Original article

Study of aerobic bacterial conjunctival flora in patients with diabetes mellitus

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Abstract

Introduction: Normal bacterial flora may be altered by a variety of factors. **Objective**: To study the aerobic bacterial conjunctival flora in patients with diabetes mellitus and to find its clinical significance by comparing the results to the conjunctival flora of non-diabetic subjects. **Materials and methods**: A total of 75 diabetic patients were included as cases and 25 non-diabetics as controls to compare the results. Specimens for the study of conjunctival flora were taken by rubbing sterile cotton-tipped swabs to the inferior palbebral conjunctiva. The conjunctival culture report of the patients with diabetic mellitus was compared to that of non-diabetic subjects. **Results:** Positive conjunctival cultures were seen in a higher percentage of patients with diabetes (unilateral and bilateral positive conjunctival cultures 34.66 % and 58.66 % respectively) compared to that in non-diabetic controls (unilateral and bilateral positive conjunctival cultures 24 % and 16 % respectively). Diabetics showed a higher proportion of coagulase negative staphylococci (45.33 %), compared to the non-diabetic group (16 %). Among the diabetic patients, positive conjunctival cultures were detected more frequently in those with diabetic retinopathy compared to those without retinopathy. A higher proportions of bilateral positive conjunctival cultures were seen in cases with proliferative diabetic retinopathy (38.63 %) in comparison to patients with no retinopathy and different stages of non-proliferative diabetic retinopathy. **Conclusion:** The conjunctival floral pattern with increased bacteria in diabetics is a predominant cause of many diabetes-related ocular infections. The presence of diabetic retinopathy is an indicator for increased colonization of conjunctiva, and its severity correlates with the severity of diabetic retinopathy.

Key-words: conjunctival floral pattern in diabetes, positive conjunctival culture

Introduction

The presence of micro-organisms in the normal human conjunctiva was established in the 19th century (Lawson, 1898). The normal microbial flora refers to population of microorganisms that dwell within the eyes of healthy individuals (Jawetz et al, 1989). The composition of the normal ocular flora plays an essential role in the healthy functioning of the eye, the maintenance of surface homeostasis, and in both prevention and causation of ocular infection. Normal ocular flora remains relatively consistent among human populations. It may however be altered by a variety of factors that include age, immunosuppression, ocular

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inflammation, dry eye, contact lens wear, antibiotic use, surgery, external exposure, climate and geography (Jesse et al, 2010).

Patients with diabetes have altered immunity at various levels and may be more susceptible to infection after ocular surgery. The patients with diabetes mellitus were more likely to have endophthalmitis caused by gram-negative organisms and appear to have a poorer visual prognosis after treatment for endophthalmitis (Phillips et al, 1994).

Diabetic patients showed confirmed growth at culture more often than non-diabetic patients. The fact that culture-positive rate is higher in diabetic patients probably reflects a more permissive environment for bacterial growth in the diabetic eye, with consequent higher bacterial density and perhaps greater number of viable organisms in the diabetic culture sample than from the non-diabetic eye (Bernard et al, 2001).

Using techniques of molecular epidemiology, the Speaker et al demonstrated that organism isolated from the vitreous was genetically indistinguishable from an isolate recovered from the patient's eyelid, conjunctiva, or nose in 82% of cases of endophthalmitis. Therefore, attention should be directed toward the external tissues and their microbial flora in the prophylaxis and prevention of postoperative bacterial endophthalmitis (Speaker et al, 1991)

This study aims to study the conjunctival flora in patients with diabetes mellitus and to find its clinical significance by comparing the results to conjunctival flora of non-diabetic subjects.

Materials and methods

The study was carried out at the Department of Ophthalmology, Hassan Institute of Medical Sciences from August 2011 to February 2012. A total of 75 diabetic patients were included as cases and 25 non-diabetics were used as controls to compare the results. The patients attending the outpatient department of ophthalmology with known history of diabetes were included in the study as cases. Non-diabetic patients with normal fasting and postprandial blood sugars admitted for cataract surgery were used as controls. Those who had clinical evidence of ocular inflammation or adnexal disease like blepharitis and conjunctivitis, history of systemic antibiotic or corticosteroid treatment 2 months before the study and use of topical eye drops within the eight weeks before the study were excluded. Written informed consent was obtained from each subject. Indirect ophthalmoscopy was performed in all and the staging of diabetic retinopathy was noted according to Early Treatment of Diabetic Retinopathy Study. The patients with hazy media preventing the visualization of retina were excluded from the study.

