Ocular Surface Bacterial Load in Intravitreal Injections: Lid Speculum vs. Manually Assisted Retraction

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ABSTRACT

Purpose: To compare the microbiological outcome of an alternative technique for intravitreal injections with the conventional method.

Materials and Methods: This is a cross sectional, prospective, case-control study. Patients undergoing intravitreal injections for retinal diseases were randomized in two groups; in Group A, the eyelids were opened with a lid speculum and in Group B, the eyelids were retracted with manual assistance. Following sample collection for microbiological evaluation, the procedure was completed in the conventional route in both groups. The groups were compared by means of demographics, diagnosis, laterality and microbiological outcome.

Results: There were 30 patients in each group. The patients had either neovascular age related macular degeneration or diabetic macular edema. The agent administered was either aflibercept or ranibizumab. The mean age was 65.9 ± 10.6 and the male/female ratio was 32/28. There was no significant difference in age, gender, diagnosis, laterality or the agent administered between groups. The manual retraction technique was associated with less culture positive cases compared to speculum (19 vs. 22, p=0.580). The culture positivity rate was significantly higher in male patients (p=0.028). No endophthalmitis or ocular adverse events were encountered.

Conclusion: Manual-assisted eyelid retraction for intravitreal injections offers improved microbiological outcome.

Keywords: Conjunctival culture; endophthalmitis; intravitreal injections; lid speculum; manual lid retraction.

INTRODUCTION

As the number of intravitreal (IV) injections for various retinal disorders grows in daily ophthalmology practice, the risk of bacterial endophthalmitis, the most devastating complication, stays aside jeopardizing the outcome. The procedure is mostly carried out in outpatient - even office-based - settings, which might make the eye more vulnerable to bacterial inoculation. Either direct inoculation of the bacteria into the vitreous cavity or passage through an entry tract is the presumed mechanism of endophthalmitis. However, the causative microorganism nearly almost arises from the ocular surface - particularly the eyelid – flora.[1-4]

Despite the lack of a global standardized protocol, numerous precautions have been carried out to diminish the rate of IV injection-associated endophthalmitis. Reported up to date, the most efficient way is use of 5% povidone-iodine (PI) which could said to be *sine qua non.*[2,5,6] Also, isolating the injection site off the eyelashes carries paramount significance to prevent ocular surface contamination; hence, use of lid speculum is the standard approach in daily practice of most retina specialists. On the other hand, despite the use of topical anesthesia, lid speculum is often bothersome for patients. This situation is reacted by the patient with squeezing the eyelids (Figure 1), which is assumed to result in increased meibomian gland secretion; thus, ocular surface bacterial load might increase.[2,7] Likewise, it is strongly recommended to avoid any compression to the eyelids during the procedure, which would induce bacterial expression from the eyelid glands.[8]

The authors declare no conflicts of interest

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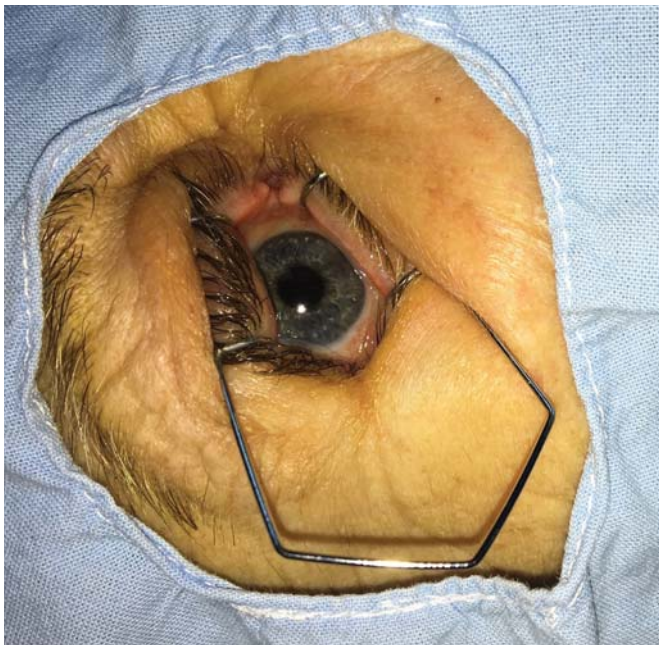

