Impact of topical moxifloxacin prophylaxis and povidone iodine on conjunctival bacterial flora in patients receiving intravitreal injections in a tertiary healthcare center in India

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ABSTRACT

Purpose: To assess the efficacy of 3 days of topical moxifloxacin in combination with povidone–iodine (PVI) versus moxifloxacin/PVI alone in eliminating conjunctival bacterial flora in patients scheduled to undergo intravitreal injections (IVI).

Patients and Methods: A prospective randomized comparative study in which 120 patients scheduled to undergo IVI at a tertiary care hospital in New Delhi were selected. Study patients were randomized into two groups. Both the groups received self-administration of moxifloxacin for 3 days prior to injection, except in Group B where it was preceded by PVI. Cultures were obtained at different time intervals, such as in Group A before and after applying moxifloxacin (for 3 days) and once again after applying PVI just before the procedure in the operating room, whereas in Group B first two samples were taken before and after applying PVI and the last sample was taken on the day of the procedure after 3 days of moxifloxacin application. Results: A statistical significance was seen between moxifloxacin prophylaxis and resistant coagulase-negative Staphylococcus (CoNS) (P = 0.0001), which implies that frequent use of antibiotic prophylaxis will ultimately lead to the formation of resistant organisms in the conjunctival flora, especially when repeated IVI are given. Conclusion: We could not establish any additional benefits of topical moxifloxacin prophylaxis with regard to a reduction in conjunctival flora when compared with PVI 5%. PVI can be used as an efficient monotherapy in patients undergoing repeated IVI.

Keywords: Intravitreal injections, moxifloxacin, povidone–iodine

Introduction

Over the last 10 years, the number of intravitreal injections (IVI) has dramatically increased owing to the efficacy of new anti-vascular endothelial growth factor agents and steroids that are used for various posterior segment diseases such as diabetic macular edema (DME), macular edema due to retinal vascular occlusions, and age-related macular degenerations (AMD).[1]

Generally, IVI are safe and well-tolerated; however, the rapidly increasing use of these injections has led to a few treatment-related complications.[2] Out of these, the most feared is endophthalmitis, which has potentially visually devastating consequences and most of the time carries a poor prognosis. Hence, minimizing the risk of endophthalmitis is of paramount importance while treating a patient with IVI.[3]
The incidence of infective endophthalmitis after IVI is estimated to be 0.2% per injection.[3] In a retrospective study, Mishra et al.[4] endophthalmitis after IVI, given from June 2009 to August 2016, reported an overall incidence of 0.131%. In a meta-analysis that included both retrospective and prospective studies, 197 cases of endophthalmitis of 350,535 IVI were identified that is 0.06% and 1/1,779 cases.[5,6]

It is believed that the most common bacteria isolated from patients with endophthalmitis after IVI are similar to the surface bacterial flora. Especially, gram-positive pathogens are responsible for 60% to 80% of cases of endophthalmitis. Among them, coagulase-negative Staphylococcus (CoNS) are the most frequent isolates. Hence, evaluating the conjunctival flora may serve as a surrogate marker for the risk of endophthalmitis.[7,8]

Pre-treatment with topical antibiotics is based on the rationale that such applications may have a synergistic effect with povidone–iodine (PVI) and enhance the natural defense system in eliminating ocular surface bacteria at the injection site.

Topical PVI is the recommended prophylaxis in many countries, and it is suggested that the application of PVI immediately before IVI with a wide-spectrum antibiotic, combined with eye draping to remove the eyelashes from the surgical field, minimizes the risk of endophthalmitis.[9]

One major distinguishing feature of IVI from almost all invasive procedures is that, the repeated nature of the injections for most patients. The repeated use of topical antibiotics significantly increases the possibility of colonization of the ocular surface with resistant organisms.[10] If PVI proved to be as effective as prophylactic antibiotics, patients undergoing any ophthalmic surgery might be spared from the disadvantages of using antibiotics such as expenses, toxicity, and allergic effects, and the growth of antibiotic-resistant organisms.[11]

Hence, the purpose of our study described herein was to determine and compare the efficacy of topical moxifloxacin and PVI individually as well as in combination with their effectiveness in reducing conjunctival bacterial flora in patients undergoing IVI.

Patients and Methods

Study design
A prospective and randomized comparative study.

Sample size
The study population consisted of 120 patients who presented to the outpatient unit of the ophthalmology department.

Inclusion criteria
1. Patients undergoing IVI.

