Risk factors and microbial colonization of soft contact lens storage cases and conjunctiva of asymptomatic lens users

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ABSTRACT

Microbial colonization of contact lens users are more than non-contact lens users due to many factors affecting natural defense mechanism. The presence of a contact lens on the cornea represents a foreign body that can alter tear flow, prevent oxygen and ionic diffusion and cause superficial alterations to the integrity of the epithelial layer. This in turn may render the cornea susceptible to microbial attachment and infection. This work was carried out to study the microbial flora (other than viruses) and factors affecting the microbial colonization of conjunctiva and contact lens storage cases. The study group comprised of randomly selected 93 asymptomatic soft contact lens users attending Medicine OPD for complaints other than eye diseases. One hundred age- and sex-matched healthy non-lens users were included as control. Two swabs each from contact lens storage cases and conjunctiva from study group and one from conjunctiva of control group were taken and inoculated for bacterial, fungal and Acanthamoeba culture. Detailed history regarding change of contact lens solution, duration of use of contact lenses per day and change of contact lens set were taken to study these factors in relation with microbial colonization of contact lens and conjunctiva. Microbial colonization was seen in 32/93 (34.41%) in both samples of the same patient. Microbial colonization was more in persons using contact lens more than 8 h and using the same contact lens for more than one year. A higher value (34.41%) of microbial colonization was recorded in asymptomatic contact lens users when compared with the Control group (4%). Culture positivity showed statistically significant correlation with factors such as duration of use of contact lenses/day and change of lenses. Use of the same contact lens for more than one year increases the microbial colonization.

INTRODUCTION

The cornea is constantly challenged by microbes, either from the normal flora of the conjunctiva and skin or from the environment. Fortunately, the surface of the cornea is protected by highly efficient natural defense mechanisms in the tear film (Brightbill, 1972). These include lysozymes (active against gram positive bacteria), lactoferin (complexes iron and deprive bacteria of an important growth factor) and secretory IgA antibody (coats microbes and hampers attachment). In association with tear film, blinking action of the eyelid prevents attachment, and wipes microorganisms from the eye surface. Due to these protective mechanisms keratitis is a rare disease and usually results from injury or surgery. However, in contact lens (CL) wearers, the risk of...
microbial keratitis is greatly increased. The presence of contact lens interferes with the ocular protective mechanisms and causes corneal trauma, dry eye, etc. (Brightbill, 1972; Coster, 1979; Coster et al., 1987). Apart from that, microbial contamination of contact lens care product is a major problem for contact lens wearers. Other factors related with contact lens uses, such as duration of use of contact lens, frequency of cleaning of contact lens, change of contact lens also reported to have the effect of microbial contamination of contact lens (Mondino et al., 1986; Weissman et al., 1987). As not much is known about the practices in contact-lens users from Nagpur, India; and the effect of these practices on microbial colonization of conjunctiva and lens, the present study was undertaken to evaluate the microbial colonization of contact lens care system and conjunctiva of asymptomatic soft lens users. Along with the factors affecting the microbial colonization like duration of contact lens use, change of contact lenses and contact lens care solution were also studied.

MATERIALS AND METHODS

Study participants and sample collection

Ninety-three (93) randomly selected asymptomatic soft contact lens users comprising of staff and patients attending Medicine OPD for problem other than eye diseases. All 93 participants belong to the age group bracket of 15-30 years and comprised of 13 males and 80 females. One hundred age- and sex-matched healthy non-lens users which comprised of 19 male and 81 female were included as Control. Mean age of study group was 23.16 years and Control group was 23.05 years (Table 1). All participants in the study group used the same cleaning solution, that is, RENU solution from Bausch and Lomb Co., USA. Persons with eye complaints, Diabetes mellitus and history of immunodeficiency conditions were excluded from the study. Institutional ethical Committee permission was taken for the study. After explaining the purpose and method, written consent of the study participants were taken. Two swabs from contents of contact lens storage cases, and two swabs from conjunctiva were taken. One swirl from each site was used for microscopy and another one for microbial culture. Conjunctival swabs were taken from Control group and processed as described for the study.

