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RESEARCH ARTICLE

EFFECTS OF DIABETES RELATED OCULAR DISORDERS ON BACTERIAL CONJUNCTIVAL FLORA

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Abstract

The aim of the present study was to investigate the pattern of conjunctival bacterial flora in diabetic patients and describing its variations according to the presence of major diabetes related ocular disorders that people with diabetes. This cross sectional study was carried out in various eye hospitals in and around Trichirappalli district, Tamilnadu, India over a period of 5 months from 1st May to 31st September 2015. Total 200 conjunctival swabs were collected from both eyes of 50 diabetic patients and 50 nondiabetic patients. Significant number of culture was positive in diabetic patients (94%) compared to that of non-diabetic individuals (78%). Significantly higher proportion of positive conjunctival culture in diabetic retinopathy patients (40%) among diabetes. Cataract patients were containing 26% of positive cultures. Patients with glaucoma were containing 10% of positive culture and Diabetic patients without any disorder were containing 18% of positive culture. *Staphylococcus epidermidis* was predominant microorganism in both study groups. *Staphylococcus aureus, Pseudomonas aeruginosa, Diptheroids, Streptococcus non-haemolytic, Moraxella sp, Escherichia coli, Enterobacter, Haemophilus influenza* were presence in various percentage of both study groups and little higher in diabetic group. We evaluated that the presence of major diabetes related ocular disorders altered the conjunctival flora. From this study we concluded that diabetes and the presence of major diabetes related ocular disorders specifically the presence of diabetic retinopathy may be a factor for a higher prevalence of bacteria in the conjunctival flora.

INTRODUCTION

Ocular regions harbors a significant number of bacteria and occasionally fungi from the external environment are called normal flora(**Hari Saxena& Promila Goswami 1971 ; Parmeshri Dass Sharma *et al*., 2013**). They play an important role in normal body functions and health by secreting antibiotics and chemical mediators to maintain surface homeostasis and immunoregulation. They also out compete pathogenic bacteria for nutrition thereby inhibiting their growth. The predominant microorganisms of conjunctiva are *Staphylococcus epidermidis, Diphtheroids, Micrococcus sp* and *Staphylococcus aureus* **(Therese KL & Madhavan HN,2004)**.In addition, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus viridans, Moraxella catarrhalis, Haemophilus influenzae, Klebsiella sp., Escherichia coli* and *Pseudomonas aeruginosa* are occasionally found. The conjunctival flora may be altered under special circumstances, as in new-borns, acquired immune deficiency patients, contact lens wearers, diabetes and patients using immunosuppressive drugs (**MillerB& Ellis P 1977; Campos M *et al* ., 1994 ; Fleiszig S 1992)**.

Enabling factors such as diabetic mellitus, may affect the growth of human conjunctival microbiota and some members of the conjunctival flora play a pathogenic role in diabetes mellitus when immune function is compromised, which may lead to serious infection (**Rich J & Lee JC 2005**). High blood sugar and poorer quality of wound healing increases the risk of eye problems from diabetes. Infact, diabetes is the leading cause of blindness in adult ages 20 to 70. The three major eye problems that people with diabetes may develop and should be aware of are retinopathy, cataracts and glaucoma (**World Health Organization 2014** ; **Michael Dansinger, MD 2015).**

The frequency of positive conjunctival cultures was significantly higher in the diabetic group than in the non diabetic group. Among diabetic patients, a significantly higher frequency of positive cultures was detected in those with diabetic retinopathy than in those without retinopathy (**Martins EN *et al., 2004 ;* Tahir Masaud Arbab *et al.,* 2010**). The presence of diabetic retinopathy is an indicator for increased colonization of conjunctiva, and its severity correlates with the severity of diabetic retinopathy (**D Karimsab** & **S K Razak 2013)**. Conditions that **increase the presence of bacteria on the ocular surface** are risk factors for the development of endophthalmitis(**Mamalis N *et al.,*2002**). Patients with diabetes mellitus known to have an impaired immune response may be at a higher risk for developing postoperative endophthalmitis (**Georges M 2012)**. Thus, the use of topical antibiotics before and after cataract surgery is justified as they are efficient in reducing and, sometimes, temporarily eliminating the conjunctival microbiota (**H. M. Kattan 1991**).There was no statistically significant difference in the proportion of conjunctival culture samples testing positive for bacterial growth in eyes undergoing glaucoma surgery compared with those undergoing cataract surgery. Glaucoma medications, or their preservatives, do not appear to significantly alter conjunctival flora. Techniques used for endophthalmitis prophylaxis prior to cataract surgery are likely appropriate for glaucoma surgery as well (**De Kaspar 2004**).

