ORIGINAL RESEARCH PAPER

Volume-7 | Issue-4 | April-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

MICROBES AS OCULAR GUARD: A PROSPECTIVE STUDY AT A TERTIARY HEALTH CARE CENTRE IN WESTERN ODISHA

Ophthalmology

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ABSTRACT

AIM: To isolate and identify the organisms from the conjunctival flora of normal population

***Methods*:** Conjunctival swabs obtained from healthy eyes of 84 patients who attended outpatient dept. of ophthalmology at VSSIMSAR, Burla for refraction and/or cataract surgery, were analyzed for growth of microorganisms.

***Results*:** The most commonly isolated organisms from conjunctival swabs of healthy eyes were coagulase-negative *Staphylococcus* (25%), fungus (21.42%), *Staphylococcus aureus* (9.5%), diphtheroids in 6 (7.14%), *Hemophilus* in 4 (4.76%) and *unidentified gram negative bacilli* in 4 (4.76%) of culture positive eyes.

***Conclusions*:** There is a high prevalence of fungal and bacterial colonization of healthy eyes in population of western odisha. A relationship may exist between resident normal flora and the etiology of ocular infections.

KEYWORDS

Conjunctival flora, Normal flora, conjunctival swabs

INTRODUCTION

Conjunctival sac is relatively free from organisms due to antibacterial action of immunoglobulins and components of the complement pathways namely lactoferrin, lysozymes and B-lysin in tears that help kill microorganisms and decrease their adherence to the ocular surface and their flushing action[1]. However, some microfloras still flourish in normal conjunctiva and have the potential to cause postoperative intraocular infection. Wide variations in composition of normal conjunctival flora are reported from different parts of world but no cause could be attributed. A recent report by Rubio[2] has blamed climate as the causative factor for this variation At birth the eyes are sterile. Soon, the conjunctival sac is invaded by various organisms which then persist throughout life. The bacterial flora of the two eyes are strikingly similar. The source of conjunctival sac flora is usually the eyelid. Lactoferrin, lysozyme and secretory IgA which are present in the tears play an important role in maintaining the normal flora. [3]

There are two types of ocular flora - Resident and Transient.

Resident ocular flora are permanent flora which represent the colonization and repeated cultures usually reveal the same organisms in large numbers. This includes Staphylococcus epidermidis, Diphtheroids, Staphylococcus aureus, Lactobacillus species and Propionibacterium species (anaerobic diphtheroid).

Transient ocular flora inhabit the eye for short periods and cannot be consistently recovered in consecutive cultures. They are influenced by a number of factors, examples of which are as follows: Increased recovery of fungi from the conjunctival sac of people living in rural areas. Increased recovery of Pseudomonas from the hospitalized patients. Increased recovery of Streptococcus, Pneumococcus and Haemophilus in children. Also in patients with certain ocular disease, there may be ocular flora different from that normally seen. Patients with keratoconjunctivitis sicca tend to harbor Proteus and Staphylococcus aureus.

Topical and systemic immunosuppressive therapy and immunosuppressive diseases like diabetes mellitus also affects the normal flora. Studies have revealed an increase in fungi recovered from

eyes on topical steroidal therapy and also an increase in the incidence of bacterial lid and conjunctival infections. [4]

The transient ocular flora include Staphylococcus species other than Staphylococcus aureus, Bacillus species, Haemophilus species, E.coli, Klebsiella species, Enterob acter species, Anaerobes : Peptococ cus, Bacteroides, Clostrid ium, Actinobacterium, Eubacterium and fungi which includes Aspergillus, Penicillium, Alternaria, Cladosporium, Candida, Helminthosporiuum, Rohodotorula and Fusarium species.

MATERIALS AND METHODS

Subjects

Eighty four normal healthy individuals of age group 15-65 years from western Odisha attending Out-patient department of VSSIMSAR, Burla from February 2017 to January 2018 for refraction or cataract surgery were selected at random after obtaining consent. The patients were divided into 3 age groups of young (15-25 years), middle aged (26-50 years) and elderly (≥51 years).

Methods

Detailed ocular and systemic examination was conducted to rule out presence of surface infection or an ocular disease with special emphasis on erythema, oedema and discharge from the eyes at least for a fortnight before. Other exclusion criteria were topical antibiotic use in the recent past and known patients of a systemic illness or an immunodeficiency disease. After obtaining the informed consent the conjunctival swabs with two moistened sterile cotton tipped applicators without touching eyelid margins or lashes were collected from both eyes of patients separately and to sent for microbiology.

