ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

THE ANALYSIS OF CONJUNCTIVAL FLORA IN DIABETIC AND NON-DIABETIC INDIVIDUALS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN

Dr. Akash Shrivastav, Dr. Brijesh Singh Yadav, Dr. Shailendra Kumar, and Dr. Pyoosh Kumar Pandey

1. Dr. Akash Shrivastav, Assistant Professor,Department of Opthalmology, RDMC, Banda, UP, India
2. Dr. Brijesh Singh Yadav, Consultant, MS Ophthalmology, Manohar Das Eye Hospital (RIO), Lucknow, UP, India, dr.brijmln@gmail.com
3. Shailendra Kumar, Consultant, MS Ophthalmology, Madan Mohan Malviya Eye Hospital Fatehpur, Fatehpur, UP, India, s.aarav100@gmail.com
4. Dr. Pyoosh Kumar Pandey, MS ophthalmology, eye surgeon, Manohar Das Eye Hospital (RIO), Lucknow, UP, India, drpyooshpandey@gmail.com

Corresponding author:

Dr. Pyoosh Kumar Pandey, MS ophthalmology, eye surgeon, Manohar Das Eye Hospital (RIO), Lucknow, UP, India, drpyooshpandey@gmail.com

Abstract

Background: The conjunctiva is a transparent mucous membrane lining the internal surfaces of the eyelids and the orbital globe. The surface is covered with stratified, non-keratinized epithelial cells and functions as a barrier against infection. The conjunctival flora is found on the ocular surface of healthy individuals and, under normal conditions, comprises noninfectious microorganisms. These microorganisms have an important role in the maintenance. **Aim and Objective:** To analyze the conjunctival bacterial and fungal flora of non-diabetic and diabetic individuals and their antibiotic sensitivity patterns. **Material and Method:** The study group consisted of 80 patients each with diabetes and non-diabetics attending the Department of Ophthalmology, Rani Durgawati Medical College, Banda, Uttar Pradesh. The conjunctival swabs were collected from both eyes and sent to the microbiology lab for smears and culture. The antibiotic sensitivity test was done using Kirby Baeur's method. **Results:** The conjunctival flora of a total of 80 patients, 40 diabetic and 40 non-diabetic, was screened. Among the 80 patients, there were 42 males and 38 females. The most common isolated bacteria in diabetic and non-diabetic groups were Staphylococcus aureus (diabetic 70%, non diabetic 87.5%). There was a statistically significant difference in microbial flora pattern between the diabetic and non-diabetic groups. There was a 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-diabetic patients (p-value <0.01).

Conclusion: The diabetic patients were more prone to ocular infection, and hence it can be concluded that they must receive a more stringent antibiotic prophylaxis.

Keywords: conjunctival flora, diabetic smellitus, gram-negative bacteria, antibiotic sensitivity

Introduction

The conjunctiva is a thin, transparent, smooth, and humid membrane that covers the sclera and inner surface of the eyelids [1]. The conjunctiva is kept moist and healthy by tears, which contain lysozyme, IgA, IgG, lysine, lactoferrin, complement, and multiple antibacterial enzymes [2]. In a healthy person, surface tissues such as skin and mucous membranes are constantly in contact with environmental organisms and becomes colonized by various micro-organisms, which are referred to as normal flora [3]. Bacteria and fungi are considered members of the normal flora of conjunctiva, whereas viruses and parasites are not considered members of the normal flora [4]. The conjunctival flora is altered under special circumstances, as in newborns, acquired immune-deficient patients, contact lens wearers, and patients using immune suppressive drugs. [5], Neisseria gonorrhoea, and Chlamydia trachomatis also infect conjunctiva. Staphylococcus epidermidis and certain corny forms,including Propionibacterium acnes, are the most common. [6]

Type 2 diabetes is a complicated disease that affects many aspects of the eye. [7] Diabetic people are at a higher risk of developing postoperative endophthalmology than non-diabetic ones [8].. The majority of pathogenic bacteria implicated in postoperative endophthalmitis are found in the conjunctiva flora, lids, and even the nasal mucosa. [9] Given the rising prevalence of diabetes, every surgeon in India is dealing with patients with diabetes for cataracts. The pathogenicity of these microorganisms forming the flora affects the development of conjunctivitis. As a harmful bacterium, S. aureus may be found in the conjunctival flora. The most common agent identified in neonatal bacterial conjunctivitis is Staphylococcus aureus. Patients with diabetes mellitus are prone to infection because glucose in the skin, urine, mucous membranes, and tears promotes the growth of microorganisms. Conjunctival flora Neisseria gonorrhoeae and Chlamydia trachomatis play a pathogenic role when immune function is compromised, which can lead to serious infection. [10-11] People with diabetes, due to potential ocular complications, represent a group at risk of ocular infections. Furthermore, Staphylococcus a saprophyticus remained the most common infection in this group, with an increase in Staphylococcus identified in cultures from individuals with proliferative diabetic retinopathy. Various studies have shown that positive conjunctival flora culture varies from 16.6% to 65% [12]. Suto et al. found that gram-positive cocci formed 67% of all isolates [13]. The conjunctival flora in diabetic subjects may differ from that in non-diabetic subjects. Therefore, this study was done to analyze the conjunctival flora of diabetic and non-diabetic individuals and their antibiotic sensitivity patterns.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

