# Investigation of expression and effects of TGF-β1 and MMP-9 in lens epithelial cells of diabetic cataract rats

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**Abstract.** Expressions and effects of transforming growth factor- 1 (TGF-β1) and matrix metalloproteinase-9 (MMP-9) in lens epithelial cells (LECs) of diabetic cataract rats were investigated. A total of 40 female Sprague-Dawley rats were randomly divided into study and control group. Rats in study group were successfully modeled diabetic cataract rats, and rats in control group were normal rats. Immunohistochemical staining was used to determine positive and negative granules in cytoplasm, and image proplus image analysis system to calculate the integral optical density of the average positive area. Quantitative analysis was performed on TGF-β1 and MMP-9 in LECs of rats in study and control groups at the 2nd and 4th weekends. There were no statistically significant differences in length and age between the two groups of rats (P>0.05). Glucose concentration in the blood of rats in study group after modeling was significantly higher than that before modeling (P<0.001), and that after modeling was significantly higher in study group than that in control group (P<0.001). The expression of TGF-β1 protein in LECs of rats in study group at T2 (the 4th weekend) was significantly higher than that at T1 (the 2nd weekend) (P<0.001), and that of TGF-β1 protein was significantly higher in study group than that in control group at T1 and T2 (P<0.001). The expression of MMP-9 protein in LECs of rats in study group at T2 was significantly higher than that at T1 (P<0.001), and that of MMP-9 protein was significantly higher in study group than that in control group at T1 and T2 (P<0.001). The TGF-β1 expression was positively correlated with the MMP-9 expression in LECs of diabetic cataract rats (r=0.825, P<001). The increased expression of MMP-9 and TGF-β1 may play an important role in the occurrence and development of diabetic cataract.

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*Key words:* transforming growth factor-β1, diabetes, matrix metalloproteinase-9, cataract

## Introduction

Diabetic cataract is a diabetic complication caused by poor glycemic control (1). Cataract usually occurs in middle-aged and elderly people, but experts say that diabetes can greatly accelerate its formation (2). In addition, people live increasingly unhealthy and irregular life, such as small daily exercise intensity, unhealthy diet, and excessive alcoholism and smoking, leading to more and more people with diabetes. Diabetic cataract has become a common multiple eye disease in chronic complications of diabetes patients (3,4), and its incidence has shown a trend of younger age (5).

Pathological changes of Lens epithelial cells (LECs) are the direct cause of diabetic cataract in diabetes patients, but the specific mechanism is not very clear (6). A large number of studies have found that transforming growth factor-β1 (TGF-β1) is closely related to the differentiation and proliferation of cataract LECs (7,8). The expression of TGF-β1 in cataract LECs is higher than that in normal cataract LECs (9). Lens capsule constitutes the extracellular matrix of LECs, and its changes have a great influence on the differentiation and proliferation of LECs. Matrix metalloproteinase (MMP) is an enzyme that decomposes the extracellular matrix of LECs (10,11). Studies have currently confirmed that MMP-9 is correlated with diabetic cataract (12). However, there are few studies on the correlation of TGF-β1 and MMP-9 with diabetic cataract. In this study, the expression of TGF-β1 and MMP-9 in LECs of diabetic cataract rats and its effect on the occurrence and development of diabetic cataract were investigated.

## Materials and methods

*Study objects*. A total of 40 female Sprague-Dawley (SD) rats (Guangdong Medical Laboratory Animal Center, Foshan, China) were fed with LAD0011 feed (Nantong Teluofei Feed Technology Co., Ltd., Nantong, China). They were randomly divided into control and study group, with 20 rats in each group. The average age of SD rats in study group was 8.45±0.39 weeks with a body weight of 200-225 g, and that in control group was 8.51±0.32 weeks with a body weight of 200-225 g. Indoor temperature 21.5±0.5˚C and humidity 45-65%, with fluorescent lighting, and unrestricted food and drink.

Table I. Comparison of general information between study and control groups of rats.

| Index | Study group (n=20) | Control group (n=20) | t | P-value |
|---|---|---|---|---|
| Length (cm) | 18.14±2.02 | 18.75±1.05 | 1.198 | 0.234 |
| Age (weeks) | 8.45±0.39 | 8.51±0.32 | 0.532 | 0.598 |
| Glucose (mmol/l) | | | | |
| Before modeling | 13.24±2.43 | 12.93±2.51 | 0.397 | 0.694 |
| After modeling | 20.12±3.48[a] | 12.76±2.46 | 7.723 | <0.001 |

[a]Glucose concentration in this group is significantly higher than that before modeling and that in control group, with statistically significant differences (P<0.001).

The study was approved by the Ethics Committee of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Patients who participated in this research had complete clinical data. The signed informed consents were obtained from the patients or the guardians.

