Analyzing the changing trend of corneal biomechanical properties under different influencing factors in T2DM patients

Tao Li
The First People's Hospital of Ziyang

Zhiwu Lin
The First People's Hospital of Ziyang

Juan Tang (✉️ 874548166@qq.com)
The First People's Hospital of Ziyang

Ying Li
The First People's Hospital of Ziyang

Xingde Liu
The First People's Hospital of Ziyang

Biao Li
The First People's Hospital of Ziyang

Xiaoli Wu
The First People's Hospital of Ziyang

Qilin Fang
The First People's Hospital of Ziyang

Chuanqiang Dai
The First People's Hospital of Ziyang

Jing Lv
The First People's Hospital of Ziyang

Guogang Liu
The First People's Hospital of Ziyang

Research Article

Keywords: T2DM, Corneal hysteresis, Corneal resistance factor, Ocular response analyzer, Diabetic keratopathy

Posted Date: April 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2819366/v1
Abstract

Objective

To analyze the changing trend of corneal hysteresis (CH) and corneal resistance factor (CRF) values under different influencing factors in T2DM patients.

Purpose

All patients underwent the ophthalmoscopy and fundus fluorescein angiography (FFA). A total of 650 patients with T2DM visited The First People's Hospital of Ziyang from February 2022 to March 2023 were included. Logistic linear regression analysis was used to evaluate the independent risk factors of gender, age, course of T2DM, hypertension, smoking, drinking, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP), fasting blood glucose (FBG), cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and hemoglobin (Hb) A1c related with T2DM. At the same time, according to the course of T2DM, FBG, DR stage, HbA1c (%) and other different pathological changes, the CH and CRF values were measured by the ocular response analyzer (ORA), and the change trend of their values was analyzed.

Results

In this study, we discovered that the course of T2DM, smoking history, BMI, FBG, DR, HbA1c, TC, TG, LDL-C were common risk factors for T2DM, while HDL-C was a protective factor, with statistically significant differences (P < 0.05). With the prolongation of T2DM, the increase of FBG, and the accumulation of HbA1C, the values of CH and CRF gradually decreased, especially in HbA1c (%) > 12, the values of CH (1.85 ± 0.33) mmHg and CRF (1.28 ± 0.69) mmHg decreased the most. Compared with the Non-DR group, the CH and CRF values gradually decreased in the mild-NPDR, moderate-NPDR, severe-NPDR and PDR groups, with the lowest CH and CRF values in the PDR group. Analysis of variance between groups showed statistically significant differences in CH and CRF values (P < 0.05).

Conclusion

In patients with T2DM, early measurement of corneal biomechanical properties by ORA so as to evaluate the change trend of CH and CRF values in different situations will help to identify and prevent diabetic keratopathy as soon as possible.

1. Introduction

Diabetes is a kind of common metabolic and endocrine diseases caused by absolute or relative insufficient insulin secretion. Its basic pathological changes are metabolic disorders which can lead to lesions in various systems and organs in the body[1, 2]. The pathogenesis of diabetes includes pathophysiological processes such as apoptosis, inflammation, neurotrophic damage and oxidative stress[3, 4]. In the field of ophthalmology, there have been extensive studies on retinopathy, glaucoma and
cataract caused by diabetes\textsuperscript{[5–7]}. In recent years, some scholars reported that more than 70% of diabetes patients can develop into diabetic keratopathy, and it had been confirmed that some patients can have the morphological change of cornea\textsuperscript{[8,10]}. Different from the traditional diabetic retinopathy, glaucoma, cataract and other diseases that easily lead to decreased vision as the main clinical symptoms, the clinical manifestations of diabetic keratopathy are often ignored. The main manifestations of patients are decreased corneal perception, delayed corneal epithelial regeneration, and bullous corneal endothelial disease, which is followed by severe lesions such as persistent corneal epithelial erosion, shallow punctate keratopathy, and even corneal ulcers. It is a potential visual threatening disease that can lead to decreased vision or permanent visual loss, especially after undergoing treatment such as cataract phacoemulsification and vitrectomy\textsuperscript{[11–13]}. Some scholars believed that long-term elevated blood sugar levels may lead to impaired endothelial "pump" function before intraocular surgery\textsuperscript{[14]}. As we konw, the cornea contains rich sensory nerve endings, so the impaired glucose metabolism will inevitably lead to sensory impairment of the cornea. However, there is still a lack of objective evaluation methods for diabetic keratopathy\textsuperscript{[15]}.

Cornea not only has biomechanical properties, but also has an important relationship with corneal structure and the development of corneal diseases. Especially after various corneal diseases and corneal surgery, the deformation and pathological changes of the cornea may lead to changes in corneal biomechanics\textsuperscript{[16,17]}. With the clinical application of Ocular Response Analyzer (ORA) in ophthalmology, it is possible to evaluate its biomechanical properties on living corneas\textsuperscript{[18,19]}. Some research reported that the measurement of corneal biomechanics by ORA had been widely used in the evaluation of corneal biomechanics of patients in the diagnosis and treatment of keratoconus, glaucoma and corneal refractive surgery\textsuperscript{[20–22]}. Therefore, this study intended to use ORA analyzer to measure the corneal biomechanics of T2DM patients and evaluate the influencing factors of corneal biomechanics in diabetes patients.

2. Materials And Methods

2.1 Study patients

This is a retrospective study designed to investigate associations between corneal biomechanical properties and potential impact factors in T2DM patients. A total of 650 patients with T2DM visited The First People's Hospital of Ziyang from February 2022 to March 2023 were included. Among them, there were 348 males and 302 females, with an average age of (54.28 ± 18.27) years old. The measurement of the central corneal thickness (CCT) and corneal curvature were detected by the ultrasonic pachymeter (Sonomed Inc.1979 Marcus Avenue Lake Success, NY 11,042, USA) and Corneal Topography (CSO, Firenze, Italy) respectively. Three measurements were taken in each eye. All eye data were collected from both eyes, and the average value was taken.
**Inclusion criteria:** 1). Symptoms of diabetes + plasma glucose level at any time ≥ 11.1 mmol/L (200 mg/dl); 2). Fasting blood glucose (FBG) ≥ 7.0 mmol/L (126 mg/dl); 3). OGTT 2 hours post meal plasma glucose ≥ 11.1 mmol/L; 4). The overall corneal thickness range of the patient was 500-520 μm, and the corneal curvature was (44.22 ± 1.43). Differences in corneal hysteresis (CH) and corneal resistance factor (CRF) caused by significant differences in corneal thickness and curvature were excluded.

**Exclusion criteria:** keratoconus, suspected keratoconus, corneal dystrophy, glaucoma, acute renal injury, type 1 diabetes, diabetes ketoacidosis, acute hyperglycemia (accompanied by severe ketonuria), and acute infection.

### 2.2 Clinical and laboratory data collection

The clinical data were obtained from medical records: age, sex, course of T2D, hypertension, a history of smoking, alcohol consumption, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP). Laboratory parameters, such as levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hemoglobin (Hb)A1c, fasting blood glucose (FBG) were measured in the morning following overnight fasting.

### 2.3 Diagnosis and staging of DR

After excluding contraindications for fundus fluorescein angiography, all patients underwent this examination. Two experienced ophthalmologists from The First People's Hospital of Ziyang staged the patient's DR lesions based on fundus fluorescence angiography images according to the international clinical DR severity scale. The fundus manifestations in diabetics were classified into the DR stages: non-DR, mild-nonproliferative (mild-NPDR), moderate-nonproliferative (moderate-NPDR), severe-nonproliferative (severe-NPDR) and proliferative (PDR).

### 2.4 Measurement of CH and CRF

The ORA measurement (Reichert Ophthalmic Instruments, Buffalo, New York, USA) was currently the main method for measuring corneal biomechanics CH and CRF. Based on the principle of dynamic bi-directional flattening, ORA collected the P1 and P2 values during the two compression processes of the cornea, and calculated the CH value using the numerical difference between the P1 and P2. The CH value mainly reflected the corneal viscosity. CRF was calculated through a specific formula which represented an indicator of evaluating corneal viscoelasticity. Both CH and CRF values were provided directly by the ORA machine.

### 2.5 Statistical analysis

Statistical management and analysis were conducted using SPSS 20.0 (SPSS Inc., Chicago, USA). Independent sample t-tests were used to assess if each parameter had a normal distribution. Categorical variables were compared using the X² test. Logistic regression analysis was performed to evaluate factors associated with T2DM. Statistical significance was set at p < 0.05.
3. Result

As we knew, the CCT and corneal curvature were the most important factors of affecting CH and CRF. Therefore, after excluding the effects of CCT and corneal curvature on CH and CRF, a total of 650 T2DM patients were included in this study. First of all, the logistic linear regression analysis was performed to evaluate influencing factors associated with T2DM (Table 1). After adjusting for gender, age, course of T2DM, a history of smoking, alcohol consumption, BMI, FBG, DR, SBP ≥ 130 mm Hg, DBP > 90 mmHg, HbA1c, TC, TG, HDL-C, and LDL-C levels, T2DM was significantly associated with course of T2DM (OR = 1.89, 95%CI: 1.43–2.46; P = 0.007), a history of smoking (OR = 1.57, 95%CI: 0.91–1.92; P = 0.031), BMI (OR = 1.59, 95%CI: 1.12–1.98; P = 0.002), FBG (OR = 1.72, 95%CI: 0.91–2.34; P = 0.047), DR (OR = 2.07, 95%CI: 1.48–2.91; P = 0.003), HbA1c (OR = 1.97, 95%CI: 1.21–3.02; P = 0.003), TC (OR = 1.71, 95%CI: 0.98–2.35; P = 0.001), TG (OR = 1.48, 95%CI: 0.65–1.72; P = 0.032), HDL-C (OR = 0.82, 95%CI: 0.43–1.23; P = 0.021), LDL-C (OR = 1.45, 95%CI: 0.78–1.92; P = 0.006). These results showed that the course of T2DM, smoking history, BMI, FBG, DR, HbA1c, TC, TG and LDL-C were common risk factors for T2DM, while HDL-C was a protective factor, with statistically significant differences (all P < 0.05).

In order to further explore the influence of T2DM course on corneal biomechanical properties, patients were divided into Group I (1 < T2DM course < 5), Group II (5 ≤ T2DM course < 10), Group III (10 ≤ T2DM course < 15), Group IV (15 ≤ T2DM course < 20) and Group V (T2DM course ≥ 20) according to the course of diabetes. As was shown in Table 2, with the prolongation of the course of T2DM, the values of CH and CRF gradually decreased. LSD-t test analysis showed that the difference between CH and CRF values was statistically significant (P < 0.05).

Then, we divided this experiment into FBG1 group (7.0mmol/L < FBG < 9mmol/L), FBG2 group (9.0mmol/L ≤ FBG < 11mmol/L), FBG3 group (11mmol/L ≤ FBG < 13mmol/L), and FBG4 group (FBG ≥ 13mmol/L) based on FBG values. Obviously, the values of CH and CRF in T2DM patients gradually decreased as FBG increased (Table 3). At the same time, we grouped this study as Group A (6.5 < HbA1c(%) ≤ 8) Group B (8 < HbA1c(%) ≤ 10) Group C (10 < HbA1c(%) ≤ 12) Group D (HbA1c(%) > 12). The results showed that in T2DM patients, with the increase of HbA1c (%), the values of CH and CRF gradually decreased, especially when HbA1c (%) > 12, the values of CH (1.85 ± 0.33) mmHg and CRF (1.28 ± 0.69) mmHg decreased the most (Table 4).

Logistic linear regression analysis showed that DR was an independent risk factor for T2DM. Therefore, the fundus fluorescence angiography examination was performed on patients, and it was found that there were 207 patients in the non DR group, 145 patients in the Mild-NPDR group, 167 patients in the Moderate-NPDR group, 89 patients in the Severe-NPDR group, and 42 patients in the PDR group. Further statistical analysis of the mean changes in CH and CRF in each group, we discovered that compared to the Non-DR group, the CH and CRF values gradually decreased in the mild-NPDR, moderate-NPDR, severe-NPDR, and PDR groups, with the lowest CH and CRF values in the PDR group (Table 5).

Table 1 Logistic linear regression analysis of influencing factors related to T2DM
Table 2
Biomechanical characteristics of cornea in T2DM patients with different diabetes course.

<table>
<thead>
<tr>
<th>Course of T2DM(year)</th>
<th>CH(mmHg) median</th>
<th>95% CI</th>
<th>CRF(mmHg) median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12.12</td>
<td>11.02, 12.34</td>
<td>12.45</td>
<td>11.32, 13.48</td>
</tr>
<tr>
<td>Group II</td>
<td>11.68</td>
<td>10.21, 12.08</td>
<td>11.92</td>
<td>10.82, 12.34</td>
</tr>
<tr>
<td>Group III</td>
<td>11.24</td>
<td>9.34, 11.23</td>
<td>11.44</td>
<td>10.93, 12.03</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.79</td>
<td>9.02, 11.08</td>
<td>10.92</td>
<td>9.78, 11.73</td>
</tr>
<tr>
<td>Group V</td>
<td>10.38</td>
<td>8.91, 10.92</td>
<td>10.33</td>
<td>9.67, 11.21</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I (1 < T2DM course < 5), Group II (5 ≤ T2DM course < 10), Group III (10 ≤ T2DM course < 15), Group IV (15 ≤ T2DM course < 20) and Group V (T2DM course ≥ 20)

Abbreviations: CH Corneal hysteresis, CRF Corneal resistance factor
### Table 3
Biomechanical characteristics of cornea in T2DM patients with different fasting blood glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>FBG1</th>
<th>FBG2</th>
<th>FBG3</th>
<th>FBG4</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH (mm Hg)</td>
<td>11.83 ± 0.79*</td>
<td>11.42 ± 0.92*</td>
<td>10.98 ± 0.77*</td>
<td>10.45 ± 0.82*</td>
<td>72.32</td>
<td>0.021</td>
</tr>
<tr>
<td>CRF (mm Hg)</td>
<td>12.32 ± 1.36*</td>
<td>11.91 ± 1.08*</td>
<td>11.24 ± 1.13*</td>
<td>10.78 ± 0.89*</td>
<td>83.23</td>
<td>0.003</td>
</tr>
</tbody>
</table>

FBG1 group(7.0mmol/L < FBG < 9mmol/L), FBG2 group(9.0mmol/L ≤ FBG < 11mmol/L), FBG3 group(11mmol/L ≤ FBG < 13mmol/L), and FBG4 group (FBG ≥ 13mmol/L)

Abbreviations: CH Corneal hysteresis, CRF Corneal resistance factor

* Comparision among the four groups each other LSD-t P < 0.05.

### Table 4
Biomechanical characteristics of cornea in T2DM patients under different HbA1c (%) conditions

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>6.5-8</th>
<th>8–10</th>
<th>10–12</th>
<th>&gt;12</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH (mm Hg)</td>
<td>12.25 ± 0.87*</td>
<td>11.87 ± 0.97*</td>
<td>11.14 ± 0.82*</td>
<td>10.78 ± 0.91*</td>
<td>58.23</td>
<td>0.001</td>
</tr>
<tr>
<td>CRF (mm Hg)</td>
<td>12.17 ± 1.18*</td>
<td>11.82 ± 1.01*</td>
<td>11.24 ± 1.04*</td>
<td>10.92 ± 0.72*</td>
<td>76.29</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Abbreviations: CH Corneal hysteresis, CRF Corneal resistance factor

* Comparision among the groups each other LSD-t P < 0.05

### Table 5
Analysis of corneal biomechanical properties under different DR stages

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-DR</th>
<th>Mild-NPDR</th>
<th>Moderate-NPDR</th>
<th>Severe-NPDR</th>
<th>PDR</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH (mm Hg)</td>
<td>12.21 ± 0.89*</td>
<td>11.82 ± 0.72*</td>
<td>11.31 ± 0.91*</td>
<td>10.94 ± 0.91*</td>
<td>10.38 ± 0.91*</td>
<td>82.31</td>
<td>0.016</td>
</tr>
<tr>
<td>CRF (mm Hg)</td>
<td>12.39 ± 0.92*</td>
<td>11.87 ± 1.04*</td>
<td>11.19 ± 0.94*</td>
<td>10.78 ± 0.87*</td>
<td>10.28 ± 0.85*</td>
<td>77.84</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: CH Corneal hysteresis, CRF Corneal resistance factor, NPDR Non proliferative diabetic retinopathy, PDR proliferative diabetic retinopathy

* Comparision among the groups each other LSD-t P < 0.05

### Discussion

The cornea is located at the outermost layer of the eyeball and has viscoelastic properties. The biomechanical properties composed of collagen fibers existing in its stromal layers play an important role in maintaining the inherent morphology of the eyeball and protecting its inner structure. Related
studies had shown that corneal biomechanical properties were closely related to factors such as corneal curvature, corneal thickness, refractive index. Therefore, it was initially applied to exclude keratoconus in corneal refractive surgery[25,26]. In recent years, with the application of ORA analyzer in corneal biomechanical measurement of patients with cataracts, glaucoma, and other diseases, research had reported that patients with primary open angle glaucoma may experience changes in corneal biomechanical parameters and the corneal biomechanical analyzer contributed to the early diagnosis and evaluation of primary open angle glaucoma[27]. Meanwhile, Giacomo reported that both coaxial micro incision Phaco and standard incision Phaco can alter corneal biomechanical characteristics, and coaxial micro incision Phaco recovered corneal biomechanical characteristics faster than standard incision Phaco which had important reference significance for selecting cataract surgery incisions[28]. ORA is a new non-contact eye response analyzer that has made it possible to evaluate the biomechanical properties of living corneas. The main indicators of ORA are CH and CRF. CH represents the corneal viscous resistance, which is the ability to absorb and disperse energy. CRF represents the overall hardness of the cornea, reflecting the cumulative effect of resistance when the cornea is compressed and deformed by airflow. This process is greatly affected by the hydration of the cornea. Many studies had shown that the oxidative stress reaction in the corneal stroma of diabetic patients was greatly affected by blood sugar, and patients often had hydration dysfunction, which further led to corneal neurotrophic disorder[29,30].

In this study, we found the course of T2DM, FBG, DR, and HbA1c were risk factors for T2DM by the logistic linear regression analysis. Therefore, we further statistically analyzed the CH and CRF values under different states of T2DM course, FBG, DR, and HbA1c. Interestingly, after excluding the effects of factors such as corneal thickness and curvature on CH and CRF, The results showed that as the course of T2DM prolonged, FBG increased, DR staging worsened, and HbA1c accumulated, CH and CRF values gradually decreased. The above results indicated that with the deterioration of the general condition in T2DM patients, the corneal biomechanics of patients gradually decreased. Markoulli reported that hyperglycemia had important effects on the morphology, physiological function, metabolism, and other aspects of corneal cells[31]. Especially in the condition of hyperglycemia, the abnormal expression of collagen fibers (type I and III collagen fibers) or protein peptides (such as keratin glycan, membrane glycan, or core proteoglycan) in the corneal stroma layer would further affect the normal cross-linking between collagen fibers which led to the changes in the number and structure of existing fibers in the corneal stroma layer, thereby affecting corneal edema and reducing the visual quality of patients. At the same time, Chang found that corneal epithelial basal cells originated from corneal limbal stem cells in T2DM patients, and he also discovered that the density of corneal epithelial basal cells decreased, while the structure and space of cells became wider[32]. As the patient's blood sugar increased, laser confocal microscopy further showed that the arrangement of corneal fibrous tissue in the corneal stromal layer was loose, and the density of collagen fibers decreased. Besides, Di G considered that the size and nucleus of corneal stroma cells in diabetic patients were large, the extracellular collagen fibers were uneven in thickness and disorderly arranged, the rough endoplasmic reticulum, mitochondria, golgi
bodies and other organelle were less, and the basement membrane was thin and discontinuous, indicating that the corneal cells in diabetes patients had low metabolism and inactive proliferation [33].

In clinical work, retinal angiography for diabetes patients is restricted by allergy, renal function, liver function and other factors. At the same time, mydriasis examination takes a long time, so not all patients with diabetes are willing to accept fundus examination. In this study, we discovered that as the DR lesion worsened, the CH and CRF values gradually decreased. Research had shown that with the increase of blood sugar and abnormal blood lipid in diabetes patients, the self-regulation of retinal vascular could be disordered and the blood flow would be abnormal. Once the blood-retinal barrier was disrupted, the retinal nerve fibers would experience nutritional disorders. Therefore, even if the fundus examination of patients was normal, patients may experience varying degrees of visual quality degradation. The cornea is one of the organs with the widest distribution of nerve endings in the body, and corneal nerve fibers play an important role in maintaining the homeostasis of the corneal epithelium. Hence, the loss of corneal nerve will lead to a decrease in the supply of neurotrophin, and patients will suffer from corneal epithelial erosion and ulcer formation, which will result in eye redness, photophobia, tears and other discomfort [34-36]. Through the analysis of CH and CRF values of patients in the different stages of DR, this study suggests that diabetes patients should be examined for corneal biomechanical properties, regardless of whether fundus angiography is feasible, which is instructive for early evaluation of corneal neuropathy in patients with type 2 diabetes, and is of great significance for early screening and preventing DR progress. The major limitations of this study is the retrospective study which is worth further exploration.

**Conclusion**

To sum up, our study investigated the association between corneal biomechanical properties measured with the ORA in T2DM patients and potential impact factors that we mentioned after excluding the influence of CCT and corneal curvature. The logistic linear regression analysis showed that the course of T2DM, smoking history, BMI, FBG, DR, HbA1c, TC, TG and LDL-C were common risk factors in T2DM patients. Besides, we discovered that as the course of T2DM prolonged, FBG increased, DR staging worsened, and HbA1c accumulated, CH and CRF values gradually decreased. Therefore, our study advised that early corneal biomechanical examination, assessment of changes in CH and CRF values, and timely intervention measures in this population may play an important role in reducing diabetes induced keratopathy. Moreover, our study concluded the corresponding CH and CRF data for clinical reference under different diabetes states.

**Abbreviations**

CH Corneal hysteresis  
CRF Corneal resistance factor  
CCT Central corneal thickness
Declarations

Acknowledgments Not applicable.

Authors' Contribution Tao Li and Zhiwu Lin were responsible for the study concept and design. Juan Tang and Ying Li did the data and project management. Xingde Liu and Biao Li was responsible for conceptualisation, funding acquisition, data acquisition. Xiaoli Wu, Qilin Fang and Chuanqiang interpreted the data and drafted the manuscript. Jing Lv and Guogang Liu was responsible for the overall content as the guarantor.

Funding This project was supported by 2022 Chengdu Medical College-The First People's Hospital of Ziyang Joint Scientific Research Fund Project(2022LHZY05).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate This study adhered to the tenets of the Declaration of Helsinki and Malaysian Guidelines for Good Clinical Practice (GCP). This study protocol was reviewed and approved by the Ethics Committee of The First People's Hospital of Ziyang(no.202100951). A signed written informed consent was obtained from all patients prior to enrolment. The authors affirm that human research participants provided informed consent for the publication of their data.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.