Two swabs from study group and one from Control group were kept in separate tubes containing sterile normal saline. For wet mount 2-3 drops of sample was taken in a clean and grease free slide and a cover slip was put on the sample and microscopy was done. For Gram staining, the drop of sample was allowed to air-dry after slightly spreading the drop. The smear was fixed by passing over the flame and then Gram staining was done. Microscopy of wet mount and Gram’s stain were also done. Culture for bacteria, fungus and Acanthamoeba were done. For bacterial isolation Blood agar, MacConkey’s agar, and Chocolate agar were used.

For fungus isolation, Sabouraud’s dextrose agar and for Acanthamoeba isolation 1.5% non-nutrient agar with E. coli culture-lawn was used. All plates were incubated at 37°C. Bacterial cultures were observed after overnight incubation. Fungal cultures were observed for 4 weeks and Acanthamoeba cultures were observed on 1st, 2nd, 3rd and 7th day under 10× magnification objectives with enhanced contrast using Nikon H600L binocular microscope. Amoebae appeared as retractile structures which were scooped from agar and suspended in small amount of Ringer solution and incubated for 2-4 h. A wet mount was observed for Acanthopodia. Identification of bacteria, fungus and Acanthamoeba was done using standard techniques (Winn et al., 2006; Chander, 1996; Edwards, 2007). All chemicals used in study were from Hi-Media Pvt. Ltd Co.

Statistical analysis

Statistical analysis were done using Chi-square test by a statistician using software STRATA. Probability ≤ 0.05 was considered statistically significant.

RESULTS

Microbial colonization of the study participants was seen in 32/93 (34.41%) cases. The microbes were the same in both specimens of the study case; the only difference was seen in the number of colonies of the organism. In contact lens sample less than 25 colonies were seen whereas in conjunctival samples scanty growth (less than 10 colonies) was seen. In Control group 4/100 (4.00%) showed microbial colonization in conjunctival samples (Table 2). This difference was statistically significant (p=0.0001).

Bacterial growth was seen in 100% in Control group, whereas it was 87.5% in study group. Bacteria in Control group were non-pathogenic e.g coagulase negative Staphylococcus (50%), Diphtheroids (25%) and non-hemolytic Streptococci (25%) as these are commensals in conjunctiva and the Control group were all immunocompetent. In the study group, the most common bacterial isolate was Pseudomonas aeruginosa (28.57%), E. coli (14.28%), coagulase positive Staphylococcus (7.14%) and Klebsiella pneumoniae, Enterococcus faecalis and Serratia marcescens (respectively, 10.71%). One sample showed mixed bacterial growth.

No fungal growth was seen in Control group. The study group showed fungal growth in 9.4% participants. One isolate (33.33%) each of Penicillium sp., Mucor sp. and...
Table 1. Demographic properties of study group.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Age in year</th>
<th>Case</th>
<th></th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 - 20</td>
<td>2 (15.38)</td>
<td>25 (31.25)</td>
<td></td>
<td>5 (26.31)</td>
<td>24 (29.26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21 - 25</td>
<td>4 (30.76)</td>
<td>32 (40.00)</td>
<td></td>
<td>3 (15.78)</td>
<td>38 (46.91)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>26 - 30</td>
<td>7 (58.33)</td>
<td>23 (28.75)</td>
<td></td>
<td>11 (57.89)</td>
<td>19 (23.45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13 (100)</td>
<td>80 (100)</td>
<td></td>
<td>19 (100)</td>
<td>81 (100)</td>
<td></td>
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</tr>
</tbody>
</table>

Values in parenthesis are percentage.

Table 2. Activity outcome of culture media.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Result of culture</th>
<th>Cases</th>
<th></th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Growth</td>
<td>32 (34.42)</td>
<td></td>
<td>4 (4.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No growth</td>
<td>61 (65.59)</td>
<td></td>
<td>96 (96.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>93 (100)</td>
<td></td>
<td>100 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis are percentage.

