MORPHOLOGICAL STUDY OF CORNEAL ENDOTHELIUM IN DIABETICS

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ABSTRACT

BACKGROUND
Diabetes is a group of metabolic diseases characterised by hyperglycaemia resulting from defect in insulin secretion, insulin action or both. Diabetic eye disease is an end-organ response to the effects of the condition on the human system. Ocular morbidity results from major abnormalities of the retina and also by alterations in the eyelids, extraocular muscles, tear-film, cornea, iris, lens and cranial nerves. The objectives of this study are- (i) To study the morphology of corneal endothelium in diabetic patients; (ii) To compare the corneal endothelial counts and thickness in patients of diabetes mellitus and controls.

MATERIALS AND METHODS
After obtaining the ethical clearance from the Institutional Ethical Committee, present study was conducted on 250 patients with 125 known cases of diabetes and 125 controls matched for age >+5 years. The study was done over a period of one and a half year. Corneal endothelial counts and thickness was then taken using Non-Contact Specular Microscope. Appropriate statistical tests were used for data analysis.

RESULTS
The endothelial cell count was found to be significantly different in both diabetic groups as compared with normal age-matched control groups. The central corneal thickness was also significantly different in diabetic patients.

CONCLUSION
In conclusion, we found an altered corneal endothelial cell density and thickness in subjects with diabetes mellitus compared with controls. This study thus suggests a higher potential for the diabetic cornea to decompensate following any stress.

KEYWORDS
Cornea, Diabetes, Endothelial.


BACKGROUND
Diabetes is the most important non-infective epidemic to hit the globe in the present millennium. Prevalence of diabetes in adults worldwide was estimated to be 4% in 1995 and is expected to rise to 5.4% by year 2025.1 Presently, it is higher in developed than in developing countries, but by year 2025 more than 75% of people with diabetes are estimated to be residing in developing countries as compared to 62% in 1995.1

Ocular morbidity results from major abnormalities of retina and also by alteration in eyelids, extraocular muscles, tear film, cornea, iris, lens and cranial nerves, but the anterior segment findings of diabetes are less extensively described than retinopathy. Diabetes causes several ultrastructural and functional changes in cornea.2

Diabetic Retinopathy (DR) is a vascular disorder affecting the microvasculature of the retina. It is estimated that diabetes mellitus affects 4 percent of the world’s population, almost half of whom have some degree of DR at any given time.3 DR occurs both in type 1 and type 2 diabetes mellitus and it has been shown that nearly all type 1 and 75 percent of type 2 diabetic patients will develop DR after 15 years’ duration of diabetes as shown in earlier epidemiological studies.4,5

While glaucoma and cataracts often occur in people without diabetes, people with diabetes are 40% more likely to be diagnosed with glaucoma and 60% more likely to develop cataracts.6 The risk of glaucoma among people with diabetes increases with age and duration of the disease. Cataracts progress faster and develop at a younger age in people with diabetes.7

Cornea is a transparent avascular watch glass like structure, which in addition to having a protective role is responsible for about 3/4th of optical power of eye, i.e. about 40 - 44 D. The normal cornea is free of blood vessels. Nutrients are supplied and metabolic products are removed mainly via aqueous humour posteriorly and tears anteriorly. The cornea is the most densely innervated tissue in the body with a subepithelial and deep stromal plexus. The adult cornea measures 11 - 12 mm horizontally and 9 - 11 mm vertically. It is approximately 0.5 mm thick in the centre and increases gradually towards the periphery of cornea, where it is about 0.7 mm thick.8
Although diabetes does not have classic corneal manifestations, several ultrastructural and functional changes occur in diabetic cornea resulting in diabetic keratopathy. Diabetic keratopathy encompasses a clinical spectrum which includes superficial punctate epitheliopathy, persistent epithelial erosion, corneal hypoesthesia, non-healing persistent epithelial defects and corneal oedema.\textsuperscript{9,10} The reported prevalence of diabetic keratopathy is around 50% - 70% among diabetic patients.\textsuperscript{11,12} Before diabetic keratopathy clinically sets in, several subclinical abnormalities develop in diabetic cornea. These abnormalities affect both structure and function of cornea. Ultrastructural changes in diabetic keratopathy include decrease in epithelial barrier function,\textsuperscript{13,14} abnormalities in shape of epithelial cells,\textsuperscript{15,16} basement membrane thickening,\textsuperscript{17,18} decreased corneal sensations\textsuperscript{19,20} and abnormalities of endothelial cells.\textsuperscript{21,22}