Conjunctival anesthesia was done by instilling 1-2 drops of 4% Xylocaine and a sample was collected by gently rubbing the conjunctiva of lower fornix from medial to lateral side with a sterilized cotton-wool swab stick moistened with a drop of sterile normal saline, taking care not to touch the lid margins and ensuring that individuals should not blink during the procedure.

On receipt of the swabs in the laboratory, one swab was inoculated in thioglycollate broth over night and gram staining was done from the broth and also cultured in blood agar and MacConkey's agar. With the other a culture on Sabouraud's agar, a smear, as also the wet preparation were made. The organsims were identified by standard microbiological procedures

These were routinely examined by a Lactophenol blue preparation and gram's staining for any evidence of fungi or bacteria.

The inoculated blood agar and Sabouraud's plates were incubated at 37° and room temperature respectively. The blood agar was rejected after 72 hrs. and the Sabouraud's after 3 weeks, if no growth was seen. Antibiotic sensitivity test wasdone using Kirby Baeur's method.

RESULT

There were 46 males (55%) and 38 females (45%) in the three age groups . Growths were obtained from 69 eyes (82.12%) and remaining 15 (17.85%) did not yield any organism . Predominantly isolated aerobes were *Staphylococcus* spp. in 29 (34.5%) followed by Fungal isolates in 18 (21.42%), diphtheroids in 6 (7.14%), *Hemophilus* in 4 (4.76%) and *unidentified gram negative bacilli* in 4 (4.76%) of culture positive eyes. Age group wise isolation rate of aerobes is shown below. *Staphylococcus epidermidis* (*S. epidermidis)* was the most common microorganism, found alone in 21 eyes (25%), followed by *Staphylococcus aureus (S. aureus)* in 8 (9.5%)

The incidence of bacteria in the normal conjunctiva, increases with age. In contrast, the fungal incidence was found to be less in younger age group.

Incidence in the sexes:

The conjunctival bacterial flora is more or less the same in either sex, but there is predominance of fungal incidence in the males. This may be attributed to the outdoor work in this predominantly agricultural area in which males take part more than females.

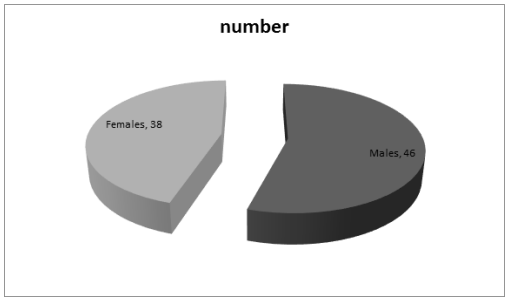

Figure 1

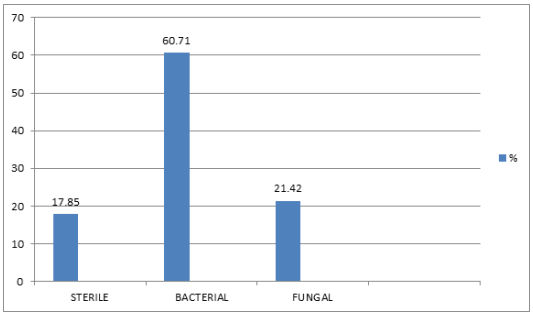

Figure 2 Percentage of isolates

Distribution of Bacterial isolates

SINGLE ISOLATES	NUMBER	PERCENTAGE (%)
Staphylococcus epidermidis	21	41.16
Staphylococcus aureus	8	15.68
Diphtheroids	6	11.76
Hemophilus	4	7.84
Neisseria	2	3.92
Micrococcus, Bacillus subtillis, Pseudomonas	1+1+1	1.96 each
Unidentified Gram negative bacilli	4	7.84

MIXED ISOLATES	NUMBER	PERCENTAGE (%)
Pseudomonas+ Coliforms	1	1.96
Diphtheroids+ Neisseria	2	3.92
TOTAL	51	

Distribution of Fungal isolates

FUNGAL ISOLATES	NUMBER	PERCENTAGE (%)
Aspergillus flavus	8	44.44
Aspergillus niger	2	11.11
Candida albicans	2	11.11
Penicillium	4	22.22
Candida albicans + Penicillium	1	5.5
Penicillium+ Aspergillus	1	5.5
TOTAL	18	

The Relation of Age to the incidence of micro-organisms

Age group	Total no. of patients	No. of cases with fungal isolates		No. of patients with bacterial isolates	
15-25 yrs	21	Number	%	Number	%
		2	9.52	12	57.14
26-50 yrs	37	10	27.02	22	59.45
>50 yrs	26	6	23.07	17	65.38

DISCUSSION

DUKE-ELDER quotes 13 authors, who have found a considerable pro¬portion of normal conjunctival sacs free from bacteria as also varying from 72% (PUTTANNA 1958-1959) to as low as 17% (KHORAZO AND THOMPSON. There are other authors who have denied this and are of opinion that practically every con¬junctiva exhibits some flora. We report around 17.85% of sterile cultures in our study population.