Exclusion criteria
1. Patients having any infection of the ocular surface or adnexa
2. Patients already on antibiotic drops.

The Ethics Committee of our medical teaching institute (VMMC and Safdarjung Hospital) approved our project for all ethical purposes. (IEC/VMMC/SJH/Thesis/October/2018-206).

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. After detailed assessment in the retina clinic, patients were selected on the basis of inclusion and exclusion criteria after explaining pertinent details of the study and obtaining valid informed consent for the same.

Sample collection
Patients were randomly divided into two groups, and each group consisted of 60 patients. Conjunctival swab samples were obtained from the inferior conjunctival fornix using Eurotubo collection swabs at three different time intervals:

In Group A, the first swab was collected on the first visit prior to antibiotic application and they were started on a 3-day routine topical prophylactic moxifloxacin 0.5%. The second swab was collected on the day of the procedure before PVI instillation and the third swab was collected 3 min after PVI disinfection.

In Group B, the first swab was collected on the first visit before PVI instillation and the second swab 3 min after PVI instillation on the same day and the patients were started on routine antibiotic prophylaxis 3 days prior IVI. The third swab was collected on the day of the procedure preoperatively before preparing the patient with PVI [Figure 1].

Extreme attention was paid to the swab, avoiding lids and lashes adjacent to the culture site. The application of PVI 3 days before the procedure is not a routine in our institution, the purpose of doing so is only to find out if there is any significant reduction in the bacterial flora on the day of the procedure compared to Group A.

Preoperatively, after the application of tropicamide with phenylephrine eye drops and local anesthetic proparacaine, the patient was scrubbed with 10% PVI over the eyelids, eyelashes, periorbital area, cheeks, and forehead. Sterile drapes and an eyelid speculum were applied to avoid the lashes. Then, the IVI was given in the inferotemporal quadrant, following which moxifloxacin eye drops were given for 1 week.

Sample processing
All the samples were processed as per conventional microbiological techniques. The swabs had been sub-cultured on blood agar and MacConkey agar and enriched in the brain heart infusion broth. After 24 h, a subculture from brain heart infusion broth was
done on blood agar and MacConkey agar. The culture plates were incubated at 37°C for 24 h. The isolates were identified by conventional microbiological techniques such as colony-forming morphology, gram staining, hanging drop method, and other appropriate biochemical reactions, wherever required. Automated identification was done on Vitek. Sensitivity was performed in accordance with the latest Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Statistical analysis**

Categorical variables are presented in number and percentage (%) and continuous variables are presented as mean ± standard deviation (SD) and median. The normality of data was tested by the Kolmogorov–Smirnov test. When the normality was rejected, non-parametric tests were used. Statistical tests were applied as follows-

1) Quantitative variables were compared using unpaired t-test/Mann–Whitney test (when the data sets were not normally distributed between the two groups.)

2) Qualitative variables were compared using the Chi-square test/Fisher’s exact test. A P value of <0.05 was considered statistically significant. The data were entered in an MS Excel spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 24.0.

**Observations and Results**

In the current study, Group A consisted of patients who were started with a 3-day pre-treatment with moxifloxacin before IVI. Group B consisted of patients who were given PVI 5% drops in the outpatient department followed by a 3-day course of antibiotics.

The distribution was as follows:

**Patient characteristics**

In the present study, age amongst Group A varied between 42 years and 74 years with a mean age of 59.9 ± 6.83 years. The age amongst group B varied between 48 years and 77 years with a mean age of 62.33 ± 6.98 years. Out of 60 subjects in Group A, 35 (58.33%) were females and 25 (43.33) were males [Table 1]. In Group B, 26 (41.67) were females and 34 (56.67) were males. Both the groups comprised a large number of patients with DME, making it the most common indication of IVI, followed by neovascular AMD and retinal vascular occlusive diseases [Table 2].

**Distribution and quantity of bacteria (comparison between two groups)**

In this study, in the first sample, all patients in Group A had no growth prior to antibiotic administration as compared to Group B where 2 patients had CoNS in their conjunctival culture and 58 had no growth prior to PVI application. We did not find any statistically significant difference in the conjunctival culture of patients of Group A and Group B (P value = 0.496) [Table 3]. In the second sample, CoNS were found in 13 patients and the rest 47 patients had no growth after application of moxifloxacin in Group A as compared to Group B where all patients had nil growth after the application of topical PVI. This result was found to be statistically significant (P value = 0.0001) [Table 4]. In the third sample, none of the patients had growth in both Group A and Group B after the application of topical PVI in Group A and after antibiotics in Group B [Table 5].

