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# Correlation between Corneal Endothelial Cell Density and Central Ocular Surface Temperature in Normal and Keratoconus Eyes

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## ABSTRACT

**Purpose:** In keratoconus (KC), an increase of the corneal back surface area may result in endothelial cell density (ECD) decrease and an increase of the corneal front surface area in ocular surface temperature (OST) decrease due to increased heat dissipation. Along with these hypotheses, we aimed to analyse the correlation between ECD and central corneal OST in patients with KC and healthy controls.

**Patients and methods:** A total of 154 eyes with KC (mean age  $36.1 \pm 12.5$  years) and 92 healthy eyes (mean age  $36.4 \pm 12.8$  years) were examined. Corneal front and back surface area at the central 5 mm corneal diameter (FSA and BSA) were calculated based on Pentacam measurement data:

$$\text{FSA or BSA} = 2 \times 3.14 \times R(R - \sqrt{R^2 - D^2})^2,$$

where R referred to corneal front or back surface radius of curvature and D to the corneal front or back surface diameter (5 mm for the present study), respectively.

ECD was determined by specular microscopy (EM-3000) and central corneal OST by thermography (TG-1000).

**Results:** ECD was significantly lower in KC ( $2498 \pm 356/\text{mm}^2$ ) patients than in controls ( $2638 \pm 294/\text{mm}^2$ ;  $p < .001$ ). FSA ( $20.35 \pm 0.26 \text{ mm}^2$  vs.  $20.17 \pm 0.03 \text{ mm}^2$ ) and BSA ( $20.84 \pm 0.58 \text{ mm}^2$  vs.  $20.45 \pm 0.08 \text{ mm}^2$ ) were significantly higher in KC patients than in controls ( $p = .001$ ;  $p < .001$ ), but the average central corneal OST did not differ significantly between both groups ( $34.2 \pm 0.6^\circ\text{C}$  vs.  $34.3 \pm 0.7^\circ\text{C}$ ;  $p = .62$ ). OST at the corneal centre correlated weakly, positively with ECD ( $r = 0.2$ ;  $p < .05$ ), but OST did not correlate with FSA ( $r = 0.045$ ) or BSA ( $r = 0.064$ ).

**Conclusions:** Endothelial cell density seems to have a mild impact on central ocular surface temperature in keratoconus and normal subjects. This effect is not correlated to the corneal front or back surface area.

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## Introduction

Thermography is a non-invasive method of measuring the surface temperature of an object. Ocular thermography was introduced by Mapstone in 1968.<sup>1</sup> Since then, many ocular thermographic parameters have been described in the literature, measured under healthy and pathological conditions. The average ocular surface temperature (OST) is  $32.5\text{--}36.5^\circ\text{C}$  in normal healthy individuals.<sup>2</sup> Pattmüller et al. found good inter- and intra-observer reliability of the TG-1000 thermograph for central corneal OST measurements, and it yielded consistent results.<sup>2</sup> According to Moussa et al.,<sup>3</sup> the central corneal OST does not change diurnally in healthy individuals.

Keratoconus (KC) was first described in 1854.<sup>4</sup> It is the most common corneal ectasia, characterised by bilateral, asymmetric corneal degeneration, which leads to thinning and protrusion of the cornea.<sup>5</sup> Although KC is defined as a non-inflammatory corneal disease, several studies have reported a potential inflammatory origin.<sup>6–10</sup> OST increases in case of inflammatory ocular conditions such as corneal ulcer,<sup>11</sup> scleritis,<sup>12</sup> atopic conjunctivitis<sup>13</sup> and dry eye syndrome.<sup>14</sup> Nevertheless, in

a previous study<sup>15</sup> central corneal OST ( $34.2 \pm 0.6^\circ\text{C}$  vs.  $34.2 \pm 0.7^\circ\text{C}$ ;  $p = .41$ ) did not differ significantly between KC and control subjects.<sup>15</sup>

The innermost layer of the cornea is the corneal endothelium, with a single layer of flat, polygonal cells that play an essential role in maintaining stromal hydration. Maintenance of this hydration gradient depends on tight junctions among endothelial cells and  $\text{Na}^+/\text{K}^+$ -ATPase and bicarbonate-dependent  $\text{Mg}^{2+}$ -ATPase pump functions.<sup>16</sup> Adequate pump function requires a minimum number of endothelial cells.