Specimens for study of conjunctival flora were taken by rubbing sterile cotton tipped swabs to the inferior palpebral conjunctiva. The lower eyelid was pulled down in order to avoid the lid margins and eye lashes to prevent contamination of the cotton swabs. Immediately the cultures for aerobic bacteria were made by direct inoculation. The media used were blood agar and chocolate agar. Culture media were incubated for aerobic bacteria at 37 degree centigrade and held for three days to look for growth. Statistical package for social sciences "SPSS-15.0"was used for data analysis. The results were presented in terms of frequencies and percentages.

Results

Males accounted for 54.66% (41) and females were 45.33% (34) of the study group. The results were compared to 25 non-diabetics who were taken as controls of which 60% were males and 40% were females. 37.33% of the diabetics were between 50-60 years and 48% of the non-diabetics were in the same age group (Table 1). In the diabetic group 10 (13.33%) had no retinopathy, 9(12%) had mild non-proliferative diabetic retinopathy (NPDR), 11 (14.66%) had moderate NPDR, 17(22.66%) had severe NPDR and 24 (32%) had proliferative diabetic retinopathy (PDR).

Negative conjunctival cultures were seen in 6.66% of diabetics compared to 60% negative cultures in non-diabetic group. Positive unilateral culture was seen in 34.66% in diabetic group compared to 24% in non-diabetic group and positive bilateral culture was seen in 58.66% of diabetic group and in16% of non-diabeticgroup (Table 2).

Staphylococcus epidermidis was the most common organism isolated in diabetic and non- diabetic groups. It was isolated in 86.66% of diabetic group as compared to 36% in non-diabetic group. Table 3 shows the other organisms isolated from conjunctiva of diabetic and non-diabetic groups and Table 4 shows the frequency of isolation of most common three organisms in positive conjunctival cultures and their breakup according to stage of diabetic retinopathy.

Diabetics showed higher proportion of coagulase negative Staphylococci (45.33%), compared to non-diabetic group (16%).

Higher proportions of bilateral positive conjunctival cultures were seen in cases with PDR (38.63%) in comparison to patients with no retinopathy and different stages of NPDR (Table 5).

Positive conjunctival culture had single organism isolation more frequently in diabetic group with no retinopathy (80%) and isolation of two or more organism isolation was more frequently seen in diabetic group with PDR (62.50%) (Table 6).

Table 1: Sex wise distribution of study group

Sex	Diabetics n =75	Non-diabetics n=25
Male	41(54.66%)	15(60%)
Female	34(45.33%)	10(40%)

Table 2: Conjunctival cultures in diabetic and non-diabetic individuals

Conjunctival cultures	Diabetics n=75	Non – diabetics n=25
Negative	5(6.66%)	15(60%)
Positive/unilateral	26(34.66%)	6(24%)
Positive /bilateral	44(58.66%)	4(16%)
Total	75	25

Table 3: Aerobic bacteria isolated by conjunctival cultures in diabetic and non-diabetic individuals

Organisms isolated	Diabetics	Non - diabetics
Staphylococcus Epidermidis	65(86.66%)	9(36 %)
Staphylococcus aureus	18(24%)	7(28%)
Diphtheroids	11(14.66%)	8(32%)
Corynebacterium sp	3(4%)	1(4%)
Staphylococcus coagulase-negative	34(45.33%)	4(16%)
Moraxella	2(2.66%)	1(4%)

Table 4: Isolation of most common three organisms in positive conjunctival cultures and their breakup according to stage of diabetic retinopathy

Organism	No retinopathy	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Staphylococcus Epidermidis	3(4.61%)	3(4.61%)	7(10.76%)	17(26.15%)	35(53.84%)
Staphylococcus coagulase-negative	1(2.94%)	4(11.76%)	5(14.70%)	7(20.58%)	17(50%)
Staphylococcus aureus	4(22.22%)	2(11.11%)	3(16.66%)	3(16.66%)	6(33.33%)

Table 5: Positive conjunctival cultures in diabetics and their breakup according to the stage of diabetic retinopathy

Positive conjunctival cultures	No retinopathy	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Unilateral n=26	6(23.07%)	3(11.53%)	4(15.38%)	6(23.07%)	7(26.92%)
Bilateral n=44	4(9.09%)	6(13.63%)	7(15.90%)	11(25%)	17(38.63%)

Table 6: Isolation of number of organisms in positive conjunctival cultures and their breakup according to stage of diabetic retinopathy

Positive conjunctival cultures	No retinopathy n=10	Mild NPDR n=9	Moderate NPDR n=11	Severe NPDR n=17	PDR n=24
Single organism	8(80%)	6(66.66%)	7(63.63%)	8(47.05%)	9(37.50%)
Two or more organisms	2(20%)	3(33.33%)	4(36.36%)	9(52.94%)	15(62.50%)

Discussion

Patients with diabetes mellitus (DM) are prone to infection because glucose in the skin, urine, mucous membranes, and tears promotes growth of microorganisms. Conjunctival flora develops soon after birth and some saprophytic conjunctival flora play a pathogenic role when immune function is compromised, which can lead to serious infection. DM is one condition that may compromise immune status (Bilen et al, 2007). Diabetics have a conjunctival flora pattern whose increased bacteria are a predominant cause of many diabetic infections (Fernández et al, 2010).

Most eyes harbour Staphylococcus epidermidis, with diphtheroids only being somewhat less common (Singer et al, 1988). This goes in consistent with our study which shows Staphylococcus epidermidis in 36% and Diphtheroids in 32% in non-diabetic controls.

Staphylococcus epidermidis was the most frequent organism isolated both in Diabetic and non diabetics in our study and this was similar to a previous study done by Walker et al (1986).

Coagulase negative staphylococci had been identified as an important causative agent in post operative endophthalmitis. In our study 45.33% of diabetic group were positive for coagulase negative staphylococci. This was in consistent with a previous study by Martins et al (2004) in which Coagulase-negative Staphylococcus was the most common microorganism isolated, and its identification was more frequent in patients with diabetic retinopathy than in those without diabetic retinopathy. The similar finding were noticed in another study by Johnson et al (1997) which showed conjunctival flora in Diabetes mellitus being associated with a higher yield of gram-positive, coagulase-negative micrococci.

Positive bilateral conjunctival cultures and isolation of two or more organisms was seen more frequently in patients with retinopathy (38.63%) as compared to patients without retinopathy (9.09%). This was in consistent with study by Martins et al (2004). Similar findings were found in another study by Tahir et al (2010) who concluded that diabetic patients have a significantly higher number of positive conjunctival cultures and the presence of diabetic retinopathy was correlated with an increase in positive cultures and a higher proportion of Staphylococcus epidermidis.

In diabetic group positive bilateral cultures seen was in higher proportion in patients with PDR compared to patients with NPDR. Similar findings were noted in a study by Lima et al (2002) whose study showed that the isolation of coagulase negative Staphylococcus was more frequent in patients with moderate, severe, very severe non proliferative diabetic retinopathy and proliferative diabetic retinopathy when compared to those without diabetic retinopathy.

Conclusion

The conjunctival floral pattern with increased bacteria in diabetics is a predominant cause of many diabetes related ocular infections. The presence of diabetic retinopathy is an indicator for increased colonization of conjunctiva and its severity correlates with the severity of diabetic retinopathy.

Diabetics and specifically those with diabetic retinopathy have higher prevalence of pathogenic bacteria in conjunctival flora plays a significant role in increased susceptibility of diabetics to infection. Ophthalmologists should be aware that conjunctival flora in diabetics differs from that in non-diabetics. This should be considered preoperatively and postoperatively, and appropriate treatment should be administered accordingly to diabetic patients.

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