Materials and Methods

The study group consisted of 80 patients each with diabetes and non-diabetics attending the outpatient ophthalmology department of the Rani Durgawati Medical College, Banda, Uttar Pradesh. After obtaining informed consent, conjunctival swabs with two-moistened sterile cotton-tipped applicators without touching eyelid margins or lashes were collected from both eyes of the patient separately and sent for microbiology, one for culture and the other for microscopy. [5] The swabs were inoculated in thioglycollate broth over night, and gram staining was done from the broth. They were also cultured in blood agar and MacConkey's agar. The organs were identified by standard microbiological procedures. The antibiotic sensitivity test was done using Kirby-Baeur's method.

Inclusioncriteria:

All genders were included, diabetic patients were included, and non-diabetic patients were included.

Exclusion criteria:

Patients with disease other than diabetes**,** To exclude infective condition of the eye and Patients not willing to give sample

Antibiotic susceptibility tests:

Antibiotic susceptibility testing was done on Mueller-Hinton agar by the Kirby-Bauer diffusion method using Clinical Laboratory Standards Institute (CLSI) guidelines. It allows the antibiotic to diffuse into the medium. If the organism is susceptible to a particular antibiotic, the growth of the organism is inhibited around the disc. This is called the zone of inhibition. When there is no inhibition, the organism is said to be resistant. The zone diameter was interpreted according to the CLSI chart. The zone diameter was measured in millimeters (mm). The results were interpreted as sensitive, resistant, and intermediately sensitive according to the CLSI chart.

Results

A total of 80 patients were screened to study the conjunctival flora in diabetic and non-diabetic individuals. The study group consisted of 40 diabetic patients and 40 non-diabetics. The age varied from 17 to 85 years. 42 were males and 38 were females, and their mean age was 53.5 (±20.5) years.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

Table 1: Culture findings of the conjunctiva flora among the study subjects

Name of the organisms	Diabetic (n = 40) (%)	Non-diabetic (n = 40) (%)	p-value
Staphylococcus aureus	28(70)	**35(87.5)**	**0.561**
Staphylococcus epidermidis	**16(40)**	**5(12.5)**	**0.005**
Streptococci sp.	**6(15)**	**4(10)**	**0.496**
Micrococcus roseus	**5(12.5)**	**3(7.5)**	**0.453**
E. coli	**2(5)**	**5(12.5)**	**0.23**
Klebsiella pneumoniae	**8(20)**	**6(15)**	**0.552**
Fungal growth	**5(12.5)**	**3(7.5)**	**0.453**

The microbial isolates are detailed in Table 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 (40%) as compared to non-diabetic (12.5%) patients (p-value = 0.005). There was a higher proportion of Staphylococcus aureus among non-diabetic patients (87.5%) as compared to diabetic patients (70%) (p-value = 0.561).

Table No. 2: Suceptibility data towards antibiotics in non-diabetic patients. Table 1 shows the average susceptibility number (%).

Antibiotics	Diabetic (n = 40) (%)	Non-diabetic (n = 40) (%)	p-value
Co-Trimoxazol	32(80)	**28(70)**	**0.303**
Doxycycline	**30(75)**	**27(67.5)**	**0.459**
Erythromycin	**14(35)**	**22(55)**	**0.718**
cefoxitin	**25(62.5)**	**22(55)**	**0.496**
ciprofloxacin	**24(60)**	**18(45)**	**0.18**

The sensitivity pattern of Staphylococci aureus showed maximum sensitivity to Co-Trimoxazol (80%) and Doxycycline (75%) in the diabetic group, whereas in the non-diabetic group it was 70% and 67.5%. Staphylococcus aureus showed maximum resistance to Erythromycin (55% in the non-diabetic group), whereas in the non-diabetic group it was 35%..

In the present study, it was found that the gram-positive Staphylococci sp. has maximum resistance against erythromycin (75%), cefoxitin (62.5%), and in the non-diabetic group, maximum resistance was seen with ciprofloxacin and doxycycline (45% and 67.5% respectively).