*Establishment of diabetic cataract rat models*

*Main reagents*. Rabbit anti-rat TGF-β1 polyclonal antibody (cat. no. ABP57257; Amyjet Scientific Co., Ltd., Shanghai, China), rabbit anti-rat MMP-9 polyclonal antibody (cat. no. E-AB-31531; Elabscience Biotechnology Co., Ltd., Wuhan, China), streptozotocin (cat. no. K0050; Shanghai BaoMan Biotech. Co., Ltd., Shanghai, China), DAB developing kit and SP immunohistochemistry kit (cat. nos. DA1010 and SP0041) both from Beijing Solarbio Science & Technology Co., Ltd., were used in this study.

*Modeling methods*. A total of 40 rats were randomly divided into study and control group, with 20 rats in each group. Rats in control group were intraperitoneally injected with the equal amount of citrate buffer solution. Rats in study group were given a single intraperitoneal injection of 2% streptozotocin (55 mg/kg), and the blood glucose concentration of the tail venous blood of rats was detected 3 days later. Lens changes of the two groups of rats after modeling were observed under a slit lamp. The lens of rats in control group was transparent; the blood glucose of rats in study group reached 16.7 mmol/l, with flocculent turbidity and vacuoles in the lens of rats, indicating successful diabetic cataract rat modeling. At T1 and T2 after the start of the experiment, 12 rats in study and control group were sacrificed by intraperitoneal injection of pentobarbital solution (50 mg/kg) 3 min after the inhalation of ether, and their eyeballs were removed. The stripped lens was immediately fixed with neutral formalin solution with a volume fraction of 10% to prepare slices. Immunohistochemistry was used to detect TGF-β1 and MMP-9 in LECs, and phosphate-buffered saline instead of primary antibody as a negative control, with a working titer of primary TGF-β1 and MMP-9 antibody of 1:100.

*Judgment of results*. The results showed that brown granules in LECs slurry were positive, while non-brown granules were negative. The Image proplus image analysis system was used to perform quantitative analysis on TGF-β1 and MMP-9 in study and control groups.

*Statistical analysis*. SPSS v.17.0 (SPSS, Inc., Chicago, IL, USA) software system was used for statistical analysis. Measurement data were expressed as mean ± standard deviation. t-test was used for comparison between the two groups, repeated measures analysis of variance for comparison at different time points in the group, with Least Significant Difference test, and Pearson analysis for correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Comparison of general information between two groups of rats*. The length and age of rats in study group were 18.14±2.02 cm and 8.45±0.39 weeks, respectively. Those in control group were 18.75±1.05 cm and 8.51±0.32 weeks, respectively. There were no statistically significant differences in length and age between the two groups of rats (P>0.05). Glucose concentrations in the blood of rats in study group before and after modeling were 13.24±2.43 and 20.12±3.48 mmol/l, respectively. Those in control group were 12.93±2.51 and 12.76±2.46 mmol/l, respectively. There was no statistically significant difference in the glucose concentration in the blood of rats between study group and control group before modeling (P>0.05). The glucose concentration in the blood of rats after modeling was significantly higher than that before modeling in study group, with a statistically significant difference (P<0.001). There was no statistically significant difference in the blood glucose concentration between before and after modeling in control group (P>0.05). The glucose concentration in the blood of rats was significantly higher in study group than that in control group after modeling, with a statistically significant difference (P<0.001; Table I).

*Comparison of TGF-β1 and MMP-9 quantitative expression between two groups after successful modeling*

*Comparison of TGF-β1 quantitative expression between two groups*. The expression of TGF-β1 protein in LECs of rats in study group was 0.046±0.003 at T1 and 0.081±0.007 at T2 and in control group was 0.005±0.002 at T1 and 0.006±0.001 at T2. The expression of TGF-β1 protein in LECs of rats in study group at T2 was significantly higher than that at T1,

Table II. Comparison of TGF-β1 quantitative expression between study and control groups.

| Index | Study group (n=20) | Control group (n=20) | t | P-value |
|---|---|---|---|---|
| T1 | 0.046±0.003 | 0.005±0.002 | 50.850 | <0.001 |
| T2 | 0.081±0.007 | 0.006±0.001 | 47.430 | <0.001 |
| t | 20.550 | 2.000 | | |
| P-value | <0.001 | 0.053 | | |

TGF-β1, transforming growth factor-β1.

Table III. Comparison of MMP-9 quantitative expression between study and control groups.

| Index | Study group (n=20) | Control group (n=20) | t | P-value |
|---|---|---|---|---|
| T1 | 0.034±0.023 | 0.001±0.001 | 6.611 | <0.001 |
| T2 | 0.061±0.012 | 0.001±0.001 | 22.730 | <0.001 |
| t | 4.654 | - | | |
| P-value | <0.001 | - | | |

MMP-9, matrix metalloproteinase-9.