Coagulase-negative Staphylococcus and gram negative bacteria was the most common microorganism isolated, and its identification was more frequent in patients with retinopathy than in those without diabetic retinopathy and they have been reported as common causes of endophthalmitis in diabetic patients(**Philips WB& Tasman WS 1994** ; **Liao HR *et al.,* 1992**). The purpose of this study is to analyze the ocular bacterial flora of diabetic patients and to compare it to that of nondiabetic patients, describing its variations according to the presence of three major diabetic related eye prooblems that people with diabetes.

MATERIALS AND METHODS

The present study was a cross sectional study carried out in various eye hospitals in and around Trichirappalli district, Tamilnadu, India over a period of 5 months from 1st May to 31st September 2015. 100 patients were participating in our studies with refractive error complaints whose ages ranged from 30-70 years. These patients were not diagnosed as any ocular infections and inflammation with slit lamp examination by an ophthalmologists. Based on the history of diabetic mellitus and glycemic level 50 patients were included in diabetic group and another 50 patients were included in nondiabetic group. Indirect ophthalmoscopy and tonometry were performed on all diabetic patients by ophthalmologists to identify the presence of major diabetes related eye problems and they were classified as normal eye, diabetic retinopathy, cataract and glaucoma on the basis of diabetes related eye problems.

With aseptic precaution and adequate light source, conjunctival swabs from both eyes were collected from all the patients by technician. The swab was moistened with sterile normal saline and rubbed two to three times over the conjunctival sac from medial to lateral side and back(**Thomson BR & Bernard KA 2007)** and this swabs were placed in Stuarts transport medium for onward transfer to microbiology laboratory.

Gram staining was performed with one swab and the second swab was inoculated onto blood agar, chocolate agar, MacConkey agar, mannitol salt agar, Haemophilus selective agar media and Robertson cooked meat medium and were incubated at 37ºC aerobically for 48 hours. Chocolate agar, Robertson cooked meat medium and Haemophilus selective agar plates were incubated in candle extinction jar. After 48 hours, all the organisms were identified by standard microbiological procedures namely colony morphology, gram staining, pigment production and relevant biochemical tests (**Cheesbrough M 2000 ; Isenberg HD 2007**). The results were presented in terms of percentages.

RESULTS

A total of 100 patients, 50 patients included in non diabetic groups and 50 patients included in diabetic groups basis on history of diabetes mellitus and glycemic level. Ophthalmoscopy and tonometry were performed by opthalmologists on 50 diabetic patients to identify and classify three major diabetic related ocular diseases and they were included in normal, diabetic retinopathy, cataract and glaucoma patients. Ten, twenty, fifteen and five numbers

of diabetic patients classified into normal eye without disorder, diabetic retinopathy, cataract and glaucoma respectively basis on diabetic related ocular disorder.

A predominance of male subjects was present in diabetic group (70%) and female subjects in nondiabetic group (60%) The mean age was 59.1 years in the diabetic group and 58.5 years in non diabetic group. There was no significant difference in age distribution among the diabetic patients with ocular diseases.

A total of 200 conjunctival swabs were studied from 100 participants. Out of total 200 conjunctival swabs, 100 were collected from both eyes of 50 nondiabetic patients and 100 from 50 diabetic patients. Significant number of culture was positive in diabetic patients (94%) compared to nondiabetics (78%). The rate of positive culture from single and both eyes was higher in diabetic patients (42.5% & 57.5% respectively) compared to the nondiabetic (30 % & 48 % respectively) (Table-1). Among the positive cultures, different bacterial species were isolated from conjunctival swabs of diabetic and non-diabetic patients. *Staphylococcuss epidermidis* was the most commonly isolated bacteria in both the study population but it was higher in diabetics (51%). Other isolated bacteria were *Staphylococcus aureus, Pseudomonas aeruginosa, Diptheroids, Enterobacter and Haemophilus influenzae* in different percentages among the both groups. *Bacillus sps* and *E.coli* was isolated from diabetic patients only (Table-2). Numbers of positive bacterial cultures among diabetic patients were varied basis on presence of major diabetes related ocular disorder. The rate of positive culture was higher in diabetic retinopathy patients than other patients (Table 3).

Table 1: culture results of conjunctival swab in diabetic and nondiabetic groups

Study groups	Total number	Negative cultures n(%)	Positive cultures n(%)	Positive/unilateral Culture n(%)	Positive/bilateral Culture n(%)
Diabetic cases	50	3(6%)	47(94%)*	20(42.5%)	27(57.5%)
Non diabetic cases	50	11(22%)	39(78%)	15(30%)	24(48%)

*Significantly higher proportion of positive conjunctival culture in diabetic subjects (93.3%)

Table 2:-Types of bacteria isolated from conjunctiva of diabetic and nondiabetic groups

Types of bacteria	Diabetic (n=47) n(%)	Non diabetic(n=39) n(%)
Staphylococcus epidermis	24(51%)*	17(43.5%)
Staphylococcus aureus	9(19%)	12(30.7%)
Pseudomonas aeruginosa	7(14%)	2(5%)
Diptheroids	1(2.12%)	3(7.69%)
Streptococcus non-haemolytic	2(4.2%)	1(2.56%)
Neisseria sp	0(0%)	1(2.56%)
Bacillus sp	1(2%)	0(0%)
Escherichia coli	1(2%)	0(0%)
Enterobacter	1(2%)	1(2.56%)
Haemophilus influenzae	1(2%)	2(5.1%)

*Significantly higher proportion of organism on culture results in diabetic patients

Table 3: culture results of conjunctival swab in diabetic patients based on presence of major eye problems.