Table 3: Stress condition sensitivity of the organisms.

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

Note: +++ very high virulence 0 same growth—moderately less virulence; ++ high virulence—less virulence; + moderately high virulence—very little virulence

The results of the stress condition sensitivity test can be represented by an 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 with respect to three stress conditions (heat, pH, and UV exposure) was considered. In this study, Staphylococcus aureus and Staphylococcus epidermidis had higher virulence as compared to Streptococcus sp., Micrococcus sp., E. coli, and Klebsiella sp. The results are documented in [Table 3].

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

Discussion

In this study, a total 80 patients (40 diabetic and 40 non-diabetic) were examined, and their conjunctival flora was compared. In this study, the conjunctival flora of diabetic patients and healthy individuals were compared. Specimens obtained for this purpose were seeded on different media. The diabetic group showed no significant difference in frequency of bacterial growth compared to the control group but yielded fewer gram-negative bacteria cultures. Although the conjunctival flora forms a defensive barrier against infection, it also includes major pathogens of ocular infections. In healthy individuals, the conjunctival flora is frequently comprised of the same microorganisms as the skin flora. [14] Gram-positive bacteria constitute the main elements of bacterial floral, though the positive culture rate and microorganisms grown show diversity. [15-16]

The present study showed that Staphylococcus aureus is the most common isolate in both the diabetic and non-diabetic groups, as found by Adam M et al. [16]. Ashtamkar S. et al. found Staphylococcus epidermidis to be the most common organism (13.2%) isolated from diabetics [17]. A study by Venkataraman M et al. showed coagulase-negative Staphylococci as the most common organism in diabetics [18]. Similar results were found in other studies [19]. Rajeshkannan R et al. found that gram-negative organisms were more common among diabetics [20], which was opposite from the present study.

This study shows the prevalence of positive culture among diabetic patients, which may be due to altered chemotaxis, adherence, phagocytosis, intracellular killing, and bactericidal activity found in diabetes [21]. A study by Rajeshkannan R et al. showed that diabetics had a positive culture of 68% as compared to non-diabetics [20]. Another study showed that the microbial growth in diabetic patients was 62.27% compared to 46.67% in non-diabetics [22].

A similar result was also found in a study by Martins EN et al. [23]. In the same study, age and gender had no effect on the 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 [24]. In the present study, Klebsiella pneumoniae was most common among gram-negative diabetic patients (20%). In a study done by Lim HW et al., the percentage was found to be 45.61% [25]. 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.

It was thought that normal ocular flora could be non-pathogenic or occasionally pathogenic. However, the pathogens of some bacterial endophthalmitis, bacterial corneal ulcers, blepharitis, conjunctivitis, and other ocular infection diseases turned out to be consistent with conjunctival isolated bacteria, and S. epidermidis has become the predisposing pathogen [26]. Although preoperative topical antibiotics can hardly make the conjunctival sacs sterile, they can effectively reduce the isolating bacteria [27]. The susceptibility of each antibiotic to different bacteria is not identical, and the regionally reported drug resistance varies widely due to different environments and the use of antibiotics [28].

Evolving bacterial resistance represents one of the most serious global public health problems, and overcoming this problem has become a great challenge. Due to different ethnic groups, environments, and antimicrobial therapies, the distribution and resistance profiles of conjunctival bacteria vary significantly from area to area. Therefore, the investigation of these subjects can be clinically useful in the primary empirical antimicrobial strategy before knowing the laboratory results.

Limitations

It was done on a limited number of patients over a short period of time. The study on The Analysis of Conjunctival Flora in Diabetic and Non-Diabetic Individuals and Their Antibiotic Sensitivity Pattern has to be done on a larger scale for better comparison. The authors plan to further continue this study on a larger scale.

Conclusion:-

A higher positive culture rate was seen in diabetic individuals as compared to non-diabetic individuals. The diabetic patients were more susceptible to ocular infections. Diabetic individuals are at a higher risk of developing other ocular infections as they are more likely to have a positive culture rate of microorganisms. The observations of the present study and various other studies infer that staphylococcal infections are common among both diabetic and non-diabetic individuals. Gram-negative bacteria Escherichia coli and Klebsiella pneumoniae were detected in higher ratios in the conjunctival flora of diabetic patients. Candida albicans was more common among diabetics as compared to non-diabetics. Conjunctival flora can become a pathogen in diabetic patients following any injury to the eye. Overuse of antibiotics should be restricted to prevent resistance among the bacteria.