**Culture sensitivity pattern**

The study also showed that the CoNS isolates were 73% resistant to ciprofloxacin, 80% resistant to erythromycin, and 53% resistant to cefoxitin, whereas it was 87% sensitive to vancomycin, 93% sensitive to gentamicin, and only 33% sensitive to cefoxitin [Table 6].

**Discussion**

Fluoroquinolones kill bacteria by interfering with the ability of the DNA to function properly resulting in abnormal protein production and cellular proliferation, whereas PVI kills bacteria on contact by disrupting the cellular membranes. Owing to their different mechanisms of action, it is reasonable to postulate that these two agents when combined could result in more potent antisepsis.

However, in our study, there was a significant rise in the culture-positive specimen after the application of topical antibiotics, whereas nil growth was reported after the application of PVI. A probable explanation for this could be the emergence of resistant strains in the conjunctiva after exposure to sub-inhibitory concentrations of antibiotics. In accordance with that we have noticed almost all the CoNS isolated were resistant to the second-generation fluoroquinolones, macrolides such as erythromycin, and even third-generation cephalosporins sometimes, whereas they were sensitive to vancomycin and gentamicin.

In a clinical research by Budzinskaya et al. in 2020 on antibiotic susceptibility patterns of ocular flora in patients undergoing IVI, they stated that conjunctival flora of patients who received 20 or more IVI along with prophylactic antibiotics showed resistance to...
a wide range of antibiotics. A study by Miller et al. demonstrated that patients diagnosed with endophthalmitis showed a greater incidence of fluoroquinolone-resistant CoNS isolates, which is almost similar to that reported in our study. Also, these resistant strains were identified to be more virulent than the sensitive ones. Kunisada et al. have demonstrated in vitro that bacteria acquire resistance to various antiseptics including chlorhexidine but not PVI. In a study by Hsu et al., they reported that the use of PVI without the use of topical antibiotics after serial IVI did not promote bacterial resistance or change the normal conjunctival flora.

Moss et al. in 2009 reported that the use of PVI alone exhibited a dramatic reduction in the number of positive cultures and any added benefits of prophylactic antibiotics were found negligible in the setting of PVI application. Another study by López et al. could not establish any additional benefits of topical moxifloxacin with regard to reduction in conjunctival flora when compared with PVI 5%.

Table 1: Comparison of age (years) and sex between Group A and Group B

| Age (years) | Group A (n=60) | Group B (n=60) | Total | P | Test performed |
|------------|----------------|----------------|-------|---|----------------|
| 41-50      | 6 (10%)        | 4 (6.67%)      | 10 (8.33%) | 0.596 | Chi square test, 1.888 |
| 51-60      | 29 (48.33%)    | 25 (41.67%)    | 54 (45%) |     |                |
| 61-70      | 22 (36.67%)    | 25 (41.67%)    | 47 (39.17%) |     |                |
| 71-80      | 3 (5%)         | 6 (10%)        | 9 (7.50%) |     |                |
| Mean±Standard deviation | 59.9±6.83 | 62.33±6.98 | 61.12±6.98 | 0.070 | Mann-Whitney test; 1455.5 |

Table 2: Comparison of diagnosis between Group A and Group B

| Diagnosis                        | Group A (n=60) | Group B (n=60) | Total | P | Test performed |
|----------------------------------|----------------|----------------|-------|---|----------------|
| ARMD with CNVM                   | 10 (16.67%)    | 8 (13.33%)     | 18 (15%) | 0.798 | Chi square test, 0.065 |
| BRVO with macular edema          | 6 (10%)        | 10 (16.67%)    | 16 (13.33%) | 0.283 | Chi square test, 1.154 |
| CRVO with macular edema          | 2 (3.33%)      | 2 (3.33%)      | 4 (3.33%) | 1 | Fisher Exact test |
| DME                              | 32 (53.33%)    | 34 (56.67%)    | 66 (55%) | 0.854 | Chi square test, 0.0337 |
| HRVO with macular edema          | 1 (1.67%)      | 0 (0%)         | 1 (0.83%) | 1 | Fisher Exact test |
| Moderate NPDR with DME           | 4 (6.67%)      | 1 (1.67%)      | 5 (4.17%) | 0.364 | Fisher Exact test |
| NSD                              | 2 (3.33%)      | 2 (3.33%)      | 4 (3.33%) | 1 | Fisher Exact test |
| PDR with vitreous hemorrhage     | 1 (1.67%)      | 0 (0%)         | 1 (0.83%) | 1 | Fisher's exact test |
| Refractory CME                   | 2 (3.33%)      | 3 (5%)         | 5 (4.17%) | 1 | Fisher's exact test |