The central corneal endothelium changes as a person ages. From age 20 to 50, the endothelial cell density remains relatively stable for most people. Beyond age 50, a slow decline begins. By age 60, most people experience a significant reduction in endothelial cell density.\textsuperscript{23}

Pleomorphism is a significant disruption in the regular hexagonal pattern of the endothelium that causes a decrease in endothelial mosaic stability. Pleomorphism occurs secondary to physiological stress from ocular disease, contact lens wear or normal ageing changes.\textsuperscript{24}

Objectives
1. To study the morphology of cornea in diabetic patients.
2. To compare the corneal endothelial counts and thickness in patients of diabetes mellitus and controls.

MATERIALS AND METHODS
The study entitled "Morphological study of corneal endothelium in diabetics" is a comparative study and was conducted on 250 patients, which included 125 known cases of diabetes and 125 controls matched for age ± 5 years after obtaining the ethical clearance from the Institutional Ethical Committee. Patients attending ophthalmology outpatient department at SMHS Hospital, Srinagar were included in the study. The study was done over a period of one and a half year. After taking written consent of the diabetics as well as controls in local language, detailed history, general physical, systemic and local examination was done. Ophthalmological evaluations performed included visual acuity, IOP measurement using non-contact tonometry, fundus examination, slit lamp biomicroscopy, Schirmer’s test, specular microscopy and pachymetry.

In this comparative study evaluation of corneal endothelial counts, thickness and assessment of morphology was done using Topcon SP-1P Non-Contact Specular Microscope.

Statistical Package for Social Sciences (SPSS- version 20.0) and Microsoft Excel software were used to carry out statistical analysis of data. Descriptive statistics of data including the mean and standard deviation for numerical variables and percentages of different categories for categorical variables was obtained. Student’s independent ‘t’ test was employed for intergroup analysis of data. Intragroup analysis was carried out with the help of paired ‘t’ test, chi-square test or Fisher exact test whichever appropriate.

RESULTS
Out of the total 125 diabetics, 16 (12.8%) males and 11 (8.80%) females suffered from Type I diabetes and 52 (41.60%) males and 46 (36.80%) females suffered from Type II diabetes.

The mean endothelial count of diabetics and controls in the age < 30 years was 3033.3 ± 89.41 cells/mm\textsuperscript{2} and 3208.1 ± 106.11 cells/mm\textsuperscript{2} respectively with a p value of < 0.001. The mean endothelial count between the age 30 - 44 years in diabetics and controls was 2662.9 ± 190.31 cells/mm\textsuperscript{2} and 3088.2 ± 131.81 cells/mm\textsuperscript{2} respectively. The mean endothelial count for 45 - 59 years was 2492.5 ± 181.65 cells/mm\textsuperscript{2} in diabetics and 2755.3 ± 111.65 cells/mm\textsuperscript{2} in controls. There was a mean endothelial count of 2243.5 ± 193.18 cells/mm\textsuperscript{2} in diabetics and 2563.8 ± 103.95 cells/mm\textsuperscript{2} in controls of age > 60 years of age. The difference was statistically significant with a p value of < 0.001.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Diabetic Mean</th>
<th>Diabetic SD</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>3033.3</td>
<td>89.41</td>
<td>3208.1</td>
<td>106.11</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>30-44</td>
<td>2662.9</td>
<td>190.31</td>
<td>3088.2</td>
<td>131.81</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>45-59</td>
<td>2492.5</td>
<td>181.65</td>
<td>2755.3</td>
<td>111.65</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>≥ 60</td>
<td>2243.5</td>
<td>193.18</td>
<td>2563.8</td>
<td>103.95</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 1. Distribution of Endothelial Cell Count in Eyes of Subjects with and without Diabetes Mellitus by Age

