Original Research Article

A comparative study of aerobic bacterial conjunctival flora in type II diabetes mellitus and non-diabetic individuals who attends to department of ophthalmology at a teaching institute

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ABSTRACT

Background: Normal conjunctival flora have important role in the healthy functioning of eye like maintenance of surface homeostasis and prevention and/or causation of ocular infection. Objective of this study is to investigate the conjunctival aerobic bacterial flora in diabetic patients and nondiabetic subjects with regard to the management of diabetes which is assessed by glycated hemoglobin levels.

Materials and Methods: A prospective study carried out to compare the conjunctiva microflora in 50 diabetes mellitus type II and age matched 50 non-diabetic healthy individuals. Swab was taken from all the participants by rubbing sterile cotton swab to inferior palpebral conjunctiva. Bacterial culture processed using blood agar, chocolate agar, eosin methylene-blue lactose sucrose agar and sabouraud dextrose agar. The bacterial flora identified using microbiology procedure.

Results: The rate of bacterial isolate determined as 40% and 34% in diabetes mellitus type II and non-diabetic healthy subjects. In these diabetic group, 30% positive for *Staphylococcus aureus*, 20% for *Escherichia coli*, 10% for *Klebsiella pneumonia*, 10% for coagulase-negative Staphylococcus and 30% for more than one bacteria. In non-diabetic group, 52.9% positive for *Staphylococcus aureus*, 23.5% for coagulase-negative Staphylococcus (CNS), 5.8% for *Klebsiella pneumoniae* and 17.6% for more than one bacteria. There was no statistical significant difference observed in isolated bacteria in diabetic and non-diabetic groups. Gram negative bacterial colonization observed high in diabetic individuals (diabetic group, $\chi2= 0.156$, p = 0.815 and in healthy non-diabetic group, $\chi2= 4.95$, p = 0.035).

Conclusion: Bacterial isolates were lower in diabetics younger than 40 years of age compared with older than 60 years of age. The conjunctival bacterial flora in diabetics differ from non-diabetic subjects. This should be considered preoperatively and postoperatively, for prophylactic and postoperative treatment should be administered accordingly to the diabetic patients.

1. Introduction

Two types of ocular flora present in resident and transient. Resident flora are permanent, which represent the colonization; repeated cultures usually reveal the same organisms in large numbers. Transient flora inhabit the eye for short periods and this cannot be consistently recovered in consecutive cultures. The composition of normal conjunctival flora have the role in the healthy functioning of the eye, surface homeostasis maintenance and prevention/causation of ocular infection. This conjunctival flora may alter by intrinsic and extrinsic changes includes age, dry eye, immunosuppression, ocular inflammation, wearing contact lens, usage of antibiotics, eye surgery, geographical diversity and climate changes.[1] The bacterial flora of healthy individuals is formed by *Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium species,* and *Propineobacterium acne.*[2,3] Diabetes mellitus typeII will affect the growth of conjunctival microbiota, which leads to increasing the

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risk of eye infection. Studies shows that *S. epidermidis, S. aureus,* and *Corynebacterium sp ecies* have been found in diabetic subjects.[4]

The current study objective is to analyse the role of diabetes type II mellitus on conjunctival bacterial growth and their variations than non-diabetic individuals.

2. Materials and Methods

2.1. Sample size

A hospital based prospective Study on 100 (50 diabetes mellitus type II and 50 age matched non-diabetic healthy individuals).

2.2. Inclusion criteria

Diabetes mellitus and Non-diabetic individuals without any pre-existing conjunctival pathology.

2.3. Exclusion criteria

Patients with pre-existing ocular diseases, dry eye or glaucoma, any antibiotic instillation use within the previous 2 months, wearing contact lens, with history of previous invasive ocular surgery and immunocompromised.

2.4. Procedure

A sterile swab specimen collected by scraping each eye's lower right fornix without local anesthesia before examination. Both group individuals underwent ophthalmologic examination; slit-lamp examination etc. After immediate transferring the swabs to microbiology, swab was cultured using blood agar; chocolate agar; eosin methylene blue lactose sucrose agar; and incubated at 37°C upto 24 hrs; swab culture on the Sabouraud dextrose agar SDA incubated at 25°C upto 2 wk. Bacterial isolates were identified using coagulase & catalase testing, gram staining.

Data was expressed as % number. Student's t test used to analyse continuous variables and chi-square test used to analyse categorical data. Spearman's correlation test used to evaluate correlation. Significance difference was set as p less than 0.05. SPSS version 21.0 statistical analysis package software.

3. Results

In diabetic group, 29 women and 21 men; In healthy control group, 26 women and 24 men were recorded. The mean age was 51.5 ± 8.5 yr (range, 39-68 yr) and 52.5 ± 10.2 yr (40-71 yr) in diabetic and non-diabetics. There was no significant differences between the groups (gender: p=0.85; age, p=0.79).

The mean duration of diabetes mellitus was 8.5 ± 5.5 years (range: 2-18 years). In these, 12 patients (24%) suffering from diabetic retinopathy (DR).

With regard to conjunctival culture it was found that 20 of the 50 diabetic patients (40%) had a positive culture for aerobic bacteria in diabetic group, and 17 (34%) in the non-diabetic patients, without statistical significant (p=0.085).

In the culture from diabetics, 6 cultures (30%) positive for *Staphylococcus aureus*; 4 cultures (20%) positive for *Escherichia coli*; 2 cultures (10%) positive for coagulase negative Staphylococci; 2 cultures (10%) positive for *Klebsiella pneumoniae* and 6 cultures (30%) for more than one bacterial species. In cultures from non-diabetics, 9 cultures (52.94%) positive for *Staphylococcus aureus*; 4 cultures (23.52%) positive for coagulase negative Staphylococci; 1 culture (5.88%) positive for *Klebsiella pneumoniae* and 3 cultures (17.64%)for more than one bacterial species. There was no statistical difference in bacterial isolates in diabetics and non-diabetic groups. ($\chi2=0.156$, p=0.815). But, gram negative bacterial colonization was higher in diabetic individuals compared to healthy individuals ($\chi2=4.95$, p=0.035).

There was no correlation observed between diabetes duration and positive culture isolates.

4. Discussion

Totally 100 subjects were screened to study the conjunctival flora both in the diabetes mellitus type II and non-diabetic healthy group in Ophthalmology of Narayana Medical college and hospital.

Diabetes is associated with reduced immune function, which contributes to more frequent isolation of organisms in diabetic individuals.

In our study, in regard to age groups, a greater number of diabetic and non-diabetic patients were found 39-68 years and 40-71 years; regardless of glycemic control (the difference was not statistically significant). Bacterial isolates were lower in diabetics younger than 40 years of age compared with older than 60 years of age, which means with advanced age being the only significant risk factor in the detection of bacterial growth.[4,5]

Our study found that age and gender had no effect on culture results.

In our study, conjunctival bacterial flora was isolated was higher in diabetes mellitus patients (40%) than the non-diabetics (34%). Whereas in previous studies, bacterial growth was observed as 94.18% and 73.3% of the cultures obtained from diabetic and non-diabetic individuals.[2]

In our study, it was noted that diabetics with higher fasting blood glucose levels were more likely to have bacterial growth in their cultures than compare with diabetics with lower blood glucose levels and also with non-diabetic patients.[2,6] The difference was not statistically significant and this might be due to small sample size.

Martin et al., identified higher rate of culture positivity in diabetics (94.18% vs. 73.33%).[2] The higher culture positivity in diabetes mellitus means more permissive

Table 1: Bacterial isolates from conjunctivae of diabetes mellitus and healthy individuals

Bacteria	Diabetes mellitus	Non-diabetic healthy	Significance
Escherichia coli Klebsiella pneumoniae	4 (20%) 2 (10%)	- 1 (5.8%)	χ2=4.95, p=0.035
Staphylococcus aureus Coagulase negative Staphylococci(CNS)	6 (30%) 2(10%)	9 (52.9%) 4 (23.5%)	
More than one species (*Staphylococcus aureus, coagulase negative Staphylococci, Escherichia coli, Klebsiella pneumoniae, diphtheroids*)	6 (30%)	3 (17.6%)	χ2=0.450, p=0.339

environment for bacterial growth.

In our study, gram positive bacteria occurs as high; among these cultures, *Staphylococcus aureus* is the most common. Martins et al., Capriotti et al., and others found CNS to be more common among the diabetics.[2,7–10]

CNS is the second common bacteria in non-diabetics; third common bacteria in diabetic groups. Martins et al, study shows CNS is the higher common bacteria in diabetic retinopathy than diabetes without diabetic retinopathy patient. These finding were noticed in another study by Johnson et al., which shows that conjunctival flora being associated with a higher yield of gram-positive, coagulase-negative micrococci in Diabetes mellitus.[11]

We didn't found significant difference in bacterial isolates between the two groups. Another factor which may affects the flora is the type of hypoglycemic therapy. Positive culture isolates were higher in insulin group than control group, without statistical significant (p=0.035).

Important findings of this study is higher incidence of gram negative bacteria in diabetics.

Klebsiella pneumoniae is the common gram negative bacteria and diabetes mellitus was the determined significant risk factor.

There was no definite cause of higher isolation rate of any bacterial species in swab of diabetics. Colonization rate of bacterial flora in diabetics is higher due to high glucose levels in tears.[12] Higher rate of bacterial colonization may weaken the immune system includes diabetes, advanced age, and usage of corticosteroids.[13]

The changes observed in flora may depends on social, personal factors, geographical area, resistance to antibiotics etc.[5] The flora undergoes constant changes, influenced by seasonal changes, temperature, environmental exposure, age, surgical trauma and host immunity.[2,3,14,15]

5. Conclusion

The Staphylococcus aureus was common isolate in both groups. *Escherichia coli* and *Klebsiella pneumoniae* were identified to be higher in diabetics. Bacterial isolates were lower in diabetics younger than 40 years of age compared with older than 60 years of age. The conjunctival flora with increased bacteria in diabetes mellitus is a predominant cause of many diabetes related ocular infections. Ophthalmologists should be aware about the conjunctival flora in diabetics differs from that in non-diabetics to be considered preoperatively/postoperatively by administering accordingly for appropriate treatment.

6. Source of Funding

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7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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