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ABSTRACT

Aim: To evaluate the influence of age on the measurements and relationships among central and peripheral IOP readings taken with a rebound tonometer.

Methods: The intraocular pressures were assessed using the ICare® rebound tonometer (Tiolat Oy, Helsinki, Finland) on the right eye of two-hundred and seventeen patients (88 males, 129 females), aged 18 to 85 years (mean ± SD, 45.9±19.8 years), at the center and at 2 mm from the nasal and temporal limbus along the horizontal meridian. Three age groups were established as being less than 30 years old (n=75), from 31 to 60 years old (n=77) and above 60 years old (n=65).

Results: There was a high correlation between central and peripheral IOP readings, with central reading being higher than peripheral ones. The higher IOP values were found within the younger group for the central location. Subjects within the older group (above 60 years of age) presented significantly lower temporal IOP readings than the remaining two groups ($p<0.001$), while no significant differences were found among groups at central and nasal locations ($p=0.099$ and $p=0.225$, respectively). There was a significant decrease in nasal and temporal IOP readings as the age increased ($p=0.011$ and $p=0.006$, respectively).

Conclusion: Older patients displayed lower IOP values than the middle-aged and younger patients in the temporal peripheral location. A negative correlation between age and IOP by rebound tonometry was found in the corneal periphery but not in its center.

INTRODUCTION

The assessment of intraocular pressure (IOP) is of major importance in glaucoma follow-up and treatment. Enormous effort has been made to develop rapid and accurate methods to measure IOP (i.e. non-contact tonometry [NCT][1,2]; dynamic contour tonometer[3], pneumotonometry[4]) in relation to classical measurements (Goldmann applanation tonometry [GAT]).

However, IOP reliability is compromised after laser corneal refractive surgery procedures due to alterations in corneal thickness and curvature (see Montés-Micó and Charman[5] for a review). New instruments to evaluate IOP not based on corneal applanation could be less affected by surgical alterations.

Rebound tonometry (Tiolat Oy, Helsinki, Finland),[6] measures IOP using the impact of a probe tip over a small area of contact, and could be useful when taking IOP readings after corneal refractive surgery. The reliability of the ICare® tonometer in healthy humans has been recently assessed against Goldmann applanation tonometry (GAT), showing good agreement for clinical purposes with mean differences in the order of 2 to 3 mmHg higher for the rebound tonometer compared to GAT in its conventional[7] and portable versions.[8]

Previous research performed in our group have shown differences in IOP measured at the center and the periphery of the cornea using rebound tonometry (lower values at corneal periphery)[9]. Topographical differences in stromal collagen package between the center and the periphery of the cornea could account for these findings being IOP measurement a reflection of different biomechanical properties depending on the corneal location [10]. Recent research pointed out that the patients' age influences the IOP measurement using GAT, NCT and pneumotonometry [11].

Then, it becomes necessary to explore the correlation of recorded IOP changes with age measured at different locations of the cornea using rebound tonometry. The goal of this study was to analyze the influence of age in the IOP measured at the center and the periphery of the cornea using rebound tonometry.

PATIENTS AND METHODS

Patients

Two-hundred and seventeen subjects (88 males, 129 females), with ages ranging from 18 to 85 years (mean ± SD, 45.9±19.8), gave their consent to participate in the study after the nature of the experimental procedures were explained. Only the values obtained on the right eyes were included in the study. The intraocular pressures were assessed using the ICare® rebound tonometer (Tiolat Oy, Helsinki, Finland).

None of the subjects exhibited any ocular condition or injury, including corneal pathology or corneal scarring, or had been previously submitted to corneal surgery, nor were taking any ocular or systemic medication likely to induce changes in IOP or corneal properties. All procedures followed the guidelines of the Declaration of Helsinki and were approved by the Scientific Committee of the School of Sciences at University of Minho (Braga, Portugal).

Three groups of patients were established according to the specifications in table 1 resulting in a similar number of subjects within the three age intervals (≤30, 31-59, >60 years).

Table 1. Age descriptive statistics corresponding to the three age groupings

	n (%)	mean±SD	Range
≤ 30 years	75 (34.6)	22.3 ± 2.94	18 – 30
31-60 years	77 (35.5)	47.78 ± 6.86	32 – 60
> 60 years	65 (29.9)	70.06 ± 5.91	61 - 85
TOTAL	217	45.9±19.8	18-85

Measurements

IOP was assessed with the ICare® after an ocular health assessment with slit lamp and fundus examination through direct ophthalmoscopy. Measurements were carried out by a trained clinician, avoiding excessive movement of the instrument as the probe hit the cornea. A new disposable probe was used for each subject. The instrument allows taking series of 6 measurements and averaged them to obtain the mean and standard deviation (SD). Three valid series were taken at center, nasal and temporal locations. Measurements at the three locations were randomly performed in order to minimize the potential effect of first readings on subsequent ones. Operating protocol followed at our group can be found elsewhere.[7] Peripheral measurements were taken at a constant distance of about 2 mm from limbus in the nasal and temporal regions of the horizontal meridian subjectively estimated by the operator as twice the thickness of the probe. For each peripheral measurement, the patient was asked to look at a peripheral fixation target on the right and left sides in front of him/her in order to take nasal and temporal (only right eye was measured). After the gaze has been re-oriented towards the peripheral target, the investigator adjusted the perpendicularity of the probe and the location where the probe was to be applied. This place was estimated as a distance that was two times the thickness of the probe (approximately 2 mm from limbus). Similar procedures were followed by previous investigators with Tono-Pen.[12]

Statistical analysis

Data were analyzed using the statistical package for social sciences SPSS version 14.0. Correlations between central and peripheral measurements were assessed

statistically as the mean of the differences compared to zero. The 95% limits of agreement (LoA = mean of the difference ± 1.96 x S.D. of the differences) were also calculated.[13] Bias was assessed statistically as the mean of the differences compared with zero. As variables did not present a normal distribution, Kruskal-Wallis test was applied to analyze the statistical significance of the differences. The level of statistical significance was established at α=0.05. Trends for differences between central and peripheral IOP readings as a function of age were assessed by regression analysis.

RESULTS

Table 2 presents the mean values of IOP registered at center, nasal and temporal locations. Despite IOP readings at corneal center were slightly superior compared to those taken at corneal periphery, those differences were not statistically significant ($p>0.05$). Since normal distribution was not present for all variables, correlations among IOP values were assessed by non-parametric tests, results of which are presented in table 3. All correlations were high and statistically significant. The stronger correlation for the whole population was found between central and nasal measurements, followed by central vs temporal and nasal vs temporal.

Table 2. Descriptive statistics for central and peripheral IOP measured with the ICare® tonometer by age group and for all the subjects considered as a whole (bold); units are mm Hg

	Age Group	Minimum	Maximum	Mean	Std. Deviation
Center	≤30	8	23	15.3	2.9
	31-60	10	25	15.3	3.1
	>60	10	23	14.4	2.5
	Total	**8**	**25**	**15.0**	**2.9**
Nasal	≤30	10	24	14.7	2.6
	31-60	10	22	14.6	2.8
	>60	9	23	13.7	2.2
	Total	**9**	**24**	**14.4**	**2.6**
Temporal	≤30	9	24	15.1	2.6
	31-60	10	23	15.1	3.1
	>60	10	22	13.9	2.3
	Total	**9**	**24**	**14.7**	**2.7**

n= 217

Figure 1 presents plots of differences between IOP readings at different corneal locations as a function of the mean value. Narrow confidence intervals were observed with 95% of the differences lying between ±2 and ±3 mmHg and mean differences close to zero. Nasal IOP values were in closer agreement with central IOP (figure 1-a) than temporal ones (figure 1-b). However, the highest agreement was observed among nasal and temporal peripheral readings as shown in figure (1-c). There was a significant trend towards higher nasal than central IOP values at higher IOP and the opposite for lower IOP ($r^2=0.107$; $p<0.001$). No significant trends were observed when we compare central and nasal IOP values ($r^2=0.013$; $p=0.099$) and nasal and temporal ones ($r^2=0.006$; $p=0.225$).

Table 2 also displays the statistics of IOP readings for the three age groups separately. Apart from the higher central values observed within the same age group, there was a trend for younger patients to present higher values of IOP for the three corneal locations where tonometric readings were taken. Box-plots in figure 2 graphically illustrate IOP values at the three corneal locations for each age grouping. As a general behavior among the three corneal locations we can highlight that the older group displays the less variability in terms of interquartile range and upper to lower bar limits range while the opposite was evident for the middle-age group. While the lower limits were

between 9 and 10 mm Hg, the upper limits of the bars present a higher variability and a higher number of outliers. Such a behavior was also found in a recent experiment involving the IOP assessment by non-contact tonometry synchronized with cardiac rhythm[14] and reflected that lower IOP values were roughly uniform while higher values present more variability, even in non-glaucomatous populations.

Table 3 presents the results of the non-parametric correlations tests between IOP values for different age groups and IOP taken at the three locations. Analyzing data by age groups, we have observed that the stronger correlation between measurements was present for the middle-age group, followed by older group and the least correlation coefficient was found in the younger group for the three combinations (central vs nasal; central vs temporal; nasal vs temporal).