Table 3: Comparison of first sample prior to antibiotic/PVI between Group A and Group B

| Prior to antibiotic | Group A (n=60) | Group B (n=60) | Total | P | Test performed |
|---------------------|----------------|----------------|-------|---|----------------|
| Coagulate-negative Staphylococcus | 0 (0%) | 2 (3.33%) | 2 (1.67%) | 0.496 | Fisher's exact test |
| No growth           | 60 (100%)      | 58 (96.67%)    | 118 (98.33%) |     |                |
| Total               | 60 (100%)      | 60 (100%)      | 120 (100%) |     |                |

Table 4: Comparison of second sample between Group A and Group B (after antibiotic/PVI respectively)

| After antibiotic | Group A (n=60) | Group B (n=60) | Total | P | Test performed |
|------------------|----------------|----------------|-------|---|----------------|
| Coagulate-negative Staphylococcus | 13 (21.67%) | 0 (0%) | 13 (10.83%) | 0.0001 | Fisher's exact test |
| No growth        | 47 (78.33%)    | 60 (100%)      | 107 (89.17%) |     |                |
| Total            | 60 (100%)      | 60 (100%)      | 120 (100%) |     |                |

Table 5: Comparison of third sample (after povidone iodine in Group A and after antibiotic in Group B)

| After povidone iodine | Group A (n=60) | Group B (n=60) | Total | P | Test performed |
|-----------------------|----------------|----------------|-------|---|----------------|
| No growth             | 60 (100%)      | 60 (100%)      | 120 (100%) | - |                |

To summarize,
- A single application of PVI demonstrates bactericidal effects, equivalent to the 3-day course of topical antibiotics.
Table 6: Distribution of antibiotic sensitivity of study subjects

| Antibiotics | Fluoroquinolones | Vancomycin | Gentamicin | Cefoxitin | Erythromycin |
|-------------|------------------|------------|------------|-----------|-------------|
| Nil result  | 2 (13.33%)       | 2 (13.33%) | 1 (6.67%)  | 2 (13.33%)| 0 (0.00%)   |
| Sensitive   | 0 (0.00%)        | 13 (86.67%)| 14 (93.33%)| 5 (33.33%)| 1 (6.67%)   |
| Intermediate| 2 (13.33%)       | 0 (0.00%)  | 0 (0.00%)  | 0 (0.00%) | 2 (13.33%)  |
| Resistant   | 11 (73.33%)      | 0 (0.00%)  | 0 (0.00%)  | 8 (53.33%)| 12 (80.00%) |

- Preservatives such as benzalkonium chloride (BAC) present in topical antibiotics have been shown to cause ocular surface irritation and disturbance of corneal epithelial barrier function.[23]
- Our study stresses the diligent use of prophylactic PVI and supports the idea of safe elimination of routine pre-injection antibiotic use before the IVI therapy. This would help to avoid the emergence of antibiotic resistance.

Conclusion

PVI remains the standard of care in antisepsis and the addition of prophylactic topical antibiotics does not seem to provide any added benefits.[22,23] To patients and physicians, this means cost reduction, alleviated patient burden, and improved practice efficiency.

Also, inappropriate and unrestricted use of antibiotics has led to the emergence of antibiotic-resistant ocular microorganisms at an alarming rate and it is regarded as a major health care challenge of this century by directly affecting treatment outcomes.[24,25] By creating awareness and understanding of antimicrobial resistance, which is difficult and expensive to treat, our study would help primary health care providers ensure rational prescription of antibiotics without compromising the health care quality.

What was known

Povidone iodine is the recommended treatment worldwide before intravitreal injections and adding prophylactic topical moxifloxacin were thought to enhance the process of eliminating ocular flora at the injection site owing to their different mechanisms of action.

What this paper adds

The repeated use of topical antibiotics significantly increases the colonization of the ocular surface with resistant organisms and causes changes in the composition of conjunctival flora with an increase in the percentage of coagulase-negative Staphylococcus, which are clinically significant. Povidone iodine 3 days prior to the intravitreal injection also provides significant reduction of conjunctival bacterial flora.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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