Original Article

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Ophthalmology Section

Assessment of Alteration in Aerobic Conjunctival and Meibomian Gland Microbial Flora after Cataract Surgery and its Association with Postoperative Meibomian Gland Dysfunction

SONIA BHARGAV[1], SURINDER KUMAR[2], TARUN ARORA[3]

ABSTRACT

Introduction: Combination of various commensal microorganism species is capable of protecting the ocular surface from colonisation by potentially pathogenic microbes. These homeostatic microorganisms can be easily altered by environmental factors like cataract surgery. These may play a role in the development of postcataract surgery Meibomian Gland Dysfunction (MGD), whose exact pathogenesis is still unknown.

Aim: To assess the change in aerobic bacterial flora of meibomian glands and conjunctiva and its association with the development of MGD after cataract surgery.

Materials and Methods: This prospective clinical study was conducted in Department of Ophthalmology at BPS Government Medical College for Women, Sonepat, Haryana, India, from June 2021 to February 2022. A total of 60 eyes of 60 patients who had undergone uncomplicated phacoemulsification surgery with intraocular lens implantation were selected. Conjunctival and Meibomian Gland (MG) secretions samples were collected preoperatively before starting antibiotics and at six weeks postoperatively. Aerobic bacteria isolated from the culture samples of patients before and after surgery were analysed for microbial flora change (MFC). At six weeks postoperative follow-up visit, the diagnosis of the MGD was made on slit lamp examination of lid margin and meibomian glands. Chi-square test was applied to assess the association between patients with MFC and patients with MGD.

Results: There were 33 males and 27 females, with a mean age of 62.41 years. Preoperative conjunctival samples of 20 patients (33.33%) and MG samples of 27 patients (45%) were culture positive for aerobic bacteria. Most common microorganism isolated from preoperative samples was Coagulase Negative *Staphylococci* (CoNS). Isolation rate of *Staphyloccus aureus* increased postoperatively in both conjunctival and MG culture samples. Postoperative aerobic Microbial Flora Change (MFC) in culture samples of either conjunctival or MG or both was present in 30 patients (50%). Total 16 patients (26.6%) developed MGD out of which 13 patients (81.25%) had MFC. The association between MFC and MGD was statistically significant (p-value=0.004).

Conclusion: There is alteration of ocular aerobic bacterial flora after cataract surgery and it has a statistically significant association with postcataract surgery MGD. Replenishment of ocular surface microflora can play a preventive and therapeutic role in the management of postcataract surgery MGD.

Keywords: Conjunctival culture, Meibomian gland culture, Ocular microbial flora

INTRODUCTION

The microbial species that inhabit the normal ocular surface are far more extensive than previously thought. There is growing evidence of the critical role of the ocular microbiome in ocular health and disease. Combination of various commensal microorganism species is capable of protecting the ocular surface from colonisation by potentially pathogenic microbes. These homeostatic microorganisms can be easily disturbed by environmental factors like cataract surgery, postoperative inflammation and the use of topical antibiotics and steroids.

The change in bacterial flora of ocular surface after cataract surgery might be one of the contributing factors in the development of Meibomian Gland Dysfunction (MGD), a common postcataract surgery morbidity whose exact pathogenesis is still elusive. Multiple studies in past have stressed on the role of bacterial flora in blepharitis and MGD [1-4]. However, none has dealt this aspect in post cataract surgery. The present study was done to assess the change in aerobic bacterial flora of meibomian glands and conjunctiva after cataract surgery and its association with the development of postcataract surgery MGD. The null hypothesis was that, the change in postoperative aerobic microbial flora was not associated with postoperative MGD.

MATERIALS AND METHODS

This prospective clinical study was conducted in the Department of Ophthalmology at BPS Government Medical College, Sonepat, Haryana, from June 2021 to February 2022. Written consent was taken from all the patients and ethical clearance was obtained from the Institutional Ethical Committee (BPSGMCW/RC/673/IEC/21/ dated 9-4-21). The patients of senile cataract who had no signs and symptoms of MGD and gave written consent for the study were included in the study.

Sample size calculation: Using proportion formula, taking prevalence of MGD as 20% at 95% confidence interval and taking margin of error as 10%, the required sample size was calculated to be 60 patients [5,6].

