Original Article

DOI: 10.7860/JCDR/2021/47457.15150

Ophthalmology Section

Analytical Study of Conjunctival Bacterial Flora in Diabetic and Non Diabetic Patients

TAMOJIT CHATTERJEE[1], SHRUTAKIRTI GHOSH[2], RINKI SAHA[3], SANDIP SAMADDAR[4]

ABSTRACT

Introduction: Conjunctiva is a transparent mucous membrane that lines inside of the eyelids and covers the sclera. It is composed of non keratinised, stratified columnar epithelium with goblet cells. Normal microbial flora constitutes organisms which are present in eyelids and conjunctiva without causing any disease. At birth eyes are sterile but they are later infected by various organisms. Gram positive organisms are mostly prevalent in ocular flora. Diabetes mellitus is a multifactorial disease that can affect all ocular structures.

Aim: To compare the microbial flora between diabetic and non diabetic patients and to study there sensitivity to antibiotics.

Materials and Methods: This was an analytical study conducted on a total of 100 patients attending Department of Opthalmology of Medical College and Hospital, Kolkata, India between June 2019 to July 2019. They were not using any topical ocular preparation. Conjunctival swab was taken from upper fornix of the patients and cultured and the results obtained were analysed using Chi-Square test.

Results: Conjunctival flora of a total of 100 patients, 30 diabetic and 70 non diabetic, were screened. Among the 30 diabetic patients, there were 15 (50%) male and 15 (50%) female. The most common isolated bacteria in diabetic and non diabetic groups were *staphylococcus aureus* (diabetic 70%, non diabetic 85.7%). There was a statistically significant difference in microbial flora pattern between the diabetic and non diabetic group. There was higher proportion of *staphylococcus epidermidis* among diabetic patients compared to non diabetic patients (p-value <0.01) and there was a higher proportion of *staphylococcus aureus* among non diabetics (p-value <0.01).

Conclusion: Present study found statistically significant difference in the microbial flora between diabetics and non diabetics. This will help the clinician to plan for the antibiotic prophylaxis before surgery for patients attending the Out Patient Department (OPD).

Keywords: Antagonism, Antibiotic sensitivity, Conjunctiva, Eye drops, Oral antibiotics, Virulence

INTRODUCTION

The conjunctiva is a transparent membrane that is present inside the eyelids and covers the sclera. Conjunctiva is composed of non keratinised, stratified squamous epithelium, adenoid layer and fibrous layer. Conjunctival glands include mucin glands like goblet cells and accessory lacrimal glands. The major function of the conjunctiva is to lubricate the eyes by tears and mucus and also act as a barrier against infection [1].

The conjunctiva that is sterile at birth becomes infected with several microbes acquired throughout life. Lactoferrin, lysozyme and Immunoglobulin A (IgA) that are present in tears play an important role in maintaining normal flora [1]. Ocular microbial flora also depend on environment, age, immunity, climate, ocular surface disease and general hygiene conditions [2]. *Staphylococcus sp.*, *Streptococcus sp. and Micrococcus sp.* that normally protects against several pathogenic organisms are prevalent in human ocular flora [3]. The majority of pathogens causing popstoperative endophthalmitis are from conjunctiva, eye lid and even nasal mucosa [4].

Diabetes mellitus is a multifactorial disease that can affect all ocular surface and even the retina [5]. Diabetic endophthalmitis patients have a slightly lower vision than non diabetic endophthalmitis patients and may require vitrectomy more often [6].

This study was aimed to compare the microbial flora between the diabetic and non diabetic patients and know their pattern of sensitivity to antibiotics. Bacterial resistance is one of the most serious problems now-a-days. Knowing the sensitivity pattern of the bacteria will help us to plan our treatment accordingly.

MATERIALS AND METHODS

This was an analytical study in which subjects were selected from patients attending OPD at Regional Institute of Ophthalmology (RIO), Kolkata, between June 2019 to July 2019 after consideration of inclusion and exclusion criteria and informed consent from the subjects. Total 100 patients (30 diabetic and 70 non diabetic) who came to RIO OPD with complaints other than eye infections were included. Ethical clearance was obtained for the same from RIO Kolkata.

Inclusion criteria: All patients without any pre existing ocular disease, not using any topical ocular preparation who were attending OPD on every Tuesday during the months of June 2019 and July 2019.

