Aerobic Bacterial Conjunctival Flora in Diabetic Patients

Tahir Masaud Arbab, Saleem Qadeer, Saeed Iqbal, Manzoor A. Mirza

Purpose: To study the aerobic conjunctival flora of diabetic patients and its relation to the presence and level of diabetic retinopathy and the duration of the disease.

Material and Methods: 80 patients form Sir Syed Diabetic Clinic with no evidence of ocular surface disease were included. These diabetic patients were compared with 50 nondiabetic subjects. All patients underwent slit-lamp evaluation, conjunctival scrapings, and indirect ophthalmoscopy.

Results: The frequency of positive conjunctival cultures was significantly higher in the diabetic group than in nondiabetic group (95% vs. 72%, p<0.001). Among diabetic patients, a significantly higher frequency of positive cultures was detected in those with diabetic retinopathy than those without retinopathy (p=0.001). Neither the duration of the diabetes nor the hypoglycemic therapy correlated with the culture results. Staphylococcus epidermidis was the most common microorganism isolated, and its identification was more frequent in patients with retinopathy than those without diabetic retinopathy.

Conclusion: Diabetic patients have a significantly higher number of positive conjunctival cultures. The presence of diabetic retinopathy was correlated with an increase in positive cultures and a higher proportion of Staphylococcus epidermidis.

The term “normal microbial flora” refers to population of microorganisms that dwell within the eyes of healthy individuals. These microorganisms play an important and specific role in maintaining health and normal conjunctival function. These bacteria, when disturbed, can promptly re-establish themselves1.

It was found that indigenous bacterial flora inhibits the establishment of foreign pathogenic bacteria by elaborating antibacterial substances and by competing for space and nutrients1.

It is an established fact that gram-positive organisms, particularly coagulase-negative Staphylococci, are the main residents of normal eye. They are the predominant cause of postoperative infections2.

The conjunctival flora may be altered under special circumstances, as in new-borns, acquired immune defiency patients, contact lens wearers, and patients using immunosuppressive drugs 3-5.

Diabetes mellitus is a multifactorial disease associated with blindness caused by retinopathy and its complications, and also other abnormalities in other parts of the eye6-9. Diabetic patients have been reported to have an increased prevalence of postoperative endophthalmitis than nondiabetic patients10, 11.

Speaker et al, demonstrated a correlation between the external flora and intraocular infection. In his study using genotypic analysis in the identification of the etiologic agent of endophthalmitis, reported that in 82% of cases the microorganism identified in the vitreous was genetically identical to an isolate recovered from the conjunctiva, lid, even the nasal mucosa of the patient12.

Gram-negative bacteria and coagulase-negative staphylococcus have been reported as common causes of endophthalmitis in diabetic patients 10, 13. However, the surface flora of the diabetic patients is not specifically addressed in the literature.
The purpose of this study is to analyze the aerobic bacterial flora of diabetic patients and to compare it to that of nondiabetic patients, describing its variations according to the presence and extent of diabetic retinopathy, and the duration of the disease.

MATERIALS AND METHODS

This study was carried out by the Department of Ophthalmology and Diabetic Clinic at Sir Syed Hospital Quyyumabad, Karachi, from January to October 2009.

A past medical history was taken. Patients with ocular symptoms, or other systemic disease were excluded. Slit-lamp examination was performed on each patient with particular attention to any evidence of dry eye, blepharitis, anterior segment infection, inflammation, or lens opacity prohibiting fundus examination.

The patients were taken from diabetic Clinic at Sir Syed hospital with a known history of diabetes mellitus for which the patient was under the care of a physician. All control patients were selected from Department of Ophthalmology, which has to undergo cataract extraction and had a normal blood sugar test on their preoperative evaluation. Only controls within the age range of the diabetic group were included.

A prior consent was obtained before taking eye culture swabs from each subject. Swabs were taken from conjunctiva of both eyes of patients. All precautionary measures were taken to avoid lid margin and eyelashes while taking the swab.