The mean central corneal thickness of diabetics and controls in the age < 30 years was 513.6 ± 6.91 µm and 503.8 ± 11.72 µm respectively. The mean central corneal thickness between the age 30 - 44 years in diabetics and controls was 530.8 ± 12.84 µm and 515.7 ± 13.16 µm respectively. The mean central corneal thickness for 45 - 59 years was 549 ± 19.86 µm in diabetics and 531.9 µm in controls. There was a mean central corneal thickness of 571.6 ± 22.43 µm in diabetics and 542.2 ± 23.7 µm in controls of age > 60 years of age. The difference was statistically significant with p value of < 0.001.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Diabetic Mean</th>
<th>Diabetic SD</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>513.6</td>
<td>6.91</td>
<td>503.8</td>
<td>11.72</td>
<td>0.012*</td>
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<tr>
<td>30-44</td>
<td>530.8</td>
<td>12.84</td>
<td>515.7</td>
<td>13.16</td>
<td>0.003*</td>
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<tr>
<td>45-59</td>
<td>549.0</td>
<td>19.86</td>
<td>531.9</td>
<td>14.87</td>
<td>&lt;0.001*</td>
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<tr>
<td>≥ 60</td>
<td>571.6</td>
<td>22.43</td>
<td>542.2</td>
<td>23.70</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 2. Distribution of Central Corneal Thickness in Eyes of Subjects with and without Diabetes Mellitus by Age

Mean value of central corneal thickness in patients with < 5 years’ duration of diabetes was 532.7 ± 20.17 µm and 540.7 ± 14.55 µm with a duration of 5-7 years. Also mean central corneal thickness was 551 ± 24.87 µm and 585.8 ± 13.22 µm in patients with 8 - 10 years and > 10 years’ duration of
diabetes respectively. The results were statistically significant with a ‘p’ value of < 0.001.

Mean value of endothelial cell counts and central corneal thickness in patients with no or mild retinopathy was 2752.5 cells/mm² and 532.6 µm respectively; it was 2517.4 cells/mm² and 542.5 µm in patients with moderate-to-severe NPDR and 2065.3 cells/mm² and 589.9 µm in patients with proliferative diabetic retinopathy/ vitreous haemorrhage. When severity of retinopathy was compared to endothelial cell count and central corneal thickness, the results were statistically significant in proliferative retinopathy.

<table>
<thead>
<tr>
<th>Retinopathy</th>
<th>ECC Mean</th>
<th>SD</th>
<th>P-value</th>
<th>CCT Mean</th>
<th>SD</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>No retinopathy to mild</td>
<td>2752.5</td>
<td>231.39</td>
<td>-</td>
<td>532.6</td>
<td>174.7</td>
<td>-</td>
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<tr>
<td>Moderate-to-severe NPDR</td>
<td>2517.4</td>
<td>158.20</td>
<td>&lt;0.001*</td>
<td>542.5</td>
<td>18.24</td>
<td>0.003*</td>
</tr>
<tr>
<td>Proliferative DR/VH</td>
<td>2065.3</td>
<td>89.35</td>
<td>&lt;0.001</td>
<td>589.9</td>
<td>9.66</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Table 3. Correlation of Severity of Retinopathy with Corneal Morphology

The mean coefficient of variation in eyes of diabetic patients was 36.2 ± 2.72 and in controls was 28.7 ± 3.10.

<table>
<thead>
<tr>
<th>CV</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>125</td>
<td>36.2</td>
<td>2.72</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Control</td>
<td>125</td>
<td>28.7</td>
<td>3.10</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Coefficient of Variation in Eyes of Subjects with and without Diabetes Mellitus

The hexagonality (%) in diabetic patients was 62.5 ± 5.04% and in controls it was 71.8 ± 3.39%.

<table>
<thead>
<tr>
<th>CCT</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>125</td>
<td>62.5</td>
<td>5.04</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Control</td>
<td>125</td>
<td>71.8</td>
<td>3.39</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Hexagonal Cells (%) in Eyes of Subjects with and without Diabetes Mellitus

DISCUSSION

Out of the diabetics, 16 (12.8%) males and 11 (8.80%) females suffered from Type I diabetes and 52 (41.60%) males and 46 (36.80%) suffered from Type II diabetes. Our results were comparable with Alan et al and Parekh et al who showed similar observations in their studies.