Table 3. Microbes isolated from culture media.

| S/N | Organism | Species | Isolates | Isolates | | | |
|-----|----------|---------|----------|----------| | | |
|     | Diptheroides | - | 1 (25) | | | |
|     | C-negative Staphylococcus | 5 (17.84) | 2 (50) | | | |
|     | C-positive Staphylococci | 2 (7.14) | - | | | |
|     | Non-haemolytic Streptococci | - | 1 (25) | | | |
| 1   | Bacterial | P. aerugenosa | 8 (28.57) | - | | | |
|     |          | E. coli | 4 (14.28) | - | | | |
|     |          | K. pneumoniae | 3 (10.71) | - | | | |
|     |          | E. faecalis | 3 (10.71) | - | | | |
|     |          | S. marscence | 3 (10.71) | - | | | |
|     | Fungal | Penicillium sp. | 1 (33.33) | - | | | |
| 2   |          | Mucor sp. | 1 (33.33) | - | | | |
|     |          | Aspergillus sp. | 1 (33.33) | - | | | |
| 3   | Protozoa | Acanthamoeba sp. | 6 (18.8) | - | | | |
| 4   | Mixed | Acanthamoeba sp. Gram negative rods | 5 | - | | | |
|     |          | 2 Gram negative rods | 1 | - | | | |

Values in parenthesis are percentage.

Aspergillus sp. were obtained from the 3 samples. Six Acanthamoeba species (18.8%) were obtained in the study group. Out of those 6, one was in pure form, whereas 5 showed concomitant growth of Gram negative Bacilli (Table 3). No parasitic growth was seen in the Control group.

We also studied the different risk factors, such as frequency of change of contact lens care solution, duration of use of same contact lens and duration of use of contact lens per day in relation to microbial colonization in study group (Table 4). It was observed that 24.1% of participants who changed solution daily,
Table 4. Correlation of risk factors with culture.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Risk factors</th>
<th>Duration (n)</th>
<th>Culture positivity (%)</th>
<th>Culture negativity (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Change of contact lens solution</td>
<td>Daily (29)</td>
<td>7 (24.1)</td>
<td>22 (75.9)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 1 day (64)</td>
<td>25 (39.1)</td>
<td>39 (60.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Duration of use of same contact lens</td>
<td>Up to 1 year (23)</td>
<td>2 (8.7)</td>
<td>21 (91.3)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 1 year (70)</td>
<td>30 (42.9)</td>
<td>40 (57.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Use of contact lens/day</td>
<td>Less than 8 h (36)</td>
<td>7 (19.4)</td>
<td>29 (80.6)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 8 h (57)</td>
<td>25 (43.8)</td>
<td>32 (56.1)</td>
<td></td>
</tr>
</tbody>
</table>

showed microbial colonization whereas 39.1% participants, who changed solution in more than one day, showed microbial colonization. This difference was not found to be statistically significant ($\chi^2=1.97$, df=1, $p=0.16$).

A total of 8.7% participants who were using same contact lens for one year, showed microbial colonization, whereas 42.9% participants using the same contact lens for more than one year showed microbial colonization. This difference was found to be statistically significant ($p=0.003$).

Similarly, relation of microbial colonization and duration of use of contact lens per day was also studied. It was observed that 19.4% persons using contact lens for less than 8 h showed microbial colonization whereas 43.8% persons using contact lens for more than or equal to 8 h showed microbial colonization. This difference was found to be statistically significant ($p=0.016$).

**DISCUSSION**

Leonardo da Vinci first described contact lenses in 1508 but it was not until 1970s, that there were reports of varying degree of microbial keratitis in contact lens wearers. There are reports of bacterial colonization as high as 24 to 45% in cultures of contact lens cases (Donzis et al., 1987; Larkin et al., 1990; Steal et al., 1992; Dovenshine et al., 1993; Midlufart et al., 1996). We found 34.3% microbial colonization similar to other studies. Gray et al. (1995) reported as high as 81% in their study group, which used single step hydrogen peroxide solution for cleaning up. As Beattie et al. (2002) had shown, multipurpose solution (MPS) though not cysticidal, kills fungi and bacteria, thereby reducing the growth chances for *Acanthamoeba*. In the present study, MPS solution was used for cleaning of lenses, which may be the reason for getting less percentage of microbial colonization.

