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Antibiotic Susceptibility Profiles of Conjunctival Flora in Diabetic and Non-Diabetic Individuals

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Abstract

The normal flora of the eye plays an important role in maintaining ocular homeostasis by various mechanisms. They primarily consist of bacteria, which under normal circumstances do not cause infection, but which can be a major source of infection following ocular surgery, trauma, or in people who are immune-compromised. Patients with diabetes mellitus (DM) are prone to infection because glucose in the skin, urine, mucous membranes and tears promotes growth of microorganism. To assess antibiotic sensitivity of conjunctival flora in diabetic and non- diabetic individuals. This case control study was carried out on 120 eyes of 60 patients, divided into 2 groups 30 diabetic patients (group A) and 30 non-diabetic patients (group B) attending ophthalmology outpatients' clinic at Al Zahraa University Hospital. History was taken and all participants had undergone a thorough ophthalmic evaluation. A specimen was taken from inferior palpebral conjunctiva of both eyes. Isolated micro-organisms were identified using routine microbiological methods. The rate of positive culture in group A was significantly higher as compared to group B. It was noted that there was a significantly higher incidence of staphylococcus epidermidis in group A as compared to group B. Maximum sensitivity was seen with fluoroquinolones (ciprofloxacin 97.4% and levofloxacin 100%). A higher positive culture rate was seen in diabetic individuals as compared to non-diabetic individuals. Fluoroquinolones (Ciprofloxacin and levofloxacin) are the drugs of choice and can be considered as routine pre-operative topical medication.

Keywords: Conjunctival flora, Diabetes mellitus, Gram- positive bacteria, Culture sensitivity, Antibiotic sensitivity, Glycosylated hemoglobin.

1. Introduction

The conjunctiva is the mucous membrane that lines the inside of the eyelids and extends all the way to the orbital globe, and the conjunctival sac is the space between the palpebral and bulbar conjunctiva [1]. Because of its constant exposure to the outside environment, the conjunctival sac can harbour both normal commensal flora and potential pathogens [2]. The normal commensal flora can protect the host either by occupying potential colonization sites of pathogens, producing antimicrobial products or stimulating an immune response that protects the host against infection [2]. Diabetes mellitus (DM) is a chronic metabolic disorder marked by high blood glucose levels caused by absolute or relative insulin deficiency in the context of cell dysfunction, insulin resistance, or both [3]. When immune function is compromised, some members of the conjunctival flora play a pathogenic role in DM, which can lead to serious infection [4]. A high glucose level creates an ideal environment for microbial growth, which may explain why diabetic patients have a diverse ocular surface microbiota [5].

Cultures are a primary diagnostic method in microbiology and are used to determine the cause of infectious disease by allowing the agent to multiply in a predetermined medium [6]. Therefore, understanding and monitoring the distribution of conjunctival bacteria are important in preoperative, peri-operative and post-operative management [6]. The spectrum of bacteria covered, the rapidity with which the antibiotic eliminates bacteria from the conjunctival surface, the duration of action, the antibiotic's penetration and toxicity, the antibiotic susceptibility pattern, and the cost all influence the choice of prophylactic topical antibiotic [7].

Up to now, the treatment and prevention of conjunctival bacterial infection are mainly based on the use of antibiotics. However, with the extensive use of antibiotics, the resistance of bacteria towards antibiotics in conjunctiva and conjunctival sac is increasing [8]. The current study was conducted to assess antibiotic sensitivity of conjunctival flora in diabetic and non-diabetic individuals.

2. Patients and Methods

A comparative, case control study was conducted at the Ophthalmology and Clinical Pathology Departments of AL-Zahraa University Hospital, Faculty of Medicine (For Girls), Al-Azhar University, Egypt, in the period from April 2022 to September 2022. This study included 120 eyes of 60 participants ranging in age from 40 to 70 years. The participants were divided into 2 groups 30 diabetic patients (group A) and 30 non-diabetic patients (group B).