Swabs were taken from conjunctiva of each patient, using sterile Stuart’s swabs, which were placed in Stuarts transport medium for onward transfer to microbiology laboratory. Swabs were streaked on culture media.

Culture media used were sheep blood agar, chocolate agar, MacConkeys agar, fungal media, mycobiotic agar and Sabouraud 4% dextrose agar.

The Gram-staining was done for each swab.

Culture media were incubated at 37 degrees C to permit bacterial growth and held for 3 days to ascertain either “growth” or “no growth”. Sabouraud 4% dextrose agar and mycobiotic agar were incubated at 25 degrees C and were held for 2 weeks to observe either growth trend.

Indirect ophthalmoscopy was performed on all subjects after culture was taken, and the patients were classified as, normal (without retinopathy), non-proliferative diabetic retinopathy, and proliferative diabetic retinopathy.

Statistical package for social sciences “SPSS-15.0” was used for data analysis. The results were presented in terms of frequencies and percentages. Chi-square test was applied to compare the study parameters between diabetic and non-diabetic groups and group of culture results. P-value ≤ 0.05 was considered statistically significant difference.

RESULTS

80 patients form Sir Syed Diabetic Clinic with no evidence of ocular surface disease were included. These diabetic patients were compared with 50 nondiabetic subjects.

A predomiance of male subjects was present in both diabetic group (70%) and nondiabetic group (60%). The mean age was 58 (± 9) years in the diabetic group and 57 (± 8.6) years in nondiabetic group. There was no significant difference in age distribution among the diabetic retinopathy subgroup.

The mean duration of diabetes mellitus was 8.7 (± 3.5) years. Sixty-one patients were taking oral hypoglycemic agents, and 19 were using insulin.

In the diabetic group, the ophthalmoscopic examination detected 21 patients (26.2%) without diabetic retinopathy, 47 patients (58.7%) with non-proliferative diabetic retinopathy, and 12 patients (15.0%) with proliferative diabetic retinopathy.

Only 4 (5%) patients of diabetic group had negative cultures compared with 14 (28%) patients in the nondiabetic group. Significantly higher (p<0.001) number of diabetics had bilateral positive culture (i.e. 75%) as shown in table 1.

A significantly higher frequency positive cultures was identified among diabetic patients with retinopathy, 60 patients (75%), compared with those without retinopathy, 16 patients (20%). Among the subjects with positive cultures in both eyes, a significantly higher proportion was identified among the patients with diabetic retinopathy (NPDR or PDR) when compared with those without retinopathy (p=0.001) (Table 2).

There were no significant differences in the prevalence of positive cultures when diabetic group was stratified by disease duration (less than 5 years versus 5 years or more), type of hypoglycemic agent, gender, or age.

The most common isolated bacteria in diabetic and nondiabetic groups were Staphylococcus epider-
midis. The other isolates are described in Table 3. The only difference among the isolates was a higher proportion of Staphylococcus epidermidis among diabetic 61 patients (80.2%) as compared to nondiabetic 22 (61.1%) patients (p=0.031).

The number of different organisms identified in the same patient differed between diabetic and nondiabetic groups (p<0.001). It was also more likely to have two or more organisms in the same subject among diabetic patients with NPDR or PDR than in diabetic patients without retinopathy (p<0.001) (Table 4).

The isolation of Staphylococcus epidermidis was also more frequent in patients with diabetic retinopathy than those without retinopathy (p=0.002) (Table 5).

Table 1: Conjunctival cultures in diabetic and nondiabetic subjects

<table>
<thead>
<tr>
<th>Conjunctival cultures</th>
<th>Diabetic n (%)</th>
<th>Nondiabetic n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>04 (5.0)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>Positive/unilateral</td>
<td>16 (20.0)</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Positive/bilateral</td>
<td>60 (75.0)*</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Total</td>
<td>80 (100)</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

*Significantly higher proportion of positive conjunctival culture in diabetic subjects (p<0.001).