Diabetic patients(n=50)				
Presence of major diabetes related disorder	Normal eye without disorder (n=10) n(%)	Diabetic retinopathy (n=20) n(%)	Cataract (n=15) n(%)	Glaucoma (n=5) n(%)
Negative culture	1(2%)	0(0%)	2(4%)	0(0%)
Positive culture	9(18%)	20(40%)*	13(26%)	5(10%)
Positive/unilateral	2(20%)	12(40%)	8(53.3%)	4 (40%)
positive/bilateral	1(20%)	11(30%)	3 (13.3%)	6(20%)

*Significantly higher proportion of positive conjunctival culture in diabetic retinopathy patients (40%)

DISCUSSION

Everyday our eyes are constantly exposed to thousands of microbes, which lodge on the outer surface of the eyes and the delicate linings that cover the eyeball. These microscopic organisms can invade the eyeball or enter into the eye from infection in the blood stream to cause sight-threatening diseases(**Fleiszig S & Efron N 1992)**.Individuals living with diabetes are more prone to infections, including bacterial infections of the eye. Diabetes is associated with reduced immune function. Polymorphonuclear neutrophils in diabetic patients show alterations in chemotaxis, adherence, phagocytosis, intracellular killing, and bactericidal activity (**Delamaire M *et al.*, 1997**). It contributes to more frequent isolation of organisms in diabetic individuals. Overall colonization rate of bacterial flora in diabetic group was higher, because they had higher levels of glucose in their tears which may contribute to the development of ocular infections. (**Najmun Nahar1 *et al.*, 2013**).

In the present study, conjunctival bacterial flora was isolated more frequently in diabetic patients (94%) than the non-diabetics (78%). Martin *et al*. (2004) also observed higher frequency of conjunctival culture positivity in diabetic group than those of nondiabetic group (94.18% vs. 73.33%) (**Martins EN *et al.*, 2004**). *Staphylococcus epidermidis* was isolated in highest percentage among all the isolates, both in nondiabetic (43.5%) and diabetic group (51%) in this present study. Though this percentage is little higher in diabetic group. *Staphylococcus aureus* was isolated in higher percentage in non diabetic group (30.7%) than the diabetic group (19%). *Pseudomonas aeruginosa* was isolated in higher percentage in diabetic group (14%) than nondiabetic group (5%).Other conjunctival bacterial flora atleast one number of positive culture in diabetic patients in comparison to non-diabetic patients. The higher culture positivity rate in diabetic patients probably reflects more permissive eye problems for bacterial growth in eyes of the diabetic patient.

The conjunctival floral pattern with increased bacteria in diabetics is a predominant cause of many diabetes-related ocular infections. Bacteria in the conjunctival sac influence the bacterial pathogens causing endophthalmitis after cataract surgery (**Speaker MG *et al.*, 1991**). Diabetes results in a prolonged and poorer quality of wound healing. Therefore, diabetics are also at a higher risk of infection following operations. In a normal patient undergoing cataract and glaucoma surgery, the risk of infection after surgery is one in 1,000 operations. This rate is slightly higher in diabetics (**Dr Clarissa Nah & Dr Yeo Kim Teck 2009)**.

In present study positive culture was isolated higher in diabetic patients with three major eye problems. Among them higher percentage of positive culture was isolated in diabetic retinopathy patients(40%). Tahir Masaud Arab *et al* (2010) also observed the presence of diabetic retinopathy is an indicator for increased colonization of conjunctiva and its severity correlates with the severity of diabetic retinopathy (**Tahir Masaud Arbab *et al.*, 2010**).

There is a significant change of conjunctival bacterial flora in diabetic patients in comparison to non-diabetic patients. *S. epidermidis* was the predominant organism in all study populations. The higher culture positivity rate in diabetic patients probably reflects the factors of diabetes related ocular disorder. The hypothesis was that diabetic patients not only might have a higher incidence of bacterial cultured from the conjunctiva but also that the presence of major diabetes related ocular disorders particularly diabetic retinopathy may be a factor for altered conjunctival flora.

CONCLUSION

From this study we concluded that diabetes and the presence of major diabetes related ocular disorders specifically the presence of diabetic retinopathy may be a factor for a higher prevalence of bacteria in the conjunctival flora.

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