2.1 Inclusion criteria:

- Both sexes were included.
- Individual’s age range 40-70 years old were included.

2.2 Exclusion criteria

Patients with active signs and symptoms of ocular infection or inflammation. Patients with history of topical or systemic antibiotics within last 1 week of inclusion date. History was taken and all participants had undergone a thorough ophthalmic evaluation. Best corrected visual acuity measurement using the Landolt visual acuity chart, which was converted to decimal notation for statistical analysis. External examination of the eye lid and conjunctiva. anterior segment examination using slit lamp biomicroscopy (Topcon, Corporation, Tokyo,Japan). Posterior-segment examination using slit lamp biomicroscopy with 90 D lens then diabetic patients were divided into nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) according to presence of neovascularization. Complete blood count (cbc) and glycosylated haemoglobin (HbA1c). investigations were done.

The specimen was taken from each participant for the study by rubbing a sterile cotton tipped swab on the inferior palpebral conjunctiva of both eyes. The lower eyelid was pulled down in order to prevent contamination of the cotton swabs by the lid margins and eyelashes.

Conjunctival samples were immediately inoculated on blood agar, macConky Figure (1), chocolate and Sabouraud

dextrose agar plates. Cultures were incubated at 37 degrees centigrade for 24 hours on blood agar, macConky and chocolate agar with use of carbon dioxide (co2) incubator for chocolate agar. Some of their growths were gram positive cocci, staphylococci Figure (2) and gram-positive bacilli, diphteroid Figure (2).
Two Sabouraud dextrose agar plates were also inoculated immediately and incubated for two weeks, one week at 25 degrees centigrade and the other at 37 degrees centigrade.
The modified Kirby bauer method was used for antibiotic susceptibility testing and interpreted according to clinical laboratory Standard institute (CLSI, 2022) guidelines [9].
The broth microdilution method was used for antibiotic susceptibility testing in case of diphtheroid isolates and in susceptibility of staphylococci to vancomycin then results interpreted according to CLSI guidelines.

2.3 Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 24 (SPSS Inc,Chicago, United States of America (USA). Quantitative data were expressed as mean ±SD. Qualitative data were expressed as frequency and percentage. Mean (average): the central value of a discrete set of numbers, specifically the sum of values divided by the number of values. Standard deviation (SD): is the measure of dispersion of a set of values. A low SD indicates that the values tend to be close to the mean of the set, while a high SD indicate that the values are spread out over a wider range.

The following tests were done:

- Independent sample T test (T): when comparing between two means (for normally distributed data).
- Mann Whitney U test (MW): when comparing between two means (for abnormally distributed data).
- Chi-square test: was used when comparing between non-parametric data.
- Probability (P-value)
  - P-value < 0.05 was considered significant.
  - P-value < 0.001 was considered as highly significant.
  - P-value > 0.05 was considered insignificant.

3. Results

As shown in Table .1, this study was done for 120 eyes of 60 patients classified into 2 groups as follows: group A (Diabetics) 30 patients and group B (Non-Diabetics)–30 patients. The average age was (55.3 ± 9.0) (range 40-70 years) in group A and (52.6 ± 9.3) (range 40-7 years) in group B. Group A included 11 (36.7 %) males and 19 (63.3 %) females, group B included 9 males (30 %) and 21 females (70 %). There were no significant differences between the groups in terms of demographic characteristics. There was a highly statistically significant (p-value < 0.001) increased HbA1C in group A (7.8 ± 0.8) when compared with group B (5.6 ± 0.4). There was a highly statistically significant (p-value = 0.001) decreased BCVA in group A 0.3 (0.1 - 0.6) (range 0.01 – 1) when compared with group B 0.6 (0.3 - 0.6) (range 0.01 – 1).
As shown in table 2 there was a statistically significant (p-value = 0.001) increased percentage of positive culture results in group A (38 patients,63.3%) when compared with group B (20 patients, 33.3%). There was no statistical significant differences (p-value > 0.05) between studied groups (group A and group B) as regard isolated organisms (*coagulase-negative staphylococci*(CONS), Staph

