOP: intraocular pressure; IOP Perk: IOP measured through Perkins applanation tonometry; IOP IC200: IOP measured with Icare200; IOPg: Goldmann-correlated IOP measured with the ocular response analyzer (ORA); IOPc: corneal-compensated IOP measured with the ORA; SD: standard deviation.

P* T student

P** Test de Mann Whitney

P+ Test X^2

1: Mean (SD)

2: Median (P25-P75)

(range: minimum- maximum)

Table 2. Differences in intraocular pressure (IOP) measurements made with the two tonometers in patients and controls.

	PCG (n=40)		**Control (n=40)**		**Difference (PCG vs. control group)**
	Mean difference (SD) (mmHg)	P*	Mean difference (SD) (mmHg)	P*	P+
IOP IC200-IOP Perkins	0.79 (0.53)	<0.001	0.80 (0.23)	<0.001	0.818
IOP IC200-IOP IOPc	-0.89 (5.15)	<0.001	1.60 (3.03)	<0.001	0.009
IOP IC200-IOP IOPg	1.48 (1.46)	<0.001	1.46 (2.48)	<0.001	0.980
IOP Perkins-IOPc	-1.58 (5.39)	<0.001	0.80 (2.61)	<0.001	0.013
IOP Perkins-IOPg	0.98 (5.46)	<0.001	0.65 (2.22)	0.001	0.719
IOPg-IOPc	-2.53 (2.07)	<0.488	0.14 (1.95)	0.899	<0.001

PCG: primary congenital glaucoma; IOP: intraocular pressure; IOP Perkins: IOP measured through Perkins applanation tonometry; IOP IC200: IOP measured with Icare200; IOPg: Goldmann-correlated IOP measured with the ocular response analyzer (ORA); IOPc: corneal-compensated IOP measured with ORA; SD: standard deviation.
* Student t-test. + t-test for paired samples

Table 3. Agreement between IOP measurements obtained by different tonometers, determined by intraclass correlation coefficient in both groups.

	PCG group		**Healthy group**	
	ICC* (IC95%)	**p**	**ICC* (IC95%)**	**P**
IOPg-IOPc	0.750 (0.580-0.857)	<0.001	0.885 (0.231-0.965)	<0.001
IOPg-IC200	0.492 (0.170-0.707)	<0.001	0.717 (0.515-0.804)	<0.001
IOPg-IOP Perkins	0.561 (0.316-0.736)	<0.001	0.644 (0.421-0.794)	<0.001
IOPc-IC200	0.283 (0.003-0.529)	0.015	0.676 (0.456-0.818)	<0.001
IOPc-IOP Perkins	0.399 (0.122-0.621)	0.003	0.602 (0.362-0.768)	<0.001
IC200-IOP Perkins	0.737 (0.492-0.882)	<0.001	0.854 (0.736-0.922)	<0.001

*Absolute agreement

ICC: Intraclass correlation coefficient; IC95%: interval confidence 95% (lower-upper limits); IOP: intraocular pressure; IOP Perkins: IOP measured through Perkins applanation tonometry; IOP IC200: IOP measured with Icare200; IOPg: Goldmann-correlated IOP measured with the ocular response analyzer (ORA); IOPc: corneal-compensated IOP measured with ORA.

Table 4. Table 4 shows the results of the univariate and multivariate linear regression models examining the effects of age, gender, CCT, CRF and CH on IOP readings made using the different tonometers in the healthy control group (Table 4a) and PCG group (Table 4b).

Table 4a.

	Adjusting variable	Healthy control group							
		Unadjusted (univariate)				Adjusted (multivariate)*			
		Slope	95% CI	p	Adjusted R-sq	Slope	95% CI	p	Adjusted R-sq
IC200	Age	0.01	-0.7-0.7	0.916	0.02	0.04	-0.003-0.10	0.243	0.31
	Gender (male)	0.89	-0.75-2,53	0.277	0.001	0.27	-1.17-1.71	0.707	
	CCT	0.05	0.03 -0.08	<0.001	0.31	0.02	-0.02-0.06	0.265	
	CRF	0.68	0.27 -1.08	0.002	0.20	1.69	0.84-2.54	<0.001	
	CH	0.38	-0.10 -0.86	0.113	0.04	-0.16	-2.05-(-0.26)	0.013	
Perkins	Age	0.19	-0.4-0.79	0.527	0.01	0.42	-0.01-0.09	0.102	0.40
	Gender (male)	0.93	-0.39-2.24	0.64	0.02	0.42	-0.72-1.56	0.456	
	CCT	0.03	0.004 -0.05	0.024	0.010	0.02	-0.01 -0.04	0.181	
	CRF	0.41	0.60 -0.76	0.022	0.10	1.48	0.81-2.15	<0.001	
	CH	0.11	-0.29 -0.51	0.574	-1.23	-1.28	-1.94 -(-0.51)	0.001	

IC200, rebound tonometer Icare 200; Perkins, Perkins applanation tonometer (handheld); CCT: central corneal thickness; CH, corneal hysteresis; CRF, corneal resistance factor; 95% CI: confidence interval (lower and upper limit); * adjusted for CH and CRF.

Table 4b.

	Adjusting variable	Primary congenital glaucoma group							
		Unadjusted (univariate)				Adjusted (multivariate)*			
		Slope	95% CI	P	Adjusted R-sq	Slope	95% CI	P	Adjusted R-sq
IC200	**age**	-0.01	-0.25-0.25	0.979	0.03	-0.11	-0.30-0.08	0.242	0.61
	Gender (male)	-5.83	-9.81-(-1.86)	0.005	0.18	-2.26	-5.45-0.94	0.160	
	CCT	0.03	0.01-0.06	0.019	0.12	-0.01	-0.03 -0.02	0.556	
	CRF	1.77	1.06 -2.48	<0.001	0.41	2.21	1.43-3.00	<0.001	
	CH	-0.01	-1.28 -1.26	0.987	-0.03	-1.73	-2.85 -0.62	0.003	
Perkins	**age**	0.08	-0.13-0.29	0.443	0.01	0.02	-0.16-0.20	0.837	0.43
	Gender (male)	-5.08	-8.42-(-1.74)	0.004	0.18	-2.86	-5.84-0.11	0.059	
	CCT	0.03	0.004 -0.05	0.025	0.10	-0.002	-0.03 -0.02	0.850	
	CRF	1.25	0.58 -1.93	0.001	0.25	1.59	0.85 -2.33	<0.001	
	CH	-0.21	-1.30 -0.89	0.697	-0.02	-1.30	-2.41-(-1.19)	0.023	

IC200, rebound tonometer Icare 200; Perkins, Perkins applanation tonometer (handheld); CCT: central corneal thickness; CH, corneal hysteresis; CRF, corneal resistance factor; 95% CI: confidence interval (lower and upper limit); *adjusted for CH and CRF.