Endothelial cell density (ECD) decreases from birth ( $3145\text{--}5013 \text{ cells}/\text{mm}^2$ ) to approximately  $2500 \text{ cells}/\text{mm}^2$  in late adulthood.<sup>17,18</sup> According to Elbaz<sup>17</sup> et al., the ECD decrease in the first 2 years of age is also related to the corneal back surface area growth. Nevertheless, after the cornea reaches the adult size, ECD decrease is getting slower.<sup>18</sup> In progressive keratoconus, there is a progressive increase in the corneal back surface area. This phenomenon may also result in endothelial cell density decrease.

Parallel to this effect, with increasing corneal front surface area in keratoconus, corneal heat dissipation may increase,

resulting in changes of ocular surface temperature, measured through ocular surface thermography.

In keratoconus (KC), an increase of the corneal back surface area may result in endothelial cell density (ECD) decrease and an increase of the corneal front surface area in ocular surface temperature (OST) decrease due to increased heat dissipation. Along with these hypotheses, we aimed to analyse the correlation between ECD and central corneal OST in patients with KC and healthy controls.

## Patients and methods

All examinations were performed following the regulations of the Declaration of Helsinki. Our study was approved by the Ethics Committee of Saarland/Germany (no. 41/18). Informed consent was obtained from all participants at Homburg Keratoconus Center (HKC).<sup>19</sup>

We excluded all patients with a history of previous ocular surgery. Eyes with tight palpebral fissure, rapid eye movements during the examination, diagnosis of pellucid marginal degeneration and keratoglobus were excluded from the study. KC was diagnosed by slit-lamp examination and corneal tomography (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany).

First, the OST was adapted to the temperature of the examination room for the subsequent OST measurement for 10 minutes. Thereafter, we examined OST using the TG-1000 Ocular Surface Thermographer (Tomey, Nuremberg, Germany). All examinations were performed by a single examiner (ON) to eliminate inter-examiner variation.<sup>2</sup> During the measurements, a standard environment was maintained in the examination room; the average room temperature was  $23.9 \pm 1.6^\circ\text{C}$  and humidity  $32.4 \pm 6.7\%$ . The examination room doors and windows were closed to minimise airflow to avoid it affecting the OST.<sup>20</sup>

OST was measured as described by Mori and associates. After the participants blinked normally, they closed both eyes for 5 seconds, and then kept their eyes open for more than 10 seconds.<sup>21</sup> During the examination, the participant's head was placed in a standard ophthalmic chin and headrest. They were instructed to look straight ahead. A sequence of 11 OST images was taken from baseline to 10 seconds after eye opening (in intervals of 1 second) for each measurement. The lateral resolution was  $70 \mu\text{m}$  in both the horizontal and vertical directions, resulting in  $320 \times 240$  data points being stored with each image. We extracted the mean OST value at the corneal centre (mean value of the OST value at the central 1 mm zone) during the 10 seconds of sustained eye opening after blinking. Data on OST 8 mm temporally from the corneal centre (conjunctival OST; measured 8 mm temporally from the corneal centre along the horizontal line projected through the TG-1000) were also collected.

All patients underwent a complete ophthalmological examination. In addition, patients were examined using a rotating Scheimpflug camera (Pentacam HR). For the present study, the topographic KC (TKC) classification (Pentacam; 0–1 to 4) was extracted for all patients. In addition, corneal front and back surface radius of curvature (RmF and RmB) were extracted and corneal front surface area (FSA) and corneal back surface area

(BSA) were calculated within a central 5 mm region, as described by Kitazawa et al.<sup>22</sup>

$$\text{FSA or BSA} = 2 \times 3.14 \times R(R - \sqrt{R^2 - D/2})^2,$$

where R refers to RmF or RmB (for calculation of FSA or BSA) and D to the corneal front or back surface diameter (5 mm for the present study), respectively.

Endothelial cell analysis and measurement of central corneal thickness (CCT) were performed using the EM-3000 (Tomey, Nuremberg, Germany) specular microscope. The values for ECD, hexagonality (6A), coefficient of variation (CV; the coefficient of variation of cell area) and CCT were extracted.