In this study when the corneal endothelial density measured with the specular microscope was compared amongst the diabetic patients and controls, a significant difference was noted in the mean and standard deviation of endothelial cell density in diabetics, which was 2538.7 ± 286.9 cells/mm² and mean and standard deviation of endothelial cell density in controls which was 2862.8 ± 254.27 cells/mm². The results were statistically significant with a ‘p’ value of < 0.001. Therefore, the mean endothelial cell density was lower in diabetic patients as compared to the controls. The results of our study were comparable to Parekh et al, where the mean endothelial cell density and standard deviation in diabetics and controls was 2562.07 ± 35.98 cells/mm² and 2852 ± 280.243 cells/mm² respectively. Hence, showing a significant decrease in the endothelial cell density in case of diabetics.

Choo et al also had similar results where the mean endothelial cell density of diabetic patients was found to be 2541.6 ± 516.4 cells/mm² and was significantly reduced as compared to the mean endothelial cell density in controls, which was 2660.1 ± 515.5 cells/mm². Results similar to our study were also obtained by Alan et al, where the endothelial cell density in diabetic patients (2578 ± 77 cells/mm²) was found significantly reduced as compared to the endothelial cell density in controls (2605 ± 66 cells/mm²). Our results were also comparable with Inoue et al, Shenoy et al and Roszkowska et al.

When the mean endothelial cell density and standard deviation was compared with respect to the age of the diabetic patients and controls, the following results were obtained. The mean endothelial cell density and standard deviation in diabetic patients and controls with < 30 years of age was 3033.3 ± 89.41 cells/mm² and 3208.1 ± 106.11 cells/mm² respectively with a ‘p’ value of < 0.001. The mean endothelial cell density and standard deviation in the age group of 30 - 44 years in diabetic patients and controls was 2662.9 ± 190.31 cells/mm² and 3088.2 ± 131.81 cells/mm² respectively. In subjects in the age group of 45 - 59 years, the mean endothelial cell density and standard deviation was 2492.5 ± 181.65 cells/mm² in diabetics and 2755.3 ± 111.65 cells/mm² in controls. While as mean endothelial cell density and standard deviation of 2243.5 ± 193.18 cells/mm² in diabetics and 2563.8 ± 103.95 cells/mm² in controls was seen in the subjects aging > 60 years of age, the difference was statistically significant with ‘p’ value of < 0.001.

The results of our study were comparable with Sudhir et al, where the mean endothelial cell density and standard deviation in both diabetic patients as well as controls decreased with respect to the increasing age of the patients. A study by Sanchis et al and Parekh et al also had similar observations in their studies.

When the endothelial cell density in the 125 diabetic patients was compared according to the duration of the diabetes, it was noted that the mean endothelial cell density and standard deviation in patients with < 5 years’ duration of diabetes was 2717.5 ± 247.40 cell/mm² and was 2621.5 ±
157.08 cells/mm$^2$ in diabetic patients with a duration of 5 - 7 years. Also, the mean endothelial cell count was $2371.7 \pm 197.16$ cells/mm$^2$ and $2105.6 \pm 99.02$ cells/mm$^2$ in patients with duration of 8 - 10 years and > 10 years respectively. The results were statistically significant (p value of < 0.001).

The obtained data demonstrates a decrease in the endothelial cell density as the duration of diabetes goes on increasing. The difference between the endothelial cell density of diabetic patients with duration < 5 years and duration > 10 years is 611.9 cells/mm$^2$. Hence, suggesting a role of duration of diabetes in the decreasing endothelial cell count.

These results were comparable with Lee et al.\textsuperscript{34} where the mean corneal endothelial cell density also decreased with the increase in duration of diabetes. Similar results were also obtained by Parekh et al.\textsuperscript{33} where the endothelial cell density and standard deviation in patients with duration < 5 years was $2724.75 \pm 267.89$ cells/mm$^2$ and in patients with duration > 10 years was $2082.86 \pm 203.56$ cells/mm$^2$. Similar results were also obtained by Briggs et al.\textsuperscript{35} where the mean endothelial cell density in patients with duration of diabetes < 10 years was $2609 \pm 217$ cells/mm$^2$ and in patients with duration > 10 years was $2429 \pm 242$ cells/mm$^2$. Hence, demonstrating a decrease in mean endothelial cell density and standard deviation with respect to increasing duration of diabetes.