Bacterial colonization was high in the present study (87.5%) compared to that reported by others (Steal et al., 1992), possibly due to other factors affecting the microbial contamination of contact lens; for example, longer use, infrequent change of solution, etc. observed in the study. But a recent study by Sharma et al. (2006) on microbiologic profile, clinical course, treatment and outcome in patients with contact lens associated microbial keratitis in the tertiary eye care centre shows that contact lenses associated microbial keratitis is a rare entity in South India. They found only 0.11% laboratory proven infectious keratitis in which 89.2% were bacterial infection; the most common being *Pseudomonas* spp.

As far as the fungal colonization is concerned, widespread range (2-24%) has been reported (Wilson and Ahearn, 1986; Wilson et al., 1990; Gray et al., 1995). The commonest isolates reported are *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. and *Fusarium* spp. We found 9.3% fungal colonization of contact lens cases and conjunctiva with *Penicillium*, *Mucor* and *Aspergillus* spp. as reported by others.

Amoebial colonization of contact lens cases was reported to be from 4-14.5% (Larkin et al., 1990; Dovenshine et al., 1993 Gray et al., 1995). Donzis did not find *Acanthamoeba* colonization in soft lens contact cases (Donzis et al., 1987).

In the present study, 18.8% of Acanthamoebal colonization was recorded. Five patients showed concomitant bacterial colonization, a state, which may be advantageous to the parasite by providing a nutrient source. They produced a symbiotic relationship that favours amoebic infection. The biofilm formed by the organism especially *Pseudomonas* increased the adsorption of Acanthamoeba to the lens (Kumar and Lloyd, 2002). An increase in Acanthamoeba keratitis is reported in contact lens users. The reason for apparent increase in the cases is not clear, although an obvious factor might be an increase in the number of contact lens wearers in recent years and a decrease in diagnostic delay as the disease has become more common (IIlingworth et al., 1995). Presence of similar bacteria,
fungi and parasites in the contact lens cases and conjunctival swabs show that the source of colonization may be the contaminated solution used for cleaning. These solutions have previously been shown to support the growth of microorganisms (Mayo et al., 1987).

Wilson and Ahearn (1986) and Wilson et al. (1990) observed that the type of disinfecting solutions used and the frequency of change of solution affects the rate of microbial contamination of contact lens cases and conjunctival surface. Frequent change of contact lens solution and cleaning of contact lens cases was suggested by them. We also tried to correlate the frequency of change of contact lens solutions with the microbial contamination. Our study shows that daily change reduces the microbial contamination rate more than the second or third day change of solution.

Wearing of same contact lens and using it for long hours of day can increase the risk of microbial contamination (p=0.016). Wilson and Ahearn (1986) also reported fungal isolates in extended wearers of soft contact lens, 2 of which developed fungal corneal ulcer. Thus in long term users of contact lenses, fungal contamination have been reported even if washed daily. Edwards et al. (2007) reported that the overnight soft contact lens use was associated with an increased risk of infection compared to daily disposable contact lens. Similarly, in the present study, it was also observed that the extended use of contact lens per day for more than 6 h, increased the chances of microbial contamination. Even the same contact lens used for longer periods, that is, more than one year has the same effect. This shows that contact lens should be changed at least once in a year. A study by Bourcier et al. (2003) to identify the risk factors of bacterial keratitis revealed contact lens wear to be the most important factor (50.3%). Failure to follow lens cleaning and lens care instructions may be stated as an important factors, apart from duration of use of contact lens.

In conclusion, even in asymptomatic soft contact lens users, microbial colonization is high. Bacterial colonization is more than fungal and Acanthamoeba, but mixed flora is common. Various factors, like use of contact lens for more than 6 h per day, change of contact lens solution after one day and use of the same lenses for more than one year increase the chance of microbial colonization. There is a need to increase awareness amongst the lens users on proper cleanliness and use of contact lenses to minimize microbial colonization.


REFERENCES