aurous, diphteroid, methicillin resistant coagulase negative staphylococci(MR-CONS)and *methicillin resistant staphylococcus aureus*(MRSA)) except for asperigellus fumigtus, there was statistically significant (p-value = 0.042) increased percentage of isolated asperigellus fumigtus in group A (4 patients, 10.5%) when compared with group B (0 patients, 0%). As shown in table 3 there was no statistical significant differences (p-value > 0.05) between NPDR and PDR patients of group A as regard isolated organisms (CONS, staph aurous, MR-CONS, and asperigellus fumigtus) except for diphteroid, there was statistically significant (p-value = 0.016) increased percentage of isolated diphteroid from PDR patients (3 patients, 37.5%) when compared with NPDR patients (1 patients, 4.3%).

As show in table 4 there was statistically significant (p-value = 0.025) increased percentage of sensitivey to ciprofloxacin in group A (37 patients, 97.4%) when compared with group B (16 patients, 80%), decreased percentage of sensitivey to doxycycline in group A (31 patients, 81.6%) when compared with group B (20 patients, 100%) and increased percentage of sensitivey to azithromycin in group A (23 patients, 60.5%) when compared with group B (6 patients, 30%).

Table (1): Comparison between studied groups as regards demographic and clinical data.

		Group A (N = 30)	Group B (N = 30)	T. test	P-value
Age (years)	Mean	55.3	52.6	MW = 373.5	0.257 NS
	±SD	9.0	9.3		
Sex	Male	11 36.7%	9 30%	X^2 = 0.3	0.584 NS
	Female	19 63.3%	21 70%		
HbA1C (%)	Mean	7.8	5.6	13.03	< 0.001 HS
	±SD	0.8	0.4		
BCVA	Median (IQR)	0.3 (0.1 - 0.6)	0.6 (0.3 - 0.6)	-3.367	0.001 HS
	Range	0.01 – 1	0.1 – 1		

T: independent sample T test, NS: p-value > 0.05 is considered non-significant and HS: p-value < 0.001 is considered highly significant.

Table (2): Comparison between studied groups as regards culture and organisms' results.

		Group A (N = 60 eye)		Group B (N = 60 eye)		X^2	P-value
Culture results	Negative	22	36.7%	40	66.7%	10.8	0.001 S
	Positive	38	63.3%	20	33.3%		
Organism	CONS	18	47.4%	11	55%	2.22	0.136 NS
	Staph aurous	6	15.8%	8	40%	0.32	0.570 NS
	Diphteroid	7	18.4%	2	10%	3.0	0.083 NS
	MR-CONS	6	15.8%	1	5%	3.7	0.051 NS
	MRSA	3	7.9%	1	5%	1.03	0.309 NS
	Aspergillus fumigtus	4	10.5%	0	0%	4.1	0.042 S

S: p-value < 0.05 is considered significant. X^2: Chi-square test., NS: p-value > 0.05 is considered non-significant, CONS: *coagulase-negative staphylococci*, MR-CONS: methicillin resistant coagulase negative staphylococci, MRSA: *methicillin resistant staphylococcus aureus*

Table (3): Relation between diabetic retinopathy and culture results.

		Diabetic Retinopathy				X^2	P-value
		NPDR (N = 23 eye)		PDR (N = 8 eye)			
Eye cultures	Neg	7	30.4%	2	25%	0.085	0.771 NS
	Pos	16	69.6%	6	75%		
Organism	CONS	6	26.1%	2	25%	0.004	0.952 NS
	Staph aurous	3	13%	0	0%	1.15	0.282 NS
	Diphteroid	1	4.3%	3	37.5%	5.8	0.016 S
	MR-CONS	4	17.4%	0	0%	1.59	0.206 NS
	MRSA	2	8.7%	1	12.5%	0.098	0.754 NS
	Asperig fumigtus	2	8.7%	0	0%	0.74	0.389 NS

S: p-value < 0.05 is considered significant, X^2: Chi-square test, NS: p-value > 0.05 is considered non-significant, CONS: *coagulase-negative staphylococci*, MR-CONS: methicillin resistant coagulase negative staphylococci, MRSA: *methicillin resistant staphylococcus aureus*

Table (4): Comparison between studied groups as regards sensitivity results.