Figure 1: *A patient who is squeezing her eyelids following placement of the lid speculum.*

In a report by Fineman and co-workers, retracting the eyelids manually was considered to cause diminished meibomian gland secretion and the authors reported very low endophthalmitis rates similar to the reports in which intravitreal injections were performed using metal lid-speculum.[1] In a recent report, Rahimy and co-workers compared both techniques and found that manual retraction caused less patient discomfort; there was no cases of endophthalmitis in both groups.[9] Both reports focused on the incidence of endophthalmitis with manual retraction technique.

In this current study we sought to investigate the rate of bacterial growth with two different techniques to separate the eyelids in intravitreal injection: the use of eyelid speculum and the manual retraction technique.

PATIENTS AND METHODS

This single-center, prospective, randomized, cross-sectional, case-control study was approved by the Institutional Review Board of Cukurova University and a written informed consent was obtained from each participant. The tenets of Declaration of Helsinki were followed.

Patients who underwent intravitreal anti-VEGF injections for neovascular type age-related macular degeneration (nAMD) or diabetic macular edema (DME) were enrolled. The anti-VEGF agents used were either ranibizumab or aflibercept. Contact lens wearers, patients with any ocular surface disorders including anterior or posterior blepharitis, patients who were on chronic use of topical medication, patients with a history of ocular surgery in the past 6 months, and patients with a known allergy to povidone-iodine were excluded. Of the pseudophakic eyes, the ones with a ruptured posterior capsule were also excluded.[10] None of patients enrolled was receiving systemic antibiotics at or around the time of culture study

Injection technique

The patients were randomized to two different techniques for eyelid opening: lid speculum (Group A) and bimanually assisted (Group B). Each patient randomly selected one of the 60 cards labeled indicating the selected technique from an enclosed box. The injection technique was as follows: the eyelids were prepared with povidone-iodine 10%. Then, in Group A, the wire lid speculum was placed; whereas, in Group B upper and lower eyelids were manually retracted by the assisting nurse (Figure 2).[1] Ocular surface samples were taken from the inferior fornix as described previously, prior to the instillation of topical anesthetic drops, care was taken not to contact the eyelid margin.[11] The following procedure was common in both groups: topical anesthetic eye drops were instilled, povidone iodine 5% was applied onto the ocular surface for 3 minutes; followed by thorough irrigation with balanced salt solution; 0.05 cc of anti-VEGF was injected via 30 gauge syringe through pars plana (3,5 mm in pseudophakic and 4 mm in phakic eyes, beyond the limbus) in the upper temporal quadrant. As the syringe was withdrawn, soft pressure was applied with a cotton tip applicator.

The procedure was conducted in the operation room in all cases. The staff wore face masks throughout the procedure

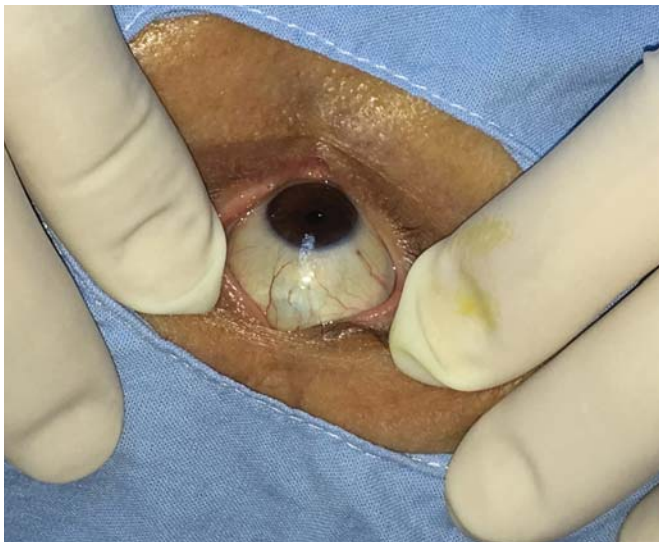

Figure 2: *The manual retraction of the eyelids by the assisting nurse.*

and care was taken to minimize speaking. Topical ofloxacin was prescribed for five days post-injection.