In this study, the endothelial cell density in diabetic patients was also compared with respect to severity of retinopathy. The mean value of endothelial cell density in patients with no or mild retinopathy was $2725.2 \pm 231.39$ cell/mm$^2$. It was statistically non-significant. However, statistically significant results were obtained when mean endothelial cell density was evaluated in patients of moderate-to-severe NPDR and proliferative diabetic retinopathy/vitreous haemorrhage. Mean endothelial cell density was $2517.4 \pm 158.20$ cells/mm$^2$ in patients with moderate-to-severe NPDR and $2065.3 \pm 89.35$ cells/mm$^2$ in patients with proliferative diabetic retinopathy/vitreous haemorrhage.

These results depict that the endothelial cell density decreases as the severity of diabetic retinopathy goes on increasing. Our results were comparable with those of Parekh et al.\textsuperscript{33} where the endothelial cell density in no or mild retinopathy moderate-to-severe NPDR and proliferative diabetic retinopathy/vitreous haemorrhage was $2748.79 \pm 229.78$ cells/mm$^2$, $2533.20 \pm 338.35$ cells/mm$^2$, and $2031.00 \pm 95.09$ cells/mm$^2$ respectively showing a decrease in endothelial cell density with increase in severity.

In this study when the central corneal thickness measured with the specular microscope was compared amongst the diabetic patients and controls, a significant difference was noted in the mean and standard deviation of central corneal thickness in diabetics, which was $545.7 \pm 25.24$ μm and mean and standard deviation of central corneal thickness in controls which was $526.1 \pm 20.46$ μm. The results were statistically significant with a 'p' value of < 0.001. Therefore, the mean central corneal thickness was higher in diabetic patients as compared to the controls. The results of our study were comparable with Alan et al.\textsuperscript{25} where the mean central corneal thickness and standard deviation in diabetics and controls was $546 \pm 7$ μm and $538 \pm 5$ μm respectively. Hence, showing a significantly increased central corneal thickness in case of diabetic patients. Sudhir et al\textsuperscript{31} and Parekh et al\textsuperscript{26} also demonstrated similar results.

In this study when the coefficient of variation of cell size was analysed and compared amongst the diabetic patients and controls, a significant difference was noted in the mean and standard deviation of the coefficient of variation. The mean coefficient of variation and standard deviation in diabetic patient was $36.2 \pm 2.72$ and the mean coefficient of variation and standard deviation in controls was $28.7 \pm 3.10$ (p value of < 0.001).

These observations depict an increased coefficient of variation of cell size amongst the diabetic patients. Our data was comparable with Lee et al.\textsuperscript{34} who also observed a significant increase in the coefficient of variation of cell size in diabetic patients ($30.2 \pm 0.4\%$) as compared to the controls ($35.4 \pm 0.6\%$). Similar results were also obtained by Sudhir et al\textsuperscript{31} and Choo et al.\textsuperscript{37}

On evaluation and comparison of hexagonality (%) amongst the diabetic patients and controls, a significant difference was noted in the mean and standard deviation of hexagonality (%). The mean and standard deviation of hexagonality in diabetic patients was $62.5 \pm 5.04\%$ and the mean and standard deviation of hexagonality in controls was $71.8 \pm 3.39\%$ (p value of < 0.001).

Our results show a decrease in the hexagonality (%) in case of diabetic patients. Hence, depicting a decrease in the number of hexagonal cells in diabetes. These results were comparable with Choo et al.\textsuperscript{37} who also observed a significant decrease in the hexagonality (%) in diabetic patients (41.1% ± 19.6) as compared to the controls (45.2% ± 20.6%). Similar results were also obtained by Sudhir et al.\textsuperscript{31}

**CONCLUSION**

In conclusion, corneal endothelial structure is affected by diabetes mellitus. Endothelial cell density is reduced, central corneal thickness is increased and pleomorphism and polymegathism are increased. These changes are also dependent on other factors such as age of the patient, duration of diabetes and severity of retinopathy.

These changes in corneal endothelial cells may manifest in disturbances in the tightness of the endothelial barrier and endothelial dysfunction. Thus, prevention of the corneal endothelium dysfunction by early detection of these changes is crucial. These changes may result in a higher potential for diabetic cornea to decompensate following any stress such as cataract extraction, trabeculotomy and vitrectomy, thus warranting proper evaluation. Non-contact specular microscopy, which evaluates endothelial morphology quickly and easily without any side effects is a useful tool for screening of corneal endothelium in diabetic patients.

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