		Group A (N = 60 eye)		Group B (N = 60 eye)		X^2	P-value
Sensitivity (sensitive)	Penicillin	10	26.3%	9	45%	3.05	0.08 NS
	Ciprofloxacin	37	97.4%	16	80%	5.01	0.025 S
	Levofloxacin	38	100%	19	95%	1.93	0.164 NS
	Doxycycline	31	81.6%	20	100%	4.2	0.041 S
	Gentamycin	34	89.5%	18	90%	0.004	0.950 NS
	Erythromycin	22	57.9%	7	35%	3.1	0.078 NS
	Azithromycin	23	60.5%	6	30%	4.88	0.027 S
	Clindamycin	31	81.6%	17	85%	0.014	0.904 NS
	Sulfa/Trimethoprim	25	65.8%	17	85%	2.03	0.154 NS
	Lienezolid	38	100%	20	100%	----	----
	Vancomycin	8	88.9%	2	100%	0.24	0.621 NS

S: p-value < 0.05 is considered significant, X^2: Chi-square test, NS: p-value > 0.05 is considered non-significant.

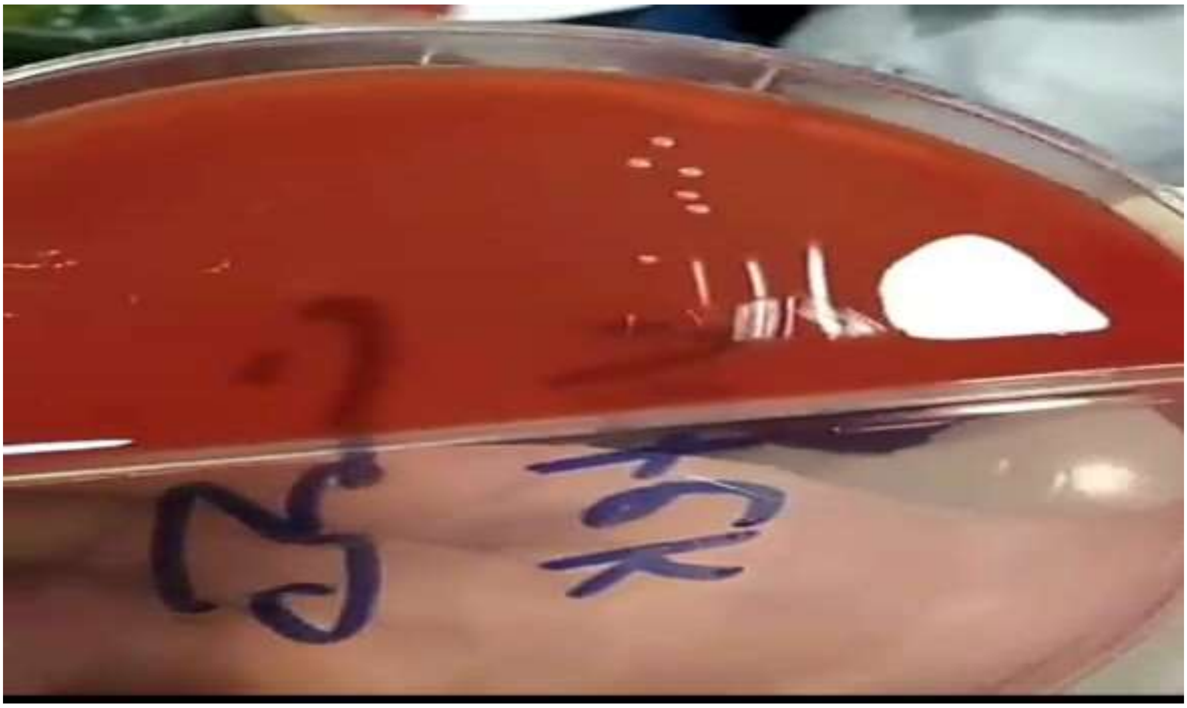

Figure (1): Growth of a few colonies of staphylococci on a blood agar plate, with no growth on MacConkey.