Exclusion criteria: Patient with pre existing ocular disease, patient using any topical ocular preparations and antibiotics, and those undergoing any major ocular surgery were excluded.

Procedure

The samples were collected from upper fornix of eyes by autoclaved swab sticks. The samples were transferred to sterile nutrient agar medium and incubated at 37°C for 3-5 days. Gram staining and Potassium Hydroxide (KOH) mount were then done for the inoculum of each plate.

Gram positive organisms found were *staphylococcus sp*, *streptococcus sp* and *micrococcus* sp. For gram positive cases catalase test and mannitol salt agar test were done.

Gram negative rods found were *Escheria coli (E coli)* and *Klebsiella*. They were further tested using phenol red lactose broth tests. Indole, Methyl Red (MR) positive but Voges-Proskauer (VP), citrate negative cases were cultured in Hi Chrome UTI Agar M1353 R.

Indole, MR negative but VP, citrate positive cases were cultured in Hi chrome UTI Agar M1418. Mucoid purple colony gives confirmatory evidence of *Klebsiella pneumonia*.

In the diabetic patients' eye swab, *staphylococcus aureus* and *Klebsiella pneumonia* were not found in same plate. So, to find out any antagonism between the two organisms, two containers of autoclaved nutrient agar were taken at a temperature of 50°C and inoculated with 50 mL broth culture of each organism. Each inoculated medium was taken in a petridish and left to solidify. Each such petridish was inoculated with 50 mL broth culture of second organism. Both plates incubated at 37°C for 24 hours.

Antibiotic sensitivity tests were done on nutrient agar plate by both antibiotic disc diffusion method (Oral antibiotics) and cup plate method (Liquid antibiotics) [7]. The zone of diameter was measured in centimeter (cm). The antibiotics used were chloramphenicol, ciprofloxacin, tetracycline, polymyxin-B, rifampicin, co-trimoxazole, cephalexin and bacitracin used as discs. Moxifloxacin, Tobramycin and Gatifloxacin were antibiotic eye drops tested.

Susceptibility of microbes towards various stress conditions like heat, pH and Ultravoilet (UV) exposure were tested. Overnight broth cultures of microbes were incubated in water bath at 50°C for 10, 20 and 30 minutes for heat treatment. For pH treatment the pH of the medium was varied from 5, 8 and 10. For UV treatment microbes were UV exposed for 5, 10 and 15 minutes.

STATISTICAL ANALYSIS

All data were collected in a predesigned data sheet. Chi-square was applied to compare the study parameters between diabetic and non diabetic groups of patients. The result was considered statically significant when p-value was less than 0.05.

RESULTS

Conjunctival flora of a total of 100 patients, 30 diabetic and 70 non diabetic were screened. Among the 30 diabetic patients there were 15 (50%) male and 15 (50%) female patients and their mean age was 53.5 (±20.5) years. The mean duration of diabetes mellitus was 18 (±15) years. The non diabetic or control group included 43 (61%) male patients and 27 (39%) female patients and their mean age was 45 (±28) years. There was no negative culture amongst diabetic patients as compared to 5(7.14 %) among non diabetic patients (p-value=0.56). There was also no significant association between positive culture and duration (<1 year or >1year) of diabetes (p-value=0.30) and age (<55 years or >55 years) of the patient (p-value=0.16).

The microbial isolates are detailed in [Table/Fig-1]. The most common isolated bacteria in both diabetic and non diabetic groups was *staphylococcus aureus*. There was a higher proportion of *staphylococcus epidermidis* among diabetic patients (43.3%) as compared to non diabetic (14.3%) patients (p-value=0.0031). There was a higher proportion of *staphylococcus aureus* among non diabetic patients (85.7%) as compared to diabetic patients (70%) (p-value=0.0043).

Name of the organisms	Diabetic (n=30) (%)	Non diabetic (n=70) (%)	Chi-square, p-value
Staphylococcus aureus	21 (70)	60 (85.7)	8.12, 0.0043
Staphylococcus epidermidis	13 (43.3)	10 (14.3)	8.73, 0.0031
Streptococci sp.	5 (16.6)	8 (11.4)	0.33, 0.56
Micrococcus roseus	4 (13.3)	5 (7.2)	0.76, 0.38
E. coli	2 (6.6)	12 (17.2)	2.27, 0.13
Klebsiella pneumoniae	7 (23.3)	9 (12.8)	1.31, 0.25
Fungal growth	4 (13.3)	5 (7.2)	0.76, 0.38

[Table/Fig-1]: Prevalence of organisms among diabetic and non diabetic patients.
p-value <0.05 considered statistically significant

Antagonism among Microorganisms

In this study, no antagonism was found between the microorganisms *Staphylococcus aureus* and *Klebsiella pneumoniae* as shown in [Table/Fig-2].