Inclusion criteria: After detailed preoperative ocular slit lamp biomicroscopic examination, all patients diagnosed with senile cataract were included in the study.

Exclusion criteria: Patients with presence of any ocular disease with special emphasis on erythema, oedema and discharge from the eyes atleast one month before surgery, use of topical antibiotic in the recent past, known systemic illness like diabetes mellitus or an immunodeficiency disease, any symptom or signs of dry eye like foreign body sensation, burning or tearing, Tear Break-Up

Time (TBUT) less than 10 second, patients with positive corneal fluorescein staining were excluded from the study.

Study Procedure

All 60 patients underwent clear corneal phacoemulsification surgery with intraocular lens implantation by the same surgeon. Postoperatively topical antibiotic and steroid combination was prescribed and tapered over four weeks.

Conjunctival and Meibomian gland (MG) secretion culture samples were taken from the same patients preoperatively before starting antibiotics and at six weeks postoperatively. Aerobic bacterial flora isolated from the samples of patients before and after surgery was analysed for change and its association with postoperative MGD incidence was studied. Microbial Flora Change (MFC) was said to have occurred if on microbial assessment of postoperative conjunctival or MG samples it was found that there was emergence of new microorganism which was not present in preoperative sample or there was absence of any microorganism previously isolated in the preoperative sample or both.

Sample collection: After instilling a drop of topical anaesthetic agent (0.5% proparacaine), conjunctival sample was collected by gently rubbing a sterile cotton-wool swab stick moistened with sterile normal saline in the lower fornix in a swirling manner avoiding lid margin or skin. The MG sample was collected after gently pressing the lid margin with the thumb to release secretions and then rubbing the gland orifices with a sterile cotton-wool swab stick moistened with sterile normal saline. Care was taken to avoid contact with lashes or skin. As a blank control, sterile swab stick moistened with sterile normal saline was exposed in the air for one minute each time the samples were being collected. The samples were put in Brain-Heart infusion and sent to microbiology laboratory where aerobic culture on blood agar, chocolate agar and MacConkey agar were done. Microbial identification was done by specific culture media, gram staining, biochemical tests [7].

At six weeks, postoperative follow-up visit, the diagnosis of the MGD was made on slit lamp examination of lid margin and meibomian glands, if two or more of the following signs were present [8]:

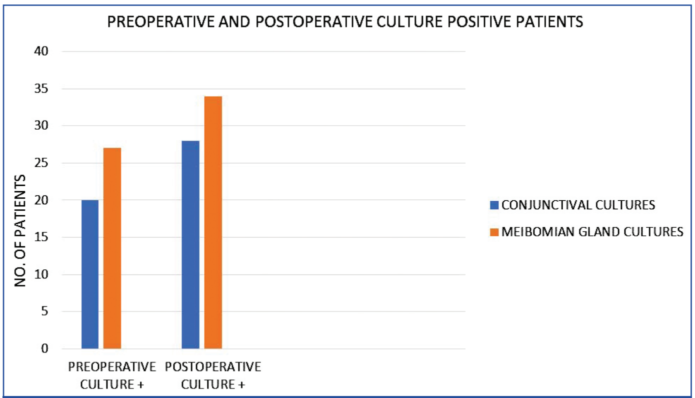
- Redness or thickening of the lid margin,
- Telangiectasia,
- Reduced or no secretions,
- Poor quality (cloudy/turbid) secretions, and
- Gland capping/plugging

STATISTICAL ANALYSIS

Statistical analysis was carried out on Statistical Package for Social Sciences (SPSS) version 25.0. Microsoft excel sheet and word was used for analysis of data. Chi-square test was applied to find association between patients with MFC and patients with MGD and to carry out other comparative analyses. A p-value <0.05 was considered as statistically significant.