All samples were taken by the same physician (SS), and all microbiological applications were made by the same physician (FK) who was blinded to group assignments. The statistics expert was also blinded during the interpretation of data.

Microbiological investigation

For conjunctival culture, the sample was obtained using a swab (pre-moistened with sterile distilled water) by rolling over the conjunctive. Then each swab was soaked in an amies transport medium. Eye specimens were sent within two hours at room temperature to the Microbiology Unit of the Cukurova University Hospital Central Laboratory.[12] The Microbiology Laboratory is certified and accredited by the Joint Commission International (JCI), a U.S. based accreditation agency.

All specimens were inoculated to a blood agar (Columbia agar with 5% sheep blood); a chocolate blood agar (Chocolate agar with PolyViteX containing factors X [hemin] and V [NAD] for isolation of fastidious strains belonging to the genera *Neisseria, Haemophilus,* and *Streptococcus pneumonia*); a Mac Conkey agar (MCK); a Schaedler agar with 5% sheep blood (SCS - an isolation medium particularly suitable for the detection of obligate and facultative anaerobic bacteria); two Sabouraud Dextrose Agar (SDA) for isolation of fungi and yeasts. Blood agar and chocolate agar were incubated at 37°C in 5% CO_2. Mac Conkey Agar and SDA were incubated at 37°C. The other SDA medium was incubated at room temperature. Schaedler agar with 5% sheep blood (SCS) was incubated in BD GasPak EZ Anaerobe Container System. During seven days, all culture media were incubated and checked for growth daily. All culture media were provided as ready to use from BioMérieux Company**.**

The isolates were identified using the standard microbiological techniques (Gram stain, colony morphology, catalase test, oxidase test, coagulase production, optochin test, 6.5% NaCl, motility, H^2S production, indole test) and also identification cards for Gram Positive and Gram Negative bacteria of the VITEK 2 system (BioMérieux, France).

Statistical analysis

Statistical analysis was performed using the statistical package *SPSS software* (version 23.0, SPSS Inc., Chicago, IL, USA). If continuous variables were normally distributed, they were described as the means± standard deviation ($p>0.05$ in Kolmogorov-Smirnov test or Shapiro-Wilk [$n<30$]) and if the continuous variables were not normally distributed, they were described as the median. Comparisons between groups were made by Mann Whitney U test when the data was not normally distributed. Categorical variables between groups were analyzed by Chi square test or Fisher's Exact test. A p value <0.05 was considered as statistically significant.

RESULTS

The cohort included 32 male (53.3 %) and 28 female patients (46.7 %), with a mean age of 65.9±10.6 (38 □ 91). There was DME in 31 patients (51.7 %) and nAMD in 29 patients (48.3%). The median of total number of injections (previous injections plus the current one) was 4, ranging between 1 to 14. The anti-VEGF agent injected was aflibercept in 31 (51.7 %) and ranibizumab in 29 (48.3%) cases. The injected eye was the left eye in 36 cases (60 %) and the right eye in 24 cases (40 %). In microbiological evaluation, no bacteria could be isolated in 19 (31.7 %) cases and 41 cases (68.3 %) revealed bacterial growth. There were 30 patients in each group (Group A – lid speculum and Group B – manual).

There was no significant difference in age, gender, total number of injections, diagnosis, laterality, and the anti-VEGF agent injected between groups. The number of cases without bacterial growth was higher in Group B; however, the difference did not reach statistical significance (n = 11 vs. 8, p=0.580) (Table 1).

When cases with positive and negative culture were compared, no significant difference was detected in age, gender, diagnosis, laterality, and the mean number of injections. The number of female patients with negative and positive culture were almost equal (13 vs. 15, respectively); nevertheless, male patients were significantly associated with bacterial growth (6 vs. 26) (p=0.028) (Table 2).

Of the 22 patients with positive culture in group 1, 12 had DM and 10 had AMD. In Group 2, diabetic patients revealed a higher rate of culture positivity (13 vs. 6).

Table 3 and 4 present microbiological evaluation results of all cases in Groups A and B. No cases of endophthalmitis or secondary uveitis with aqueous and vitreous cells occurred in any patient enrolled.

DISCUSSION

The wider the use of intravitreal injections – particularly the anti-VEGFs – has further raised concerns on adverse effects – in particular endophthalmitis - associated with the procedure. The rate of post-injection endophthalmitis differs from 0.03% to 0.06%.[6] Prevention has mainly

Table 1. *Age, gender, total number of injections, diagnosis, laterality, the type of anti-VEGF agent injected, and bacterial growth rate comparison between groups.*

		Group A		Group B		
		Mean ± sd	**Median (min-max)**	**Mean ± sd**	**Median (min-max)**	**p**
	Age	66.2±10.5	67 (38-91)	65.7±10.8	64 (46-90)	0.867
	Injection	5±3.6	4 (1-14)	4.8±2.9	4 (1-11)	0.876
		n	**%**	**n**	**%**	
Gender	**Male**	14	46.7	18	60	0.438
	Female	16	53.3	12	40	
Diagnosis	**nAMD**	15	50	14	46.7	1.00
	DME	15	50	16	53.3	
Laterality	**L**	20	66.7	16	46.7	0.430
	R	10	33.3	14	53.3	
Agent	**aflibercept**	15	50	16	46.7	1.00
	ranibizumab	15	50	14	53.3	
Culture	**Negative**	8	26.7	11	36.7	0.58
	Positive	22	73.3	19	63.3	

Sd: standard deviation, **min:** minimum, **max:** maximum**, injection:** total number of injections including all previous ones and the current one, **nAMD:** neoavascular type age related macular degeneration, **DME:** diabetic macular edema, **L:** left eye, **R:** right eye

Table 2. *Comparison of age, gender, total number of injections, diagnosis, and laterality between culture negative and positive cases.*

		Culture negative		Culture positive		
		Mean ± sd	**Median (min-max)**	**Mean ± sd**	**Median (min-max)**	**p**
	Age	68.3±9.5	67 (56-91)	64.9±11.1	66 (38-90)	0.505
	Injection	5.3±3.7	4 (1-14)	4.7±3.0	4 (1-11)	0.248
		n	**%**	**n**	**%**	
Gender	**Male**	6	31.6	26	63.4	**0.028**
	Female	13	68.4	15	36.6	
Diagnosis	**nAMD**	12	63.2	17	41.5	0.166
	DME	7	36.8	24	58.5	
Laterality	**L**	13	68.4	23	56.1	0.410
	R	6	31.6	18	43.9	

sd: standard deviation, **min:** minimum, **max:** maximum, **injection:** total number of injections including all previous ones and the current one, **nAMD:** neovascular type age related macular degeneration, **DME:** diabetic macular edema, **L:** left eye, **R:** right eye

focused on the use of antiseptic agents like PI, chlorhexidine, etc. The injection is rather a standardized procedure, with minimal modification reported up to date.[1] The rate of lid speculum use for intravitreal injections was reported to be 92% amongst retina specialists.[13] In this current study, we sought to evaluate a modified intravitreal injection technique by means of bacterial growth.

Of note, the patients are subject to numerous injections lifetime. Hence, the technique applied should be easy to perform, efficient to prevent infection, and comfortable for the patient. Repeated applications ought to result in the same outcome. It is currently known that post-injection antibiotic use fails to prevent endophthalmitis; ironically, it is associated with increased rates of antibiotic resistance in conjunctival bacterial flora. In particular, the repeated use – several times a year – is likely to multiply the risk.[14] Post-injection antibiotics were also proposed to cause resistance in the nasopharyngeal flora to cause pneumonia.[3]

As mentioned above, eyelid flora is the main cause of endophthalmitis in IV injections. Meibomian gland

Table 3. *Microbiological evaluation of patients in Group A.*

Patient	Growth		Patient	Growth	
	No. of Colonies	**Isolated bacteria**		**No. of Colonies**	**Isolated bacteria**
1	6	*Staphylococcus epidermidis*	**16**	10 20 10 12	*Staphylococcus aureus* *Staphylococcus epidermidis* Diphtheroid rods *Staphylococcus hominis*
2	None	No growth	**17**	6	*Staphylococcus epidermidis*
3	1 500	*Erysipelothrix rhusiopathiae* Diphtheroid rods	**18**	2 1 1 50	*Staphylococcus epidermidis* *Granulicatella elegans* *Staphylococcus hominis* Diphtheroid rods
4	1	*Staphylococcus epidermidis*	**19**	None	No growth
5	None	No growth	**20**	1 10 5	*Staphylococcus aureus* *Staphylococcus epidermidis* Diphtheroid rods
6	21	*Staphylococcus hominis*	**21**	1 10	*Staphylococcus epidermidis* Diphtheroid rods
7	10	*Staphylococcus capitis*	**22**	4	*Staphylococcus epidermidis*
8	None	No growth	**23**	10	*Staphylococcus epidermidis*
9	3 1	Diphtheroid rods *Staphylococcus epidermidis*	**24**	20 50	*Staphylococcus epidermidis* Diphtheroid rods
10	None	No growth	**25**	10 100	*Staphylococcus epidermidis* Diphtheroid rods
11	None	No growth	**26**	3 10 20	*Staphylococcus hominis* *Staphylococcus epidermidis* Diphtheroid rods
12	4 4	*Staphylococcus epidermidis* *Staphylococcus hominis*	**27**	2 20	*Staphylococcus epidermidis* Diphtheroid rods
13	20 20 1	*Staphylococcus epidermidis* *Staphylococcus hominis* *Leuconostoc mesenteroides*	**28**	None	No growth
14	2	*Staphylococcus epidermidis*	**29**	3 3	*Staphylococcus capitis* *Staphylococcus epidermidis*
15	None	No growth	**30**	1 1 20	*Staphylococcus hominis* *Staphylococcus epidermidis* Diphtheroid rods

excretions are the source of conjunctival bacteria.[2] Hence, in the standard injection technique, a lid speculum is used to keep the eyelids open and keep the eyelashes off the injection site.[15] Fineman et al. described the bimanual assisted eyelid retraction technique to prevent contact with the eyelids and the eyelashes.[1] In this retrospective review, their endophthalmitis rate was 0.03%, which is in concordance with the literature. The authors claimed that, placement of the speculum might induce release of bacteria from the eyelids onto the ocular surface, causing endophthalmitis. This was also supported by Friedman and co-workers with their concern of putting pressure on the lids with speculum could result in increased meibomian gland discharge.[2] In a more recent report, in which manual eyelid retraction and speculum use was compared by means of patient discomfort, the manual assisted technique was associated with significantly higher self-reported patient comfort and preference.[9] Additionally, corneal abrasion developed in 3 (8.3%) of speculum eyes, which did not occur with the manual technique. No cases of endophthalmitis developed with both techniques.

Lid speculum placement is reported to be the most unpleasant step of the intravitreal injection procedure.[2,15] This maneuver would directly compress lids – basically meibomian glands - resulting in increased discharge; moreover, the discomfort due to the speculum would push

Table 4. *Microbiological evaluation of patients in Group B.*

Patient	Growth		Patient	Growth	
	No. of Colonies	**Isolated bacteria**		**No. of Colonies**	**Isolated bacteria**
1	None	No growth	**16**	3	*Staphylococcus epidermidis*
2	1	Diphtheroid rods	**17**	2	*Staphylococcus epidermidis*
3	25 3 4 7 300	*Streptococcus mitis* *Staphylococcus lugdunensis* *Serratia marcescens* *Staphylococcus haemolyticus* Diphtheroid rods	**18**	None	No growth
4	4 200	*Staphylococcus aureus* Diphtheroid rods	**19**	None	No growth
5	1	*Staphylococcus hominis*	**20**	None	No growth
6	2	*Staphylococcus epidermidis*	**21**