Table 2: Positive conjunctival cultures in diabetic subjects according to the level of diabetic retinopathy

<table>
<thead>
<tr>
<th>Level of retinopathy</th>
<th>Absent n (%)</th>
<th>NPDR n (%)</th>
<th>PDR n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive conjunctival cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>9 (56.2)</td>
<td>6 (12.2)</td>
<td>1 (9.0)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>7 (43.7)</td>
<td>43 (87.7)*</td>
<td>10 (90.9)*</td>
</tr>
<tr>
<td>Total</td>
<td>16 (100)</td>
<td>49 (100)</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

*Significantly higher proportion of bilateral conjunctival culture in NPDR and PDR subjects (p=0.001).

Table 3: Bacteria isolated from conjunctiva of diabetic and nondiabetic subjects

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Diabetic (n=76) n (%)</th>
<th>Nondiabetic (n=36) n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>61 (80.2)*</td>
<td>22 (61.1)</td>
<td>0.031</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>17 (22.3)</td>
<td>12 (33.3)</td>
<td>0.216</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10 (13.1)</td>
<td>4 (11.1)</td>
<td>0.760</td>
</tr>
<tr>
<td>Streptococcus non-hemolytic</td>
<td>2 (2.6)</td>
<td>1 (2.7)</td>
<td>0.999</td>
</tr>
<tr>
<td>Moraxella</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
<td>0.999</td>
</tr>
<tr>
<td>Staphylococcus coagulase-negative</td>
<td>2 (2.6)</td>
<td>1 (2.7)</td>
<td>0.999</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

*Shows significantly higher proportion at 5% level of significance.

Table 4. Conjunctival culture results according to the number of different organisms isolated from the same subject

<table>
<thead>
<tr>
<th>Retinopathy Level</th>
<th>Culture results</th>
<th>Non Diabetic (n=50) n (%)</th>
<th>Diabetic (n=80) n (%)</th>
<th>Absent (n=21) n (%)</th>
<th>NPDR (n=47) n (%)</th>
<th>PDR (n=12) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>14 (28.0)</td>
<td>4 (5.0)</td>
<td>4 (19.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>One organism</td>
<td>19 (38.8)</td>
<td>46 (57.5)*</td>
<td>15 (71.4)</td>
<td>27 (57.4)</td>
<td>4 (33.3)</td>
<td></td>
</tr>
<tr>
<td>2 or more organism</td>
<td>14 (34.0)</td>
<td>30 (37.5)</td>
<td>2 (9.5)</td>
<td>20 (42.5)</td>
<td>8 (66.6)*</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly higher proportion of at least 1 organism on culture result in diabetic subjects (p<0.001).

DISCUSSION

In the present study, we evaluated the conjunctival aerobic bacterial flora in diabetic patients, stratified by the presence and extent of retinopathy, versus nondiabetic subjects. The hypothesis was that diabetic
patients not only might have a higher incidence of bacterial pathogens cultured from the conjunctiva but also that the severity of diabetes mellitus might be a predictor for bacterial colonization.

**Table 5: Identification of staphylococcus epidermidis according to the presence of diabetic retinopathy**

<table>
<thead>
<tr>
<th>Diabetic Retinopathy</th>
<th>Conjunctival Cultures</th>
<th>Absent n (%)</th>
<th>Present n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td>8 (38.0)</td>
<td>5 (8.4)</td>
</tr>
<tr>
<td>Positive/unilateral</td>
<td></td>
<td>7 (33.3)</td>
<td>16 (27.1)</td>
</tr>
<tr>
<td>Positive/bilateral</td>
<td></td>
<td>6 (28.5)</td>
<td>38 (64.4)*</td>
</tr>
</tbody>
</table>

*Significantly higher proportion of bilateral conjunctival culture in patients with diabetic retinopathy (p=0.002).