RESULTS

A total of 60 eyes of 60 patients were selected as per the inclusion and exclusion criteria defined in the study. There were 33 males and 27 females, with a mean age of 62.41 years. Preoperatively conjunctival samples of 20 patients (33.33%) and MG samples of 27 patients (45%) were culture positive [Table/Fig-1]. Most common microorganism isolated from preoperative samples was Coagulase Negative *Staphylococcus* (CoNS) which was present in conjunctival samples of 7 patients (11.6%) and MG samples of 12 patients (20%). *Staphylococcus aureus, Micrococcus, Bacillus, Diphtheroids, Streptococcus, E.coli, Acinetobacter* were the other species isolated [Table/Fig-2]. Cultures of all blank control samples were sterile.



[Table/Fig-1]: Graph showing number of preoperative and postoperative culture positive samples of conjunctival and meibomian gland.

Microorganism isolated	Preoperative conjunctival culture	Postoperative conjunctival culture	Preoperative meibomian gland culture	Postoperative meibomian gland culture
CoNS	7	10	12	8
S.aureus	5	8	6	12
Micrococcus	4	5	5	8
Diphtheroids	0	2	1	1
E.coli	2	0	1	1
Bacillus	1	2	2	3
Streptococcus	0	1	0	1
Acinetobacter	1	0	0	0
Total no. of patients	20	28	27	34

[Table/Fig-2]: Aerobic bacteria isolated from preoperative and postoperative conjunctival and meibomian gland culture samples among the patients.
*CoNS: Coagulase Negative Staphylococcus

In the postoperative period at six weeks, culture positivity increased to 28 patients (46.7%) in the conjunctival sample and 34 patients (56.7%) in the MG samples [Table/Fig-1]. The increase in culture positivity of conjunctival and MG samples was not found to be statistically significant (p-value=0.136 and p-value=0.201, respectively). Compared to preoperative period, the isolation rate of *Staphylococcus aureus* increased postoperatively in both conjunctival culture samples {8 patients (13.3%) compared to 5 patients (8.3%) preoperatively} and MG culture samples {12 patients (20%) compared to 6 patients (10%) preoperatively} which was not statistically significant (p-value=0.378 and p-value=0.327, respectively) [Table/Fig-2].

Total 22 patients (36.66%) had sterile culture of both conjunctival and MG samples throughout the preoperative and postoperative period. Postoperative aerobic MFC in culture samples of either conjunctival or MG or both was present in 30 patients (50%).

Total 16 patients (26.6%) which included eight male and eight female patients developed postoperative MGD out of which 13 patients (81.3%) had shown MFC. This was significantly higher than the overall rate of MFC which was 50%, statistically significant association was found between MFC and MGD p-value=0.004 [Table/Fig-3] and null hypothesis was, thus, rejected.

Microflora change	MGD present	MGD absent	Total patients
Present	13	17	30
Absent	3	27	30
Total patients	16	44	60

[Table/Fig-3]: Number of patients with microbial flora change and Meibomian Gland Dysfunction (MGD) present or absent (p-value=0.004), (Chi-square test significant at p-value <0.05).

Eight patients (50%) out of 16 patients developing MGD, had positive conjunctival cultures and 13 patients (81.2%) had positive MG cultures postoperatively. This is higher than the overall postoperative culture positivity rates of 46.7% and 56.7% in conjunctival and MG cultures respectively. When comparing postoperative culture positivity in MGD and non MGD patients, the MG culture positivity was found to be statistically significantly higher in MGD patients (p-value=0.03) [Table/Fig-4]. Whereas, higher conjunctival culture positivity in MGD patients was not statistically significant (p-value=0.370) [Table/Fig-5]. [Table/Fig-6] shows aerobic bacteria isolated from culture samples of patients developing meibomian gland dysfunction.

Meibomian gland dysfunction	MG culture positive	MG culture negative	Total patients
Present	13	03	16
Absent	21	23	44
Total patients	34	26	60

[Table/Fig-4]: Association between postoperative MG culture positivity and MGD.
Chi-square test (significant), p-value=0.03

Meibomian gland dysfunction	Conjunctival culture positive	Conjunctival culture negative	Total patients
Present	09	07	16
Absent	19	25	44
Total patients	28	32	60

[Table/Fig-5]: Association between postoperative conjunctival culture positivity and MGD.
Chi-square test (non significant), p-value=0.370