A total of 154 eyes in 90 patients with KC and 92 eyes from 46 controls were examined. Patients with a TKC between two stages (e.g., “0–1”) were classified as the more advanced stage. Patient age was  $36.1 \pm 12.5$  years (range 14–67 years) in KC patients and  $36.4 \pm 12.8$  years (range 18–78 years) in controls at the time of the examination, with no significant difference in patient age between the two groups ( $p = .923$ ). The KC group included 34.1% females and 53.6% left eyes, and the control group 52.4% females and 47.6% left eyes. According to TKC, 24 eyes were classified as stage 1 (TKC 1; 15.6%), 55 eyes as stage 2 (TKC 2; 35.7%), 51 eyes as stage 3 (TKC 3; 33.1%) and 24 eyes as stage 4 (TKC 4; 15.6%). Ninety-two control eyes were classified as “TKC 0”.

Statistical analyses were performed using SPSS software (SPSS version 20, IBM, New York).  $P \leq 0.05$  was considered statistically significant. A non-parametric Mann–Whitney U-test was performed to compare measurement data between KC patients and controls. Correlations were tested using the Spearman Rho test. The strength of the correlation was determined to be ‘very strong’ with  $1.0 \geq r \geq 0.8$ , ‘strong’ for  $0.8 > r \geq 0.6$ , ‘moderate’ for  $0.6 > r \geq 0.4$ , ‘weak’ for  $0.4 > r \geq 0.2$ .<sup>23</sup>

## Results

ECD, hexagonality, the average endothelial CV, CCT, OSDI score, and central and conjunctival OST are provided in Table 1. RmF, RmB, FSA and BSA are shown in Table 2.

ECD ( $2498 \pm 356$  vs.  $2638 \pm 294/\text{mm}^2$ ) and CCT ( $475 \pm 50$  vs.  $529 \pm 35 \mu\text{m}$ ) were significantly lower ( $p < .001$ ;  $p < .001$ ) and CV ( $48.2 \pm 18.3$  vs.  $47.3 \pm 52.3$ ) was significantly higher ( $p = .001$ ) in KC patients than in healthy controls, but hexagonality ( $42.8 \pm 22.7$  vs.  $38.9 \pm 20.8$ ) did not differ significantly between the two groups ( $p = .69$ ).

The average central corneal OST was  $34.2 \pm 0.6^\circ\text{C}$  in KC patients and  $34.3 \pm 0.7^\circ\text{C}$  in controls and did not differ significantly ( $p = .62$ ). Conjunctival OST ( $34.4 \pm 1.0^\circ\text{C}$  vs.  $34.6 \pm 0.8^\circ\text{C}$ ) also did not significantly differ between the two groups ( $p = .21$ ). Using a Kruskal–Wallis test, we found no difference in OST between less and more advanced stages of KC; therefore, we did not proof for differences between TKC groups.

RmF ( $6.9 \pm 0.8$  vs.  $7.7 \pm 0.2$ ) and RmB ( $5.6 \pm 0.8$  vs.  $6.3 \pm 0.2$ ) were significantly lower ( $p < .001$ ;  $p < .001$ ), FSA ( $20.35 \pm 0.26$  vs.  $20.17 \pm 0.03$ ) and BSA ( $20.84 \pm 0.58$  vs.  $20.45 \pm 0.08$ ) were significantly higher in KC subjects ( $p < .001$ ;  $p < .001$ ), compared to controls.

**Table 1.** Measurements in different topographic keratoconus classification (TKC) stages. Data are given as mean±SD (minimum-maximum). ECD, endothelial cell density; CV, coefficient of variation of the corneal endothelial area; CCT, central corneal thickness; OSDI, Ocular Surface Disease Index; OST, ocular surface temperature (central: at corneal centre, conjunctival: 8 mm temporally from the corneal centre).