Reference

1. Eye Health. Conjunctiva. American Academy of Ophthalmology, March-28, 2018.
2. Tears. Medline Plus, October-23, 2018.
3. Labtests online. Normal Flora. American Association for Clinical Chemistry, July-10, 2017.
4. UK ESSAYS. Normal Flora and their Benefit Feb- 22, 2017.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

5. Coskun M, Kocak AG, Simavli H, et al. ormal conjunctival flora and detecting antibiogram sensitivity to fluroquilones and penicillin derivatives. Glo-Kat. 2007; 2: 167-70.
6. Najmun Nahar, Ripon Baura, Sultana Razia, et al. Conjunctival Bacterial flora in healthy individuals and health care workers. (HCWs). Bangladesh J Med Microbiol 2012; 06:15- 19.
7. Jawetz E, Melnnick LJ, Adelberg AE. Medical Microbiology (18th) ed. Prentice Hall International, USA. 1989; 18: 275–278.
8. Kowalski PR and Roat IM. Normal flora of the human conjunctiva. In: Tasman W. and Jaeger E.A., eds. Duane's Ophthalmology. Lippincott William and Wilkins, Philadelphia 2006; Volume 2.
9. Coşkun M, Koçak AG, Simavlı H, et al. Analyzing normal conjunctival flora and detecting antibiogram sensitivity to flouroquinolones and penicillin derivates. Glo-Kat. 2007; 2:167–170.
10. Starr MB, prophylactic antibiotic for ophthalmic surgery, Surv of Ophthalmology, 1983; 27:353–73.
11. MillerB, Allis p. conjunctival flora in patients receiving immunosuppressive drugs, arch opthalmol 1977; 95: 2012-4.
12. Kumar MA, Kurien SS, Selvaraj S, Devi SU, Selvasundari S. Comparison of different techniques of cataract surgery in bacterial contamination of the anterior chamber in diabetic and non-diabetic population. Indian J Ophthalmol 2012;60:41-4.
13. Suto C, Morinaga M, Yagi T, et al. Conjunctival sac bacterial flora isolated prior to cataract surgery. Infect Drug Resist. 2012;5:37-41. doi: 10.2147/IDR. S27937. Epub 2012 Jan 24.
14. McNatt J, Allen SD, Wilson LA, Dowell VR., Jr Anaerobic flora of the normal human conjunctival sac. Arch Ophthalmol. 1978;96:1448–1450.
15. Manav G, Bilgin L, Gezer A. Conjunctival flora in normal population. Turk J. Ophthalmol. 1992; 12:121–124.
16. Coşkun M, Koçak AG, Simavlı H, et al. Analyzing normal conjunctival flora and detecting antibiogram sensitivity to flouroquinolones and penicillin derivates. Glo-Kat. 2007; 2:167–170.
17. 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.
18. Ashtamkar S, Maheshgauri R, Vadodaria B, et al. Analysis of conjunctival flora in diabetic and non-diabetic individuals and their antibiotic sensitivity patterns. Indian Journal of Clinical and Experimental Ophthalmology. 2020;6(1):138-44.
19. Venkataraman M., Krishnagopal S., and Ramachandran RA. Spectrum of conjunctival bacterial flora in non-insulin-dependent diabetics Indian J Sci Stud. 2015;3(1):133-36.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 05, 2024

20. 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.
21. Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, and Genetet B. Impaired leucocyte functions in diabetic patients. Diabet Med. 1997;14(1):29–34.
22. Muralidhar C. A., Khaja Moinuddin S., and Anadi V. The significance of normal conjunctival flora in diabetic versus healthy individuals. Trop J Ophthalmol Otolaryngol (internet). 2019;4(1):55-59.
23. Martins EN, Alvarenga LS, Höfling-Lima AL, et al. Aerobic bacterial conjunctival flora in diabetic patients. Cornea. 2004;23(2):136-42.
24. Zhang Z, Adappa ND, Lautenbach E, Chiu AG, Doghramji L, Howland TJ, et The effect of diabetes mellitus on chronic rhinosinusitis and sinus surgery outcome. Int Forum Allergy Rhinol. 2014;4(4):315-20.
25. Lim HW, Shin JW, Cho HY, et al. Endogenous endophthalmitis in the Korean population: A six-year retrospective study. Retina. 2014;34(3):592-602.
26. Carreras B. Bacteriological analysis in the management of conjunctivitis. Comparison of antibiotic resistance between 1982 and 2008. Arch. Soc. Esp. Oftalmol. 2012;87, 107–111
27. Mshangila B., Paddy M., Kajumbula H., et al. External ocular surface bacterial isolates and their antimicrobial susceptibility patterns among preoperative cataract patients at Mulago National Hospital in Kampala, Uganda. BMC Ophthalmol. 2013 Nov 15; 13:71.
28. Muluye D, Wondimeneh Y, Moges F, et al. Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. BMC Res Notes. 2014 May 12;7:292.