Microorganism isolated	Preoperative conjunctival culture	Postoperative conjunctival culture	Preoperative meibomian gland culture	Postoperative meibomian gland culture
CoNS	0	2	1	0
S.aureus	2	3	2	4
Micrococcus	2	2	3	3
Diphtheroids	0	1	1	1
E.coli	0	0	1	1
Bacillus	1	0	2	3
Streptococcus	0	0	0	1
Acinetobacter	1	0	0	0
Total culture positive patients	6	8	10	13

[Table/Fig-6]: Aerobic bacteria isolated from culture samples of patients (n) developing meibomian gland dysfunction.
*CoNS: Coagulase negative *Staphylococcus*

Staphylococcus aureus was the most commonly isolated aerobic bacteria in MG samples postoperatively which was present in culture samples of 12 patients (20%) and also in MGD patients with 4 patients (25% MGD patients) showing *Staphylococcus aureus*.

Isolation rate of Coagulase Negative Staphylococcus (CoNS) which is a normal commensal of ocular surface decreased postoperatively in MG culture samples, from 12 patients preoperatively to eight patients postoperatively [Table/Fig-2]. None of the MGD patients had CoNS isolated from MG culture samples [Table/Fig-4].

Mean age of patients developing MGD and MFC was 67.81 years and 66.03 years, respectively, which is higher than overall mean age of 62.41 years, indicating that older age is a risk factor for postoperative MGD (p-value=0.197, OR=1.66).

DISCUSSION

There are variety of microorganism species living in and over our body. Their number far exceeds the number of human cells by multiple times. The human genome consists of approximately 20,000 genes but the microbiome associated with the human body collectively consists of approximately 8 million genes. These microbes live with us in a homeostatic environment and are involved in various vital functions like absorption of nutrients, defending against pathogens, and maintaining the immune system in check. On the ocular surface too, commensal microorganisms co-exist in harmonious relationship with the eye [9]. This fine homeostatic balance may get disturbed during ocular surgery and with the use of topical antibiotics and steroids, may result in inflammation and disease.

Kuklo P and Grzybowski A, evaluated conjunctival bacterial flora and antibiotic susceptibility profile in patients undergoing phacoemulsification, before surgery and 20 minutes post-surgery. They concluded that, preoperative chemotherapy changes the profile of conjunctival bacteria and their susceptibility to antibiotics [10]. In the immediate postoperative period, changes in microbial isolation and profile are expected due to the use of prophylactic antibiotics and betadine. In the present study, postoperative samples were collected after six weeks of surgery and two weeks after cessation of topical medications to give enough time for eye to heal and ocular microflora to recover from the trauma of surgery and topical antibiotics.

In the present study there was 40% increase in conjunctival and 26% increase in MG culture positivity rate postoperatively which indicates microbial changes on ocular surface even six weeks after cataract surgery.

After the cataract surgery the normal commensal population of microbes is altered to more pathogenic ones. This was demonstrated in the present study, which showed increased isolation rate of *Staphylococcus aureus* and decreased isolation rate of CoNS postoperatively. The cells of ocular surface produce inflammatory response on exposure to these newly inhabited pathogenic bacteria [11,12]. This inflammatory state may lead to the vicious cycle of dry eye and MGD.

The ocular surface microbiome has been studied in various eye diseases like dry eye and MGD, allergic conjunctivitis, Stevens-Johnson Syndrome, Sjogren's Syndrome, trachoma, chronic limbal stem cell deficiency, chronic ocular graft vs host disease, and Behçet disease. The 16S rRNA sequencing shows more diversity in the ocular surface microbiota of people with type 2 diabetes mellitus [9]. To eliminate this confounding factor, diabetic patients were excluded in the present study.

Various studies report increased incidence and aggravation of MGD after cataract surgery. There is thickened lid margin and inflammation and deterioration of meibum quality and expressibility leading to significant ocular discomfort and patient dissatisfaction after ocular surgery [13-15]. In the present study, 26.7% patients developed MGD postoperatively.

There is also enough evidence available in literature suggesting that MGD patients show more diversity of bacterial communities. Using traditional cultivation methods, Zhang SD et al., concluded in their study that the rate of positive bacterial culture of the conjunctival sac in patients with MGD is significantly higher than those without MGD [2].