| TKC                   | ECD (/mm <sup>2</sup> ) | Hexagonality (6A, %) | CV in %              | CCT (µm)           | OSDI                   | OST central (°C)       | OST conjunctival (°C)  |
|-----------------------|-------------------------|----------------------|----------------------|--------------------|------------------------|------------------------|------------------------|
| TKC 0 (n = 92)        | 2638 ± 294 (1813–3244)  | 38.9 ± 20.8 (0–70)   | 47.3 ± 52.3 (30–137) | 529 ± 35 (477–627) | 19.3 ± 18.3 (0–79.6)   | 34.3 ± 0.7 (32.4–35.4) | 34.6 ± 0.8 (31.6–35.8) |
| TKC 1–TKC 4 (n = 154) | 2498 ± 356 (1208–3245)  | 42.8 ± 22.7 (0–100)  | 48.2 ± 18.3 (23–126) | 475 ± 50 (366–563) | 32.3 ± 22.6 (0–88.9)   | 34.2 ± 0.6 (32.4–35.4) | 34.4 ± 1.0 (30.8–36.3) |
| P value*              | <b>&lt;0.001</b>        | 0.69                 | <b>0.001</b>         | <b>&lt;0.001</b>   | <b>&lt;0.001</b>       | 0.62                   | 0.21                   |
| TKC 1 (n = 24)        | 2592 ± 262 (2101–2988)  | 41.7 ± 19.6 (0–65)   | 40.6 ± 7.9 (30–58)   | 491 ± 33 (408–538) | 30.1 ± 19.3 (4.2–60.4) | 34.3 ± 0.7 (32.4–35.4) | 34.3 ± 1.0 (31.9–35.9) |
| TKC 2 (n = 55)        | 2528 ± 350 (1595–3245)  | 49.1 ± 20.9 (0–100)  | 47.6 ± 16.4 (30–106) | 471 ± 44 (370–557) | 31.7 ± 25.2 (0–87.5)   | 34.2 ± 0.6 (32.4–35.4) | 34.5 ± 0.9 (30.8–36.2) |
| TKC 3 (n = 51)        | 2408 ± 334 (1303–3047)  | 37.9 ± 23.9 (0–100)  | 50.7 ± 16.4 (30–115) | 447 ± 45 (366–527) | 29.4 ± 18.5 (4.2–88.9) | 34.3 ± 0.7 (32.4–35.4) | 34.5 ± 1.2 (31.1–36.3) |
| TKC 4 (n = 24)        | 2380 ± 397 (1208–3039)  | 35.3 ± 24.1 (0–75)   | 59.1 ± 27.7 (23–126) | 449 ± 62 (373–563) | 41.6 ± 25.9 (0–88.9)   | 34.2 ± 0.6 (32.9–35.0) | 34.5 ± 0.9 (32.0–36.0) |

\*Difference between normal cornea (= TKC 0) and all eyes in TKC 1–TKC 4 groups. Patients with a TKC between two stages (e.g., 0–1) were always classified as the more advanced stage.

**Table 2.** Measurements in different topographic keratoconus classification (TKC) stages. Data are given as mean±SD (minimum-maximum). Corneal front and back surface radius of curvature (RmF/RmB) and corneal front and back surface area at a central 5 mm region (FSA/BSA) are shown.

| TKC                   | RmF (mm)            | RmB (mm)            | FSA (mm <sup>2</sup> )     | BSA (mm <sup>2</sup> )     |
|-----------------------|---------------------|---------------------|----------------------------|----------------------------|
| TKC 0 (n = 92)        | 7.7 ± 0.2 (7.1–8.5) | 6.3 ± 0.2 (5.6–7.2) | 20.17 ± 0.03 (20.07–20.29) | 20.45 ± 0.08 (20.25–20.72) |
| TKC 1–TKC 4 (n = 154) | 6.9 ± 0.8 (4.3–8.4) | 5.6 ± 0.8 (3.2–7.4) | 20.35 ± 0.26 (20.09–21.69) | 20.84 ± 0.58 (20.23–24.13) |
| P value*              | <b>&lt;0.001</b>    | <b>&lt;0.001</b>    | <b>&lt;0.001</b>           | <b>&lt;0.001</b>           |
| TKC 1 (n = 24)        | 7.5 ± 0.2 (7.2–8.0) | 6.1 ± 0.3 (5.6–6.8) | 20.20 ± 0.04 (20.14–20.27) | 20.53 ± 0.09 (20.33–20.72) |
| TKC 2 (n = 55)        | 7.3 ± 0.4 (6.5–8.4) | 5.9 ± 0.5 (4.9–7.4) | 20.25 ± 0.08 (20.09–20.43) | 20.62 ± 0.20 (20.23–21.07) |
| TKC 3 (n = 51)        | 6.8 ± 0.6 (5.4–8.4) | 5.4 ± 0.6 (4.0–6.9) | 20.34 ± 0.14 (20.09–20.81) | 20.85 ± 0.33 (20.32–22.05) |
| TKC 4 (n = 24)        | 5.8 ± 1.0 (4.3–7.7) | 4.6 ± 1.0 (3.2–6.9) | 20.75 ± 0.44 (20.19–21.69) | 21.66 ± 0.97 (20.32–24.13) |