The incidence of sterile sacs was more in young than adult age groups decreased in elderly, meaning thereby that latter have more tendency to harbor micro-organisms.

AGARWAL AND KHOSLA [5] in their series found that in the urban population only 23% of conjunctival sacs were sterile, as compared to 30% in rural population. This differs from the findings of HAMMAKE [6]where more positive results are from the rural areas. AGARWAL et alt explain this difference as due to the indiscriminate use of corticosteroid and antibiotic eye, ointment by the urban people.

ROHATAG1[7] in his series of 50 normal conjunctiva found bacteria in all of them *Staphylococcus Albus Coagulase Negative* in 16 out of 50 cases, and *B. Xerosis* in 13 out of 50.

KHORAZA AND THOMPSON, as quoted by GIVNER [8] made cultural studies of the normal conjunctiva in 1122 patients. They found staphylococci were present in 64 per cent of them, diphtheroids in 36 per cent, alpha haemolytic strepto-cocci in 3-4 per cent, streptococcus pyogenes in 1.2 per cent, pneumococci 2.6 per cent, sarcinae 1 per cent. More than one organisms were present in 25 per cent, and no organism was present in 17 per cent.

Staphylococcus epidermidis is the commonest bacterium in normal conjunctival sacs reported universally. In the present study also, this was the commonest organism found in 25% eye followed by *S. aureus* in 9.5%. Rajvanshi *et al*[9]have reported higher (53%) incidence for *S. epidermidis* and (14.7%) for *S. aureus*. But Park *et al*[10] found S. epidermidis accounting for 60.6% of isolates and Liu *et al*[11] reported much higher incidence (76.07%) of *S. epidermidis* as well as of *S. aureus* at 20.51%. However, *S. aureus* was reported much lower-only 3.4% by Saxena and Goswami[12] and 2.9 % by Sinha and Das[13] respectively.

We also observed that *Haemophilus* spp. Was more prevalent in younger than middle aged individuals and were conspicuous by their absence in elderly. Conversely, diphtheroids were more prevalent in latter age group.

Surprisingly few investigations have been done on the mycotic flora of the healthy conjunctiva, but those that are available indicate that quite a proportion of apparently normal conjunctiva are contaminated in this way. Thus in Central Europe FAZAKAS[14],[15]found in 24-257 (of cases; MITSUI AND HANABUSA[16] found 18.5% in Japan. HAMMEKE AND ELLIS[6] found Fungi in 10.3% of the normal eyes of the adults in ARKANSAS 5 % of children and 1.0% of new born infants. HINLEY and SMITH (England) found positive cultures for fungus in 27.9 per cent of normal conjunctiva. The most common are blastomycosis particularly Penicillium. In contrast with this, we found predominant population of Aspergillus spp in our study (11.90%)

It would seem that most of these are air-born and it is interesting that their occurrence depends greatly on the occupation, since many of them live amidst vegetation (plants and vegetable material) they are found most commonly in the rural population. FAZAKAS [14] for example as quoted by DUKE-ELDER isolated fungi from the conjunctival sac, the lids and the lacrimal passages in 83% of harvesters and threshers. BALAKRISHNAN[17]opines that in primary keratomycosis, infection is carried from the soil to the cornea due to minor injuries.

WILLIAMSON, et al[18] from Great Britain isolated fungi from 2.9% of the healthy conjunctival sac examined, the incidence being higher in older subjects. Most species isolated were non pathogenic transchient aerial contaminants, but some potential pathogens were found.

The limitations of this study are-a hospital based survey which does not truly represent a cross section of the population, and possibility of inclusion of an asymptomatic patient of diabetes mellitus, AIDS or any other disease leading to lowering of immunity, though apparently only healthy individuals were enrolled.

In conclusion, normal conjunctival flora shows considerable variation in its composition. Although *S. epidermidis* is the predominant aerobe, occurrence of diphtheroids is much less. *E. coli* was not found as a single isolate in any of the conjunctival sacs in this study. Fungi were isolated in about 21% of cases, probably due to outdoor exposure. Common commensals of conjunctiva are also known to cause ocular infections after intraocular surgery, hence it is important to administer broad spectrum antibiotics for a few days prior to surgery as a preventive measure.

FOOTNOTES

No conflict of interest

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