With the use of modern 16S rDNA (Recombinant Deoxyribonucleic Acid) gene sequencing, Dong X et al., confirmed that both the bacterial imbalance in the conjunctival sac of patients with MGD and the composition of bacterial community on the ocular surface of patients with severe MGD changed significantly. There was a positive correlation between the abundance of *Staphylococcus* and the degree of meibomian gland loss in these patients [3].

Jiang X et al., concluded in their study that MGD might be correlated with bacterial changes which is characterised by higher isolation rate, a greater number of bacterial species, and a higher grade of bacterial severity [4].

The results of the present study done on cataract surgery patients are consistent with the findings of above studies. In patients who developed MGD, bacterial alteration was more pronounced as shown by number of patients with MFC. In patients developing MGD, the postoperative culture positivity rate of MG samples was statistically significantly higher compared to those in non MGD patients. Also, the isolation rate of *Staphylococcus aureus* was higher and of CoNS was lower in MG samples of MGD patients.

The MGD meibum contains distinct microbial profile whose immune evasive virulence is much stronger than that in the healthy controls. These microbe profiling alterations are responsible for immune cells getting activated to elicit an inflammatory response in tears and in glandular tissue, a key link in inducing pathological changes [16].

The results of the present study prove the association of MGD and MFC after cataract surgery but their aetiological correlation is still debatable. Whether, MGD precedes MFC or is it the latter which causes MGD or probably, it is a vicious cycle in which one favours the other. On one hand, invasion of meibomian glands by pathogenic bacteria which release specific factors (e.g., lipases) can lead to meibomian gland inspissation, plugging and disruption of normal gland and eyelid physiology [1]. And on the other hand, MGD itself can cause significant alterations of meibum causing dry eye which can act as potential predisposing factor for ocular surface bacterial proliferation and microflora alteration.

The present study is unique for two reasons. Firstly, the change in ocular surface bacterial flora, especially meibomian gland flora, six weeks after cataract surgery has never been studied before. Secondly, there are many studies which indicate role of altered bacterial flora in MGD, but, none, so far has analysed this aspect in postcataract surgery MGD. The present study was the first, to do so.

The present study also provides a possible therapeutic clue to the post operative MGD management which currently seems ineffective in majority of cases. The therapeutic approach to MGD should also focus on reversal of microbial alteration and replenishment of commensal population to prevent growth of pathogens. Therapeutic microbial alteration has been explored in the past in the treatment of dry eye with some success. A four weeks treatment with Lactobacillus probiotic in eye drops has been associated with a modest reduction in signs and symptoms of patients with mild to moderate vernal keratoconjunctivitis [17]. Prebiotics which are non digestible fermentable oligosaccharides, such as Fructo-Oligosaccharides (FOS) and galacto-oligosaccharides can act as an important adjuncts in promoting the growth of healthy microbiota [18]. Disaccharides like trehalose containing eye drops is already being used in treatment of dry eye [19].

Limitation(s)

Due to Institutional constraints, only aerobic bacterial flora could be cultured and studied which does not truly represent the whole normal ocular surface microbiome. The use of conventional culture techniques for bacterial isolation was another limitation of this study. In future studies, the use of more advanced gene sequencing techniques for microbial flora isolation which can also include anaerobes, viruses and fungi can give us a better understanding of behaviour of commensal microbiome after cataract surgery and its association with MGD.

CONCLUSION(S)

Postcataract surgery, there is alteration of conjunctival and meibomian gland aerobic bacterial flora and it has a statistically significant association with development of MGD after cataract surgery. This demonstrates the importance of commensal microflora of the ocular surface. Ocular surface commensal microflora replenishment should be explored as a possible therapeutic option in MGD management.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Ophthalmology, BPS Government Medical College for Women, Sonepat, Haryana, India.
2. Professor, Department of Microbiology, BPS Government Medical College for Women, Sonepat, Haryana, India.
3. Senior Consultant, Department of Ophthalmology, Centre for Sight Group of Eye Hospitals, New Delhi, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sonia Bhargav,
A3/69, FF, Janakpuri, New Delhi, India.
E-mail: soniabhargav@yahoo.co.in

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