Plate No.	First organism	Second organism	Result
1.	*Staphylococcus aureus*	*Klebsiella pneumoniae*	Small bacterial colonies were found within the agar media
2.	*Klebsiella pneumoniae*	*Staphylococcus aureus*	Lawn culture of organism was found over the surface of the medium

[Table/Fig-2]: Antagonism among organisms.

Antibiotic Sensitivity Test

In this study, *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from the diabetic patients were more resistant than the same isolated from the non diabetic patients. *Streptococci sp., Micrococcus roseus, E. coli* isolated from the non diabetic patients were more resistant than the same isolated from the diabetic patients [Table/Fig-3,4].

Stress Condition Sensitivity Test

The results of stress condition sensitivity test can be represented by arbitrary scale analysis. From this analysis, we can compare the virulence of the microbes isolated from diabetic and non diabetic cases. In this analysis, virulence level in respect to 3 stress conditions (heat, pH and UV exposure) was considered. In this study, *Staphylococcus aureus* and *Staphylococcus epidermidis* has higher virulence as compared to *Streptococcus sp., Micrococcus sp., E. coli, Klebsiella sp.* The results are documented in [Table/Fig-5].

Antibiotics	*Staphylococcus Epidemidis*	*Staphylococcus Aureus*	*Streptococcus Sp.*	*E. Coli*	*Micrococcus Roseus*
Chloramphenicol	1.3	1.3	1.5	1.7	1.5
Ciprofloxacin	1.2	1.7	1.8	1.8	2.2
Tetracycline	1.4	1.2	1.1	1.3	1.1
Polymyxin B	0.9	0.7	0.6	0.6	0
Rifampicin	0.9	0.7	1	0.8	1
Cotrimoxazole	0	0	0	1.5	0
Cephalexin	1.5	1.7	1.5	1.2	1
Bacitracin	0.5	0.7	0.6	0.7	0.4
Moxifloxacin	2.8	2.2	2.3	2.55	2.7
Tobramycin	2.4	1.8	1.9	2.2	1.7
Gatifloxacin	2	1.8	2.1	2.4	2.1

[Table/Fig-3]: Susceptibility data towards antibiotics in diabetic patients.
Table shows the average susceptibility number (%)

Antibiotics	*Staphylococcus Epidemidis*	*Staphylococcus Aureus*	*Streptococcus Sp.*	*E. Coli*	*Micrococcus Roseus*
Chloramphenicol	1.4	1.6	1.4	0.8	1.4
Ciprofloxacin	1.7	1.8	1.7	0.6	2
Tetracycline	1.2	1.3	0.9	0.6	1.8
Polymyxin B	0.85	1	0.7	0.65	0.7
Rifampicin	0.8	0.75	0.8	1.1	1.2
Cotrimoxazole	0	0	0	1.3	0
Cephalexin	1.6	1.8	1.2	1.3	1.2
Bacitracin	0.6	0.5	0.45	0.75	0.6
Moxifloxacin	2.4	2	2.6	2.2	2.2
Tobramycin	2.6	2.1	1.95	2	2.2
Gatifloxacin	2.2	1.7	2.4	2.2	1.9

[Table/Fig-4]: Susceptibility data towards antibiotics in non diabetic patients.
Table shows the average susceptibility number (%)

Organisms	Heat	UV exposure	pH	Total virulence level	Virulence status
Stapylococcus aureus	+	0	0	+	Moderately high
Staphylococcus epidermidis	+	+	0	++	High
Streptococci sp	0	0	-	-	Moderately less
Micrococcus roseus	0	-	-	--	Less
E. coli	_	-	-	---	Very less
Klebsiella pneumoniae	0	0	-	-	Moderately less

[Table/Fig-5]: Stress condition sensitivity of the organisms.
+++ very high virulence 0 same growth - moderately less virulence; ++ High virulence -- less virulence; + Moderately high virulence --- very less virulence

DISCUSSION

In this study, total 100 patients (30 diabetic and 70 non diabetics) were examined and their conjunctival flora was compared. The present study showed that *staphylococcus aureus* is the most common isolate in both the diabetic and non diabetic group, as is found by Adam M et al., [8]. Ashtamkar S et al., found *Staphylococcus epidermidis* to be the most common organism (13.2%) isolated from diabetics [9]. Study by Venkataraman M et al., showed coagulase negative Staphylococci as the most common organism in diabetics [10]. Similar results were found in other studies [11-14]. Rajeshkannan R et al., found that gram negative organisms were more common among diabetics [15] which was opposite with the present study.