\*Difference between normal cornea (= TKC 0) and all eyes in TKC 1–TKC 4 groups. Patients with a TKC between two stages (e.g., 0–1) were always classified as the more advanced stage.

ECD weakly positively correlated with central corneal OST ( $r = 0.2$ ;  $p < .05$ ) and did not correlate with conjunctival OST ( $r = 0.043$ ). CV weakly negatively correlated with central corneal OST ( $r = -0.212$ ;  $p = .001$ ) and did not correlate with conjunctival OST ( $r = -0.075$ ). Hexagonality did not correlate with the OST within the regions of interest ( $r > 0.013$ ). CCT also did not correlate with central corneal OST ( $r = 0.048$ ).

Figures 1–2 present the scatter plot analysis of central corneal OST and ECD; CV; hexagonality; CCT; OSDI; FSA and BSA in normal (TKC 0) and keratoconus eyes (TKC 1–TKC 4), with regression lines for TKC 0 and TKC 1–TKC 4 groups.

RmF, RmB, FSA and BSA did not correlate with central corneal OST ( $r = 0.015$ ;  $r = -0.024$ ;  $r = 0.045$ ;  $r = 0.064$ ). FSA and BSA did not correlate with ECD ( $r = -0.186$ ;  $r = -0.170$ ), hexagonality ( $r = -0.131$ ;  $r = -0.142$ ) and CV ( $r = 0.133$ ;  $r = 0.102$ ).

OST at all examined regions did not correlate with patient age ( $r < -0.158$ ). ECD correlated weakly inversely with patient age ( $r = -0.365$ ;  $p < .001$ ), whereas CV and hexagonality did not correlate with age ( $r < 0.104$ ).