The prevalence of positive cultures in nondiabetic subjects found in this study is similar to that previously reported in nondiabetic patients. However, the proportion of positive cultures in the diabetic group (95.0%) was significantly higher and was similar to cultures in immunodeficient patients.

All immunodeficient patients studied by Friedlander, in 1980, demonstrated positive conjunctival cultures. Comeric-Smith et al analyzed the lids of HIV patients and obtained growth in 100% of cultures compared with only 33% of the non-HIV subjects. In contrast, Gritz et al, studying the HIV patients flora, did not detect a significant difference in positive cultures when HIV patients were compared with controls.

In our study, only the presence of diabetic retinopathy correlated with the prevalence of positive cultures. No other variables (hypoglycemic therapy, age, and disease duration) correlated with the culture results. We recognize that the accuracy of disease duration may have been compromised by inaccurate information provided by the patient.

Studies of the number of different bacterial species in diabetic patients are not addressed in the literature. In patients with diabetic retinopathy, the identification of two or more organisms was significantly more frequent, indicating that the presence of retinopathy might be a marker for altered conjunctival flora.

Staphylococcus epidermidis was the most frequent organism isolated from diabetic and nondiabetic groups, consistent with the previous reported of Walker and Claoue.

The frequency of Staphylococcus epidermidis isolated from the conjunctiva of nondiabetic subjects (61.1%) in our study was similar to that reported from nondiabetic patients.

Akhter Jamal Khan studied the normal conjunctival flora in Karachi and obtained cultures from 800 patients. His results showed Staphylococcus epidermidis (57.7%) was the most common bacteria, followed by Diphtheroids Sp (26.6%).

Shehla Rubab compared the indigenous microbial flora of the eye to that found in conjunctival and corneal infections at Al-Shifa trust hospital at Rawalpindi. She found out that in the control group of 700 eyes, the microorganism detected included Staphylococcus epidermidis in 57.7%, Staphylococcus aureus in 22.5%, Streptococcus pneumoniae in 8.3% and Diphtheroids in 3.3% of cases.

In our study, among the diabetic patients, the isolation of Staphylococcus epidermidis was significantly more frequent (85.0%). Table 5 shows that the presence of retinopathy was also a marker for a high frequency of Staphylococcus epidermidis isolation.

The importance of coagulase-negative staphylococcus in the flora is that it has frequently been identified as a causative agent of endophthalmitis. Several authors have described series of endophthalmitis cases caused by Staphylococcus epidermidis. In 1997, Johnson et al detected a higher frequency of coagulase-negative staphylococcus in diabetic patients with endophthalmitis when compared with nondiabetic subjects.

Assuming that a significant number of endophthalmitis cases are related to resident flora, Staphylococcus epidermidis as a causative agent may be linked to the high frequency of this microbe in conjunctival flora. The presence of retinopathy may indicate a more significant risk for this infection. This study has demonstrated that it was associated with higher frequency of Staphylococcus epidermidis identification (91.5%).

If our findings are correct, the presence of diabetic retinopathy is correlated with a higher prevalence of Staphylococcus epidermidis on the conjunctival...
surface. As such, retinopathy may signal an increased risk of endophthalmitis.

Coagulase-negative staphylococcus and gram-negative bacteria are described as the most frequent etiologic agents in diabetic subjects in various endophthalmitis series\(^ {10, 13, 24}\).

Despite the fact that the incidence of postoperative infection is small relative to frequency of ophthalmic surgery, infection is most common in diabetic patients, and it may predict a poor visual outcome \(^ {10, 11, 24}\). The high prevalence of organisms in the conjunctival flora of diabetic patients, as demonstrated in this paper, may play a role in the higher susceptibility to postoperative infection.

This study demonstrates that diabetes, and specifically the presence of diabetic retinopathy signals a higher prevalence of potentially pathogenic bacteria in the conjunctival flora.

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**REFERENCE**