This study shows the prevalence of positive culture among diabetic patients that may be due to altered chemotaxis, adherence, phagocytosis, intracellular killing and bactericidal activity found in diabetes [16-18]. Study by Rajeshkannan R et al., showed that diabetics had a positive culture of 68% as compared to non diabetics [15]. Another study showed that the microbial growth in diabetic patients was 62.27% compared to 46.67% in non diabetics [19]. Similar result was also found in study by Martins EN et al., [14]. In the same study, age and gender had no effect on frequency of culture.

It was found that the frequency of gram negative bacteria was four times higher in diabetics with chronic rhinosinusitis than in non diabetic patients [20]. In the present study, *Klebsiella pneumoniae* was most common among gram negative in diabetic patients (23.3%). In study done by Lim HW et al., the percentage was found to be 45.61% [21].

Previously several authors carried out antibiotic sensitivity tests. Long C et al., did antibiotic susceptibility analysis of microbes isolated between different time periods and showed that ciprofloxacin was the most effective against bacterial isolates, followed by cefoperazone during the first decade (1990-1999), but during the second decade (2000-2009) ceftazidime showed the greatest level of activity against most bacterial isolates, followed by cefuroxime. They also showed several gram negative organisms like *P aeruginosa, B. proteus* and *E. coli* were multidrug (gentamycin, neomycin, chloromycetin, ofloxacin) resistant [22]. Study by Ashtamkar S et al., found *Staphylococcus epidermidis* was sensitive to gentamycin, vancomycin, linezolid [9].

Rajeshkannan R et al., studied antibiotic resistance to erythromycin, ampicilin, cephalosporin. They found that diabetics were more resistant to all three antibiotics (22.7%) compared to non diabetics (16.6%). Resistance of diabetics to erythromycin and ampicilin is 47.7% compared to non diabetics that is 36.6% [15]. In another study, it was found that the gram positive *staphylococci sp.* has maximum resistance against erythromycin (38%) among the diabetic group and in the non diabetic group maximum resistance was seen with ampicillin (30%). The gram negative bacilli such as *pseudomonas* showed maximum resistance against ampicillin in both the groups (56%) [1]. In the present study, usually topical antibiotics were preferred for ocular application due to its greater local concentration. Also, drops were more preferred over ointments normally as ointments were sticky and may hamper vision just after applying it. Hence, use of eye drops of antibiotics like moxifloxacin can be potential in clinical settings.

Limitation(s)

It was done on a limited number of patients over a short period of time. The study on the susceptibility of the organisms to stress conditions has to be done on a larger scale for better comparison. Authors plan to further continue this study on a larger scale.

CONCLUSION(S)

The diabetic patients were more prone to ocular infection and hence it can be concluded that they must receive a more stringent antibiotic prophylaxis. Secondly, the study showed that the organism isolated from both the diabetic and the non diabetic patients were more sensitive to moxifloxacin, tobramycin and gatifloxacin. Hence, authors recommend the use of these drugs in antibiotic prophylaxis of patients before planned surgery.

REFERENCES

[1] Suresha K, Mandava K, Kindo AJ. Conjunctival flora in diabetics & normal population-a comparative study. SRJM. 2014;7:01-04.
[2] Manav G, Bilgin L, Gezer A. Conjunctival flora in normal population. Turk J Ophthalmol. 1992;12:121-24.
[3] Coskun M, Kocak AG, Simavli H, Anayol MA, Toklu Y, Celikbilek N. Analyzing normal conjunctival flora and detecting antibiogram sensitivity to fluroquilones and penicillin derivatives. Glo-Kat. 2007;2:167-70.
[4] Speaker M, Milch FA, Shah MK, Eisner W, Kreiswirth BN. Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology. 1991;98(5):639-49.
[5] Cohen SM, Flynn HW JR, Murray TG, Smiddy WE. Endophthalmitis after pars plana vitrectomy, the post vitrectomy endophthalmitis study group. Ophthalmology. 1995;102(5):705-12.