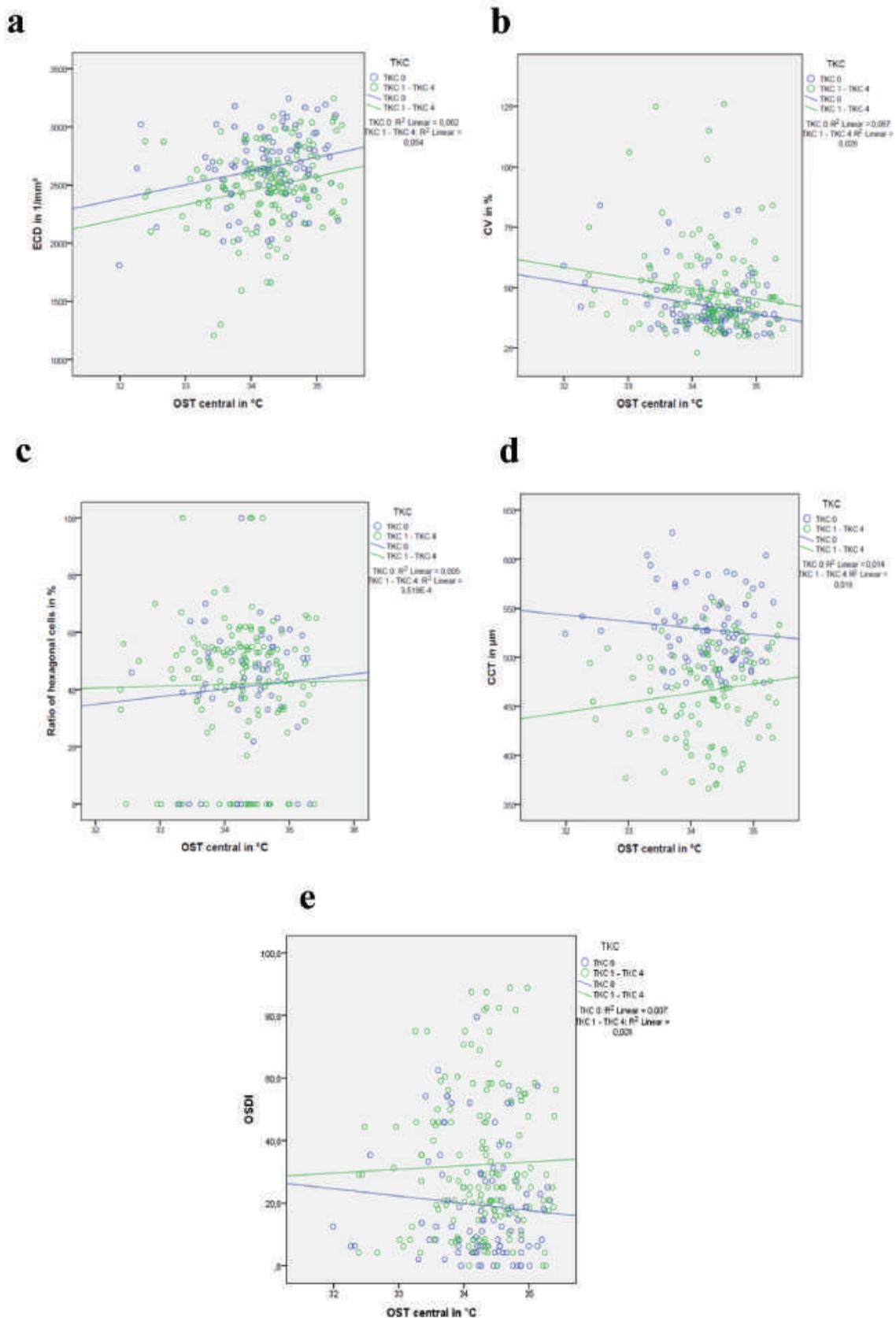
## Discussion

The most conspicuous finding of our study is that OST at the corneal centre correlates weakly positively with ECD and weakly negatively with CV. Interestingly, we demonstrated these weak correlations in both keratoconus and healthy eyes. Nevertheless, no correlation between RmF, RmB, FSA, BSA and central corneal OST could be verified in our present study. These results rather show that ECD has a special role in the regulation of OST, without an influence of the corneal front/back surface curvature and area.

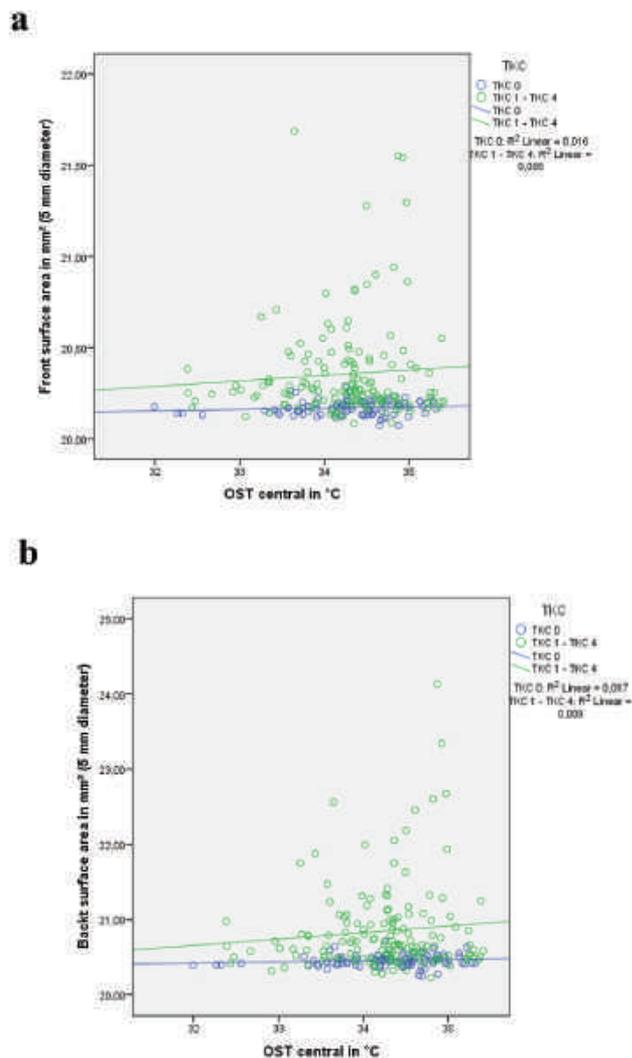
We hypothesized that an increase of the corneal back surface area results in ECD decrease and parallelly, an increase of the corneal front surface area results in OST decrease due to increased heat dissipation in keratoconus eyes. Although there was a significantly lower ECD (and higher CV) in keratoconus eyes than in controls, a significant difference in OST could not be shown between the patient groups, so this fact contradicted our hypothesis.