Table 3. Nonparametric correlations between central and peripheral ICare® IOP readings within each age group and for all subjects considered as a whole (bold); units are mmHg

	Age Group	**Spearman's Correlation**	**Significance (*p*)**
Central vs Nasal	≤30	0.832(**)	<0.001
	31-60	0.924(**)	<0.001
	>60	0.833(**)	<0.001
	Total	**0.869(**)**	**<0.001**
Central vs Temporal	≤30	0.787(**)	<0.001
	31-60	0.879(**)	<0.001
	>60	0.837(**)	<0.001
	Total	**0.835(**)**	**<0.001**
Nasal vs Temporal	≤30	0.744(**)	<0.001
	31-60	0.913(**)	<0.001
	>60	0.806(**)	<0.001
	Total	**0.826(**)**	**<0.001**

(**) Correlation was significant at the 0.01 level (2-tailed)

According to table 4, statistically significant differences in temporal IOP were found between the older group (>60) and the remaining two groups (p=0.011), differences only approach significance for nasal IOP values (p=0.052) while definitively not significant differences were found for central IOP measurement (p=0.201).

Table 4. Paired comparisons of ICare® IOP readings between age groups

	Center	**Nasal**	**Temporal**
<30 vs 31-60	-0.019	0.122	0.042
<30 vs >60	0.823	1.045	1.258*
31-60 vs >60	0.842	0.923	1.216*

*The mean difference was significant at the 0.05 level by Kruskall-Wallis test

Figure 3 shows a scatter-plot of different IOP readings as a function of age and a negative correlation between both parameters was observed. This trend towards lower IOP values as a function of age was statistically significant for the temporal ($r=0.185$; $p=0.006$) and nasal readings ($r=0.173$; $p=0.011$) as obtained from ANOVA Curve-Fit analysis. No significant trend existed for central IOP measurement as a function of age ($r=0.126$; $p=0.059$).

DISCUSSION

In the present study, we have found evidences that age could play a significant role on the resistance of the peripheral cornea to the impact of a rebound tonometer. Although we have not performed measurements of corneal thickness, this parameter it is not likely to be responsible for the significant trends towards lower IOP with age as this parameter has demonstrated not to vary significantly as a function of age.[15] For reference purposes, we can consider the values of ultrasonic CT data obtained in a more recent study (*unpublished data*) carried out in 64 right eyes of patients ranging from 18 to 44 years of age. According to those data, central thickness for a normal average cornea was 532±37 μm at center, and 623±40 and 597±46 μm at 4 mm from center in the nasal and temporal regions, respectively. These correspond to a distance from limbus of approximately 2 mm which was the place where ICare peripheral measurements were taken in the present study.

Tonnu *et al*. found a significant trend for GAT and ocular blood flow tonometer (OBF) to overestimate IOP compared to Tono-Pen in eyes of older subjects, this is, Tono-Pen gives lower values than GAT and OBF in older eyes.[11] Also, Eisenberg et al, in a study comprising patients from 4 to 85 years of age found that Topo-Pen measured lower values in older patients than the portable version of GAT.[16] Those finding agree in some way with our trend for lower IOP with ICare as age increases.

A potential explanation for these findings would involve the biomechanical properties and the histological arrangement of the normal cornea and how they change with age.

The macroscopic arrangement of the stromal collagen lamellae seems to be the basis of the shape, strength and transparency of the corneal tissue.[17] The stroma of the human cornea represents 90% of its total thickness and is primarily constituted of collagen fibrils arranged in approximately 200 to 300 parallel lamellae.[17] Despite the increase in the number of lamellae at limbus, a constant number across the transparent portion of the cornea has been generally assumed by the scientific community. Recent studies have confirmed that the increase in collagen diameter and larger interfibrillar spacing could account for the increased peripheral corneal thickness in the normal cornea.[10] Considering that this collagen network would be responsible for the cornea's mechanical strength, there is room for the hypothesis that the response of central and peripheral cornea to rebound tonometry could be influenced by differences in the histological arrangement of the stroma.

Boote *et al*. have demonstrated that collagen fibrils were more closely packed in the prepupillary region compared to peripheral corneal areas; they also found that fibril diameter increase significantly at a distance of 3 to 4 mm from corneal center towards limbus resulting in different optical and biomechanical properties across corneal topography.[10] More interestingly for the purpose of the present study was that previous studies using X-ray diffraction have demonstrated that collagen fibrils increase in diameter with age.[18]

The influence of biomechanical properties of the cornea on GAT is well known[15,19,20] acquiring special relevance when measuring IOP after refractive surgery,[21-24] as well as in the diagnosis and management of glaucoma with normal tension glaucomatous and ocular hypertensive eyes displaying significantly different values of

central and peripheral corneal thickness.[25] More reliable measurements of the IOP were obtained in the temporal part of the cornea after refractive surgery.[12]

From our previous experiments with peripheral rebound tonometry, we have observed that central and peripheral readings reflected what we considered a paradoxical behavior of IOP as with lower IOP values at periphery (despite being thicker) than at center (despite thinner thickness). The higher central values of IOP and the correlations between central and peripheral ICare IOP measurements were in agreement with those found in a previous work carried out at our group on a more limited sample.[9]

The results of Boote *et al*, showed a mean collagen interfibrilar separation 5 to 7% larger in the periphery compared to the central 3 mm of the cornea and could in part support the assumption that central cornea, despite thinner than peripheral cornea could display higher resistance to tonometric devices.[10]

An expansion of the work of Boote *et al*.[10] to corneas of younger and ageing subjects could answer this question regarding the stromal organization a corneal periphery as a function of age. If such differences in ICare IOP readings are related to changes on the biomechanical behavior of the human cornea with age, and if such changes could vary from corneal center to periphery need to be addressed in other specific studies using appropriate instrumentation to quantify such properties. A peripheral thinning in the aging cornea[28] could be involved in some way on changes of corneal response to rebound tonometry at these places. However, because of the apparent insensibility of ICare IOP to large differences in corneal thickness, other hypothesis could not be ignored.

Another potential explanation that should also help to clarify why differences between central and peripheral rebound tonometry measurements vary among age groups could be related to corneal and/or ocular rigidity.

Pallikaris *et al*. concluded that ocular rigidity increases with age; however, despite statistical significant, their results displayed large scatter.[26] On the other hand, Grabner *et al*.[27] found that the corneal resistance to indentation was positively correlated to IOP values (higher resistance with higher IOP) and corneal thickness (higher resistance in thicker corneas) but inversely correlated to age (younger patients presented higher resistance). So, contrary to the trends of apparent increased ocular rigidity in older patients, younger patients could have more rigid corneas than older patients. Changes in hydration control in elderly could be partially responsibly for such a behavior and could explain, at least in part the lower values of IOP found in older patients in the present study whose trends were statistically significant for temporal and nasal readings.

Several points with significance to clinical practice and basic research could be highlighted from the present study. Correlations between central and peripheral IOP readings were different for different age groups. Contrary to other instruments, when using ICare rebound tonometer, lower values of IOP could be expected when readings will be taken at peripheral locations. Peripheral temporal IOP taken with ICare rebound tonometry in older subjects were significantly lower than those taken at the same location in younger subjects. Differences between central and peripheral IOP measurements could increase as a function of age, as peripheral readings have demonstrated a trend to decrease significantly with age while central rebound IOP measurements did not.

In conclusion, we have shown that the ICare® tonometer can conveniently measure central and peripheral intraocular pressure. Thus, the ICare® tonometer is promising diagnostic modality for the objective assessment of central and peripheral IOP.

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Table 1. Age descriptive statistics corresponding to the three age groupings

Table 2. Descriptive statistics of central and peripheral IOP measured with the ICare® tonometer by age group and for all subjects considered as a whole (bold); units are mm Hg

Table 3. Nonparametric correlations between central and peripheral ICare® IOP readings within each age group and for all subjects considered as a whole (bold); units are mm Hg

Table 4. Paired comparisons of ICare® IOP readings between age groups

Figure 1. Plots of differences among IOP readings as a function of the mean value for center vs nasal (1a), center vs temporal (1b) and nasal vs temporal (1c).

Figure 2. Box plot showing IOP values by location and age grouping.

Figure 3. Regression analysis of IOP distribution as a function of age for central, peripheral nasal and peripheral temporal.

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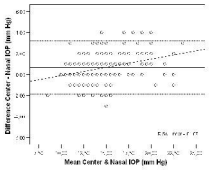
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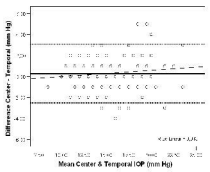
Mean Center & Nasal IOP (mm Hg)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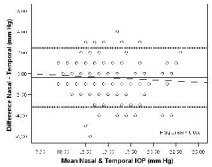
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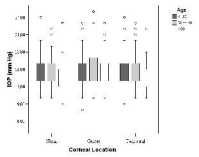
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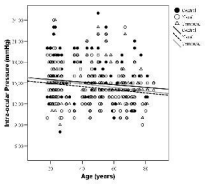
Age
Corneal Location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