[6] Doft BH, Wisniewski SR, Kelsey SF, Groer-Fitzgerald S. Endophthalmitis Vitrectomy Study Group. Diabetes and post cataract extraction endophthalmitis. Curr Opin Ophthalmol. 2002;13(3):147-51.
[7] Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized disc method. Amer J of Clin Path. 1966;45(4):493-96.
[8] Adam M, Balci M, Bayhan HA, Inkaya AC, Uyar M, Gurdal C. Conjunctival flora in diabetic and non diabetic individuals. Turk J Ophthalmology. 2015;45(5):193-96.
[9] Ashtamkar S, Maheshgauri R, Vadodaria B, Motwani D, Sharma A. Analysis of conjunctival flora in diabetic and non diabetic individuals and their antibiotic sensitivity pattern. Indian J of Clinical and Experimental Ophthalmology. 2020;6(1):138-44.
[10] Venkataraman M, Krishnagopal S, Sivaraman U, Ramachandran RA. Spectrum of conjunctival bacterial flora in non-insulin dependent Diabetics. Indian J Sci Stud. 2015;3(1):133-36.
[11] Tahir MA, Saleem Q, Saeed I, Manzoor AM. Aerobic bacterial conjunctival flora in diabetic patients. Pak J Ophthalmol. 2010;26(4):177-81.
[12] Nahar N, Anwar S, Miah M. Conjunctival bacterial flora in diabetic patients. Ibrahim Medical College Journal. 2014;7(1):05-08.
[13] Capriotti J, Pelletier J, Shah M, Caviano DM, Ritterband DC. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. International Ophthalmology. 2008;29(2):81-84.
[14] Martins EN, Alvarenga LS, Höfling-Lima AL, Fretas D, Zorat-Yu MC, Farah ME. Aerobic bacterial conjunctival flora in diabetic patients. Cornea. 2004;23(2):136-42.
[15] Rajeshkannan R, Venkatesan MJ, Ezhilvendhan K, Rao AVR. A study to compare the conjunctival flora of non diabetic individuals with that of diabetic patients. Tro J Ophtha & Oto. 2019;4(1):48-54.
[16] Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. Diabet Med. 1997;14(1):29-34.
[17] Cason L, Winkler CH. Bacteriology of the eye. AMA Arch Ophthalmol. 1954;51(2):196-99.
[18] Smith CH. Bacteriology of healthy conjunctiva. Br J Ophthalmol. 1954;38(12):719-26.
[19] Muralidhar C A, Khaja Moinuddin S, Anadi V. Significance of normal conjunctival flora in diabetic versus healthy individuals. Trop J Ophthalmol Otolaryngol (internet). 2019;4(1):55-59.
[20] Zhang Z, Adappa ND, Lautenbach E, Chiu AG, Doghramji L, Howland TJ, et al. The effect of diabetes mellitus on chronic rhinosinusitis and sinus surgery outcome. Int Forum Allergy Rhinol. 2014;4(4):315-20.
[21] Lim HW, Shin JW, Cho HY, Kim HK, Kang S, Song SJ, et al. Endogenous endophthalmitis in the Korean population: A six-year retrospective study. Retina. 2014;34(3):592-602.
[22] Long C, Liu B, Xu C, Jing Y, Yuan Z, Lin X. Causative organisms of post-traumatic endophthalmitis: A 20-year retrospective study. BMC Ophthalmol. 2014;14:34.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Ophthalmology, Raiganj Government Medical College, Raiganj, West Bengal, India.
2. RMO Cum CT, Department of Ophthalmology, Burdwan Medical College and Hospital, Burdwan, West Bengal, India.
3. Associate Professor, Department of Ophthalmology, KPC Medical College and Hospital, Kolkata, West Bengal, India.
4. Associate Professor, Department of Ophthalmology, Burdwan Medical College and Hospital, Burdwan, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sandip Samaddar
179,28/C, Santosh Roy Road, Kolkata-700008, West Bengal, India.
E-mail: drsandipSamaddar@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 30, 2020
- Manual Googling: May 21, 2021
- iThenticate Software: Jun 09, 2021 (9%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 26, 2020**
Date of Peer Review: **Dec 28, 2020**
Date of Acceptance: **May 25, 2021**
Date of Publishing: **Jul 01, 2021**