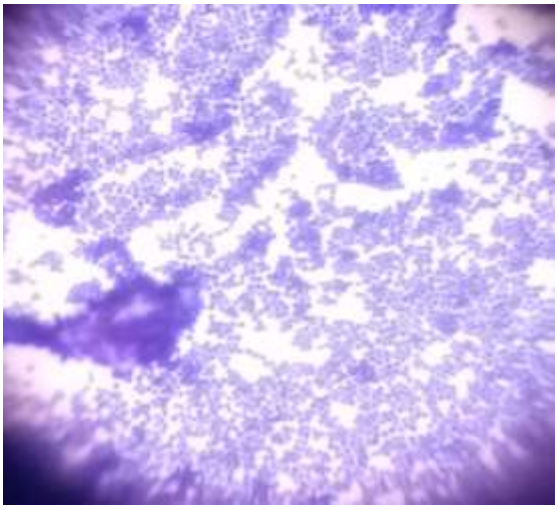

Figure (2): Gram positive cocci in cluster (staphylococci)

Figure (3): Gram positive bacilli, diphtheroid

4. Discussion

Diabetic patients are prone to develop eye infections such as blepharitis, conjunctivitis, keratitis, stye, chalazion and orbital cellulitis. It was found that these patients have an increased quantity of glucose present in their tears in comparison to non-diabetics which may be a factor in the development of ocular infections [10]. Due to constant blinking which clears the conjunctiva at regular intervals, the tears wash away any foreign bodies and bacteria. Bacteriostatic substances like lysozyme, IgA and IgG, lower temperature of conjunctiva because of evaporation of tears and moderate blood supply hamper the growth of bacteria. However, unchecked use of antibiotics lately has led to changes in the normal flora as well as pathogenic bacteria [2]. Tears also act as an antimicrobial defense, by washing away pathogens by the mechanical action of the eyelids and also contain the enzyme lysozyme which has antimicrobial properties. This therefore results in preventing the overgrowth of a particular microorganism and infection [10]. This study differentiates between antibiotic sensitivity in diabetic and non-diabetic individuals, and it was conducted on 120 eyes of 60 patients, 30 diabetic patients (group A) and 30 non-diabetic patients (group B), patient's age ranged 40-70 years old and there was no statistically significant difference found between the two studied groups regarding age of the patient and gender.