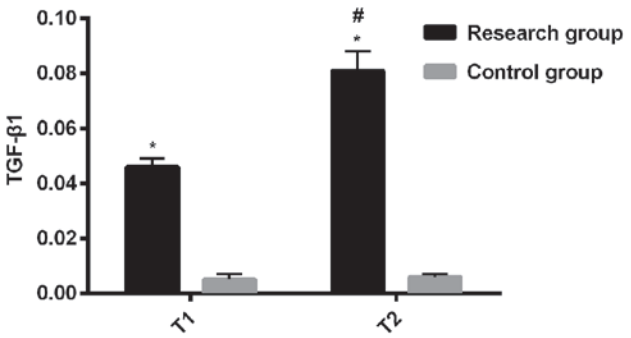


Figure 1. Comparison of TGF-β1 quantitative expression between two groups after successful modeling. The expression of TGF-β1 protein in LECs of rats in study group at T2 was significantly higher than that at T1, with a statistically significant difference (#P<0.001). Expression was significantly higher in study group than that in control group at T1 and T2, with a statistically significant difference (*P<0.001). TGF-β1, transforming growth factor-β1; LECs, lens epithelial cells.

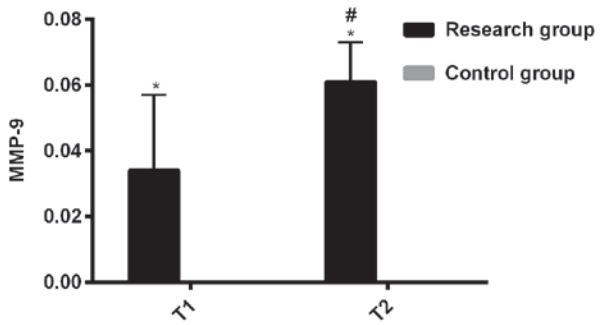


Figure 2. Comparison of MMP-9 quantitative expression between two groups. The expression of MMP-9 protein in LECs of rats in study group at T2 was significantly higher than that at T1, with a statistically significant difference (#P<0.001). There was no statistically significant difference in that at T1 and T2 in control group after successful modeling (P>0.05). Expression was significantly higher in study group than that in control group at T1 and T2, with a statistically significant difference (*P<0.001). MMP-9, matrix metalloproteinase-9; LECs, lens epithelial cells.

with a statistically significant difference (P<0.001). There was no statistically significant difference in that at T1 and T2 in control group (P>0.05). Expression in LECs of rats was significantly higher in study than that in control group at T1 and T2, with a statistically significant difference (P<0.001). It is indicated that the expression of TGF-β1 protein in LECs of diabetic cataract rats was higher in study group than that in normal control rats at the same time points, which increased with time, showing an upward trend (Table II and Fig. 1).

*Comparison of MMP-9 quantitative expression between two groups*. The expression of MMP-9 protein in LECs of rats in study group was 0.034±0.023 at T1 and 0.061±0.012 at T2. That in control group was 0.001±0.001 at T1 and T2. The expression of MMP-9 protein in LECs of rats in study group at T2 was significantly higher than that at T1, with a statistically significant difference (P<0.001). There was no statistically significant difference in that at T1 and T2 in control group after successful modeling (P>0.05). Expression in LECs of rats was significantly higher in study group than that in control group at T1 and T2, with a statistically significant difference (P<0.001). It is indicated that the expression of MMP-9 protein in LECs of diabetic cataract rats was higher in study group than that in normal control rats at the same time points, which increased with time, showing an upward trend (Table III and Fig. 2).

*Correlation analysis of TGF-β1 expression with MMP-9 expression in LECs of diabetic cataract rats*. The TGF- 1 expression was positively correlated with the MMP-9 expression in LECs of diabetic cataract rats (r=0.825, P<001; Fig. 3).

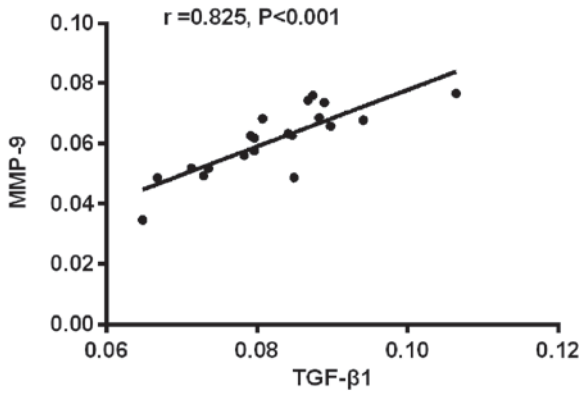


Figure 3. Correlation analysis of TGF-β1 expression with MMP-9 expression in LECs of diabetic cataract rats. The TGF-β1 expression was positively correlated with the MMP-9 expression in LECs of diabetic cataract rats (r=0.825, P<001). TGF-β1, transforming growth factor-β1; LECs, lens epithelial cells; MMP-9 matrix metalloproteinase-9.

## Discussion

Posterior capsular turbidity is the most common in lens turbidity types of diabetic cataract. This is due to pathological changes of anterior and posterior lens capsule caused by abnormal differentiation of LECs with rapid lesion progression (13). MMPs, main mediators of extracellular matrix degradation, are a class of proteolytic enzymes. They are present in the form of zymogens, and their main physiological role is to degrade extracellular matrix (14,15). Gelatinase B (MMP-9) is a matrix hydrolase that degrades type IV collagen (16). Luo *et al* found that TGF-β1 promotes the expression of extracellular matrix such as collagen and fibronectin, which is beneficial to cell repair and embryo development. TGF-β1 is generally produced by cell autocrine and paracrine (17). TGF-β1 produced by normal cell autocrine or paracrine needs activation to exert its effect, and most of TGF-β1 are present in a potentially inactive form (18). There are currently few studies on the correlation of TGF-β1 and MMP-9 with diabetic cataract. In this study, the expression of TGF-β1 and MMP-9 in LECs of diabetic cataract rats and its effect on the occurrence and development of diabetic cataract were investigated.

Experimental rats were randomly divided into study group and control group. Rats in study group were successfully modeled diabetic cataract rats, and rats in control group were normal rats. First, the general information of the two groups of rats was compared. The results showed that there were no statistically significant differences in length and age between the two groups of rats, demonstrating that the two groups of rats are comparable. The glucose concentration in the blood of rats in study group after modeling was significantly higher than that before modeling, and that after modeling was significantly higher in study group than that in control group, with statistically significant differences. Long-term hyperglycemia in diabetes will lead to various chronic complications in different tissues, such as chronic damage and dysfunction in the heart, blood vessels and eyes. Among them, cataract and retinopathy are the most common in eye complications of diabetes (19,20). Then, the quantitative expressions of TGF-β1 and MMP-9 between the two groups were compared at T1 and T2 after modeling. The results showed that the expression of TGF-β1 protein in LECs of rats in study group at T2 was significantly higher than that at T1, and it was significantly higher in study group than that in control group at T1 and T2, with statistically significant differences. Therefore, it is hypothesized that the expression of TGF-β1 protein in LECs of diabetic cataract rats was higher in study group than that in normal control rats at the same time points, which increases with time, showing an upward trend. Studies have shown that the expression level of TGF-β1 is significantly increased in diabetes patients in the early stage, suggesting that high glucose environment may be the main cause of TGF-β1 production and activation (21,22). The expression of MMP-9 protein in LECs of rats was significantly higher in study group than that in control group at T1 and T2. Therefore, it is speculated that the expression of MMP-9 protein in LECs of diabetic cataract rats was higher in study group than that in normal control rats at the same time points, which increases with time, showing an upward trend. In recent years, a large number of studies on MMPS have shown that MMP-9 is highly expressed in LECs of diabetic cataract, but less expressed in LECs of non-diabetic cataract (11,23,24), which is similar to findings in this study and supports our results. Finally, Pearson analysis was used to perform correlation analysis on the expression of TGF-β1 and MMP-9 in LECs of diabetic cataract rats. The results showed that the TGF-β1 expression was positively correlated with the MMP-9 expression in LECs of diabetic cataract rats. Therefore, it is believed that the TGF-β1 expression and the MMP-9 expression in LECs of diabetic cataract rats have a certain mutual regulation effect. Xu *et al* (9) reported that the interaction between TGF-β1-induced cells and extracellular matrix comes into play through regulating the transcriptional activity of MMP-9 in LECs.

In this study, due to the insufficient number of rats enrolled, there may be some contingency in some results. In order to improve study results, the number of experimental rats will be increased later in a further study.

In summary, the expression of MMP-9 protein in LECs of diabetic cataract rats is higher than that in normal rats. It is believed that the specific mechanism of diabetic cataract may be the that hyperglycemia causes TGF-β1 activation, resulting in the increased expression of MMP-9, and the extracellular matrix degradation in LECs of diabetic cataract leads to the abnormal proliferation and differentiation of LECs, causing cataract. The increased expression of TGF-β1 and MMP-2 proteins are correlated with the occurrence and development of diabetic cataract.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

KL and WA conceived and designed the study. WA helped with establishment of diabetic cataract rat models, and KL interpreted the data by using Pearson analysis. Both authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Patients who participated in this research had complete clinical data. The signed informed consents were obtained from the patients or the guardians.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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