Kitazawa et al.<sup>24</sup> described the ratio of the front and back corneal surface area in keratoconus smaller than in normal eyes. Therefore, a significant difference in ECD, without a significant difference in OST between KC and normal eyes (as corneal front surface increases less than the corneal back surface in KC and there is less heat dissipation), could probably be explained. Nevertheless, that RmF, RmB also did not correlate with OST in the examined KC and control subjects, contradicts again our hypothesis. Therefore, we rather assume, that ECD has a mild role in OST regulation, without an influence of the corneal front/back surface curvature and area, which should be further explored in the future.

The corneal epithelial and endothelial barrier plays a major role in the maintenance of corneal transparency.<sup>25</sup> If corneal endothelium is compromised, the cornea becomes thicker, oedematous and loses its transparency.<sup>26</sup> Acting as a permeability barrier, the endothelial monolayer restricts the flow of aqueous humour and solutes into the hydrophilic stroma.<sup>25</sup> In addition, there is endothelial active ion transport<sup>27</sup> from the stroma to the aqueous humour. This mechanism corresponds to a combined leaky barrier and



**Figure 1.** Scatter plot of the central corneal ocular surface temperature (OST central) and endothelial cell density (ECD) (a)/coefficient of variation (CV) (b)/ratio of hexagonal cells (c)/central corneal thickness (CCT)(d)/Ocular Surface Disease Index (OSDI)(e) in normal corneas (TKC 0; n = 92, blue circles and blue regression line) and keratoconus corneas (TKC 1–TKC 4; n = 154, green circles and green regression line). Patients with a TKC between two stages (e.g., TKC 0–1) were always classified as the more advanced stage. R<sup>2</sup> refers to Spearman's coefficient of determination.



**Figure 2.** Scatter plot of the central corneal ocular surface temperature (OST central) and corneal front and back surface area (FSA/BSA) (**a**, **b**) in normal corneas (TKC 0;  $n = 92$ , blue circles and blue regression line) and keratoconus corneas (TKC 1–TKC 4;  $n = 154$ , green circles and green regression line). Patients with a TKC between two stages (e.g., TKC 0–1) were always classified as the more advanced stage.  $R^2$  refers to Spearman's coefficient of determination.

fluid pump.<sup>25</sup> The maintenance of corneal thickness and transparency is based on a balance of fluid inflow leaking into the stroma and outflow being actively pumped out from the stroma by the endothelium.<sup>28</sup> As we have proven ECD to be weakly correlated with central corneal temperature, it would be important to analyse patients with endothelial pathologies such as bullous keratopathy or Fuchs' dystrophy. OST may be capable to detect early endothelial dysfunction in such cases.

Long-standing corneal oedema also predisposes individuals to complications, such as corneal vascularisation, infection and scarring.<sup>29</sup> Elevated cytokine levels have been reported in the aqueous humour of eyes with bullous keratopathy and low endothelial density, which reflects inflammation in this condition. Elevated cytokine levels have also been described in tears of keratoconus patients, but as OST positively correlated with ECD in keratoconus patients of our present study, this contradicts an inflammatory hypothesis.<sup>30</sup> If elevated cytokine levels are related to an OST increase, also requires further analysis.

Endothelial cell morphology reflects the quantitative corneal properties and provides a qualitative description of the functional status, in regards to variation in the cell area and shape. The quality of corneal endothelium may not be assessed by cell density measurements alone, but by quantification of the CV, the percentage of hexagonal cells and the CCT.<sup>31</sup> Although our KC patients had significantly lower ECD and higher CV than controls, we could not find a difference in the hexagonality of the cells between both groups. Nevertheless, plenty of other factors such as dry eye syndrome or type 2 diabetes mellitus may decrease ECD/function and change hexagonality.<sup>32,33</sup> Similar to our present study, according to Goebels et al.<sup>34</sup> in KC, corneal thickness and ECD are significantly decreased and endothelial CV significantly increased with the progression of KC severity.