In the present study, the rate of positive culture in group A was significantly higher as compared in group B (63.3% vs. 33.3%, respectively). The difference was statistically significant. This agrees with the study by Ashtamkar et al. [11] showed that the rate of positive culture in group A was significantly higher as compared to group B (21.7% vs. 4.3%, respectively). The difference was statistically significant. In the study by *Suresha* et al. [12] it was shown that the normal conjunctival flora of non-diabetics with that of diabetics and identifying the organisms from the conjunctival flora and their antibiotic sensitivity pattern reported total positive cultures in 148 from 100 patients (diabetic=72 and non-diabetics=74). 20 patients had negative cultures. Similarly, Adam et al. [13] showed that the aerobic bacterial conjunctival flora in diabetic patients and comparing it to non-diabetics reported growth in 38.5% of diabetics and 34.9% of non-diabetic individuals. In the current study, the mean HbA1c level of patients in group A and group B was 7.8 ± 0.8 and 5.6 ± 0.4 respectively. The mean difference was statistically significant. This agrees with the study by Ashtamkar et al. [11] that showed the mean HbA1c level of patients in group A and group B was 5.40 ± 0.92% and 4.85 ± 0.22%, respectively. The mean difference was statistically significant. In the present study, the incidence of staphylococcus epidermidis was higher in female patients (34.2 %) as compared to male patients (22.7 %), whereas, of diphteroid was higher in male patients (27.3 %) as compared to female patients (2.6 %). There was no significant association of bacterial isolates and the sex of patients in group A. This disagree with the study by Ashtamkar et al. [11], that showed the incidence of staphylococcus epidermidis was present equally in male and female patients (6.6%). The incidence of Staphylococcus aureus was higher in male patients as compared to female patients (4.4% vs. 2.2%). The incidence of klebsiella was seen in a female patient. There was no significant association of bacterial isolates and sex. The current study showed that the incidence of staphylococcus aureus in the age group of 51-60 years was higher as compared to 40-50 years. The incidence of MRSA was seen in patients in the age group of 61-70 years. No significant association of bacterial isolates and age of patients in group A. This is concordant to the study by Ashtamkar et al. [11], the association of bacterial isolates and age showed that the incidence of staphylococcus epidermidis was higher in the age group of 61-70 years (8.8 %) as compared to 40-50 years (2.2 %) and 51-60 years (2.2 %). Also, the incidence of staphylococcus aureus was higher in the age group of 61-70 years (4.4%) as compared to 51-60 years (2.2%). The incidence of klebsiella was seen in a patient in the age group of 51-60 years. There was no significant association of bacterial isolates and age. In the current study, the most common bacteria isolated in group A were staphylococcus epidermidis (47.4%) followed by diphteroid (18.4%) and staphylococcus aureus (15.8%). The most common bacteria isolated in group B was staphylococcus epidermidis (55%) followed by staphylococcus aureus (40 %) and diphteroid (10 %). It was noticed that there was a significantly higher incidence of staphylococcus epidermidis in group A as compared to group B. This is concordant to the study by Ashtamkar et al. [11]

showed the most common bacteria isolated in group A was staphylococcus epidermidis (13.2%) followed by staphylococcus aureus (6.6%) and klebsiella (2.2%), the most common bacteria isolated in group B was staphylococcus epidermidis (2.2%) and stap hylococcus aureus (2.2%). There was a significantly higher incidence of staphylococcus epidermidis in group A as compared to group B.

In our study the antibiotic sensitivity of bacteria isolated in patients
in group A showed that maximum sensitivity was seen with levofloxacin (100%), linezolid (100%), ciprofloxacin (97.4%), gentamycin (89.5%) and vancomycin (88.9%), while resistance was seen with pencillien (77.8%), erythromycin (33.3%) and azithromycin (33.3%).

This is concordant to the study by Ashtamkar et al. [11] showed that maximum sensitivity was seen with gentamicin (100%), vancomycin (100%), clindamycin (100%), linezolid (100%) and cotrimoxazole (100%) while resistance was seen with ciprofloxacin (33.3%), erythromycin (33.3%) and oxacillin (33.3%).

The limitations of the current study were the sample size was relatively small, the traditional culture method was used which may have compromised the growth of the cultivable microbiome from the ocular surface and missed some nonculturable microbes and we did not use the local anaesthetic which brought local pain to the subjects, because this eye drops had antimicrobial effects.

So,we recommend: 1-increase sample size, 2-modified methods such as genetic analysis and 16S rRNA sequencing, may lead to a much more rapid, precise and complete analysis but these techniques cannot distinguish viable and nonviable bacteria, and the bacteria obtained by traditional culture methods are living and the dominant bacteria and 3- tell subjects about minimal local pain.

5. Conclusion

Diabetic individuals had a higher positive culture rate than non-diabetic individuals. Diabetics are more likely to develop postoperative endophthalmitis and other ocular infections as they are more prone to have a positive culture rate of microorganisms.

Fluoroquinolones (Ciprofloxacin 97.4% and Levofloxacin 100%) showed maximum sensitivity and can be considered as a routine pre-operative topical medication.

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