During aging, corneal ECD decreases by 0.3% to 1% per year.<sup>35</sup> This phenomenon is also observed in our data set, as ECD correlated with patient age.

Pattmüller et al.<sup>36</sup> analysed the correlation of OST and ECD at the corneal centre in 61 Caucasian healthy adults (mean age  $24.9 \pm 6.7$ ), also using the TG-1000 thermographer. They concluded that in young healthy adults the average ocular surface temperature does not correlate with ECD, which contradicts our present study. However, for their examination, all dry eye subjects have been excluded and the patient age was 10 years lower compared to our present study population. In addition, air humidity was different during their examination ( $41.83 \pm 4.19\%$  vs.  $32.4 \pm 6.7\%$ ). Changes in environmental factors may have a significant impact on OST measurements. An additional drawback of both studies is that body temperature has not been measured immediately before OST measurements.

Earlier studies of Morgan et al.<sup>37</sup> found decreasing OST with increasing corneal thickness. In contrast, Efron et al.<sup>38</sup> and Alió et al.<sup>39</sup> found increasing OST from the centre of the cornea to the periphery which was explained with the proximity of the limbal vessels. The lower temperature of the central cornea was explained by its distance from the corneoscleral limbus, by the regional corneal thickness profile, the depth of the anterior chamber and by the differences of the local tear film.<sup>21</sup> Nevertheless, varying measurement methods and environmental conditions could also have an impact on these contradictory results. We could not verify a correlation of CCT and OST in our normal and keratoconus patients.

Schroeter et al.<sup>40,41</sup> analysed the impact of temporary hypo- and hyperthermia on corneal endothelial cell survival during organ culture preservation. The exposure of organ-cultured porcine corneas to 4°C for 12 hours and 21°C for 48 hours did not compromise the endothelial cell density of donor corneas in a clinically relevant manner.<sup>40</sup> Exposure of the porcine corneas to 40°C or 42°C for 12 h also did not induce endothelial cell loss.<sup>41</sup> Nevertheless, exposure to 44°C and 50°C led to total necrosis of the endothelial cell layer.<sup>41</sup> These findings show that temperature changes of the ocular surface below 40°C may not have an impact on the endothelial cell layer. This is especially important concerning corneal organ cultures and corneal storage temperature. Nevertheless, measurement series with a longer exposure time should further strengthen this hypothesis as in our

in vivo study, ECD weakly correlated with the ocular surface temperature.

OST was measured under different ophthalmological conditions. Tai-Yuan Su et al.<sup>42</sup> found that the eyelid margin temperature is higher in cases of Meibomian gland dysfunction than in healthy controls. Furthermore, according to Morgan et al.<sup>14</sup> the mean OST is larger in dry eye than controls, and larger variation in temperature was measured across the ocular surface in the dry eye group. The OST is also higher when a corneal ulcer is present<sup>11</sup> and in corneal immunological transplant rejection<sup>43</sup> compared to normal eyes. The OST has been investigated to analyse bleb function after glaucoma surgery,<sup>44</sup> after corneal refractive surgery,<sup>45</sup> following cataract surgery,<sup>46</sup> and in ocular blood flow evaluation.<sup>47</sup> However, to the best of our knowledge, this is the first study to analyse the relationship between OST and corneal ECD in a corneal pathology. It would be interesting to analyse in future studies whether, in pathological conditions (e.g., blepharitis, dry eye, corneal ulcer, corneal transplant rejection, glaucoma, following corneal refractive or cataract surgery),<sup>11,14,43-47</sup> ECD also has an impact on OST.

In summary, endothelial cell density seems to have a mild impact on central ocular surface temperature in keratoconus and normal subjects. This effect is not correlated to the corneal front or back surface area or curvature. The exact reason for this phenomenon remains unclear.

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There is no conflict of interest to declare.

## Disclaimer

The views expressed in the submitted article are the author's own and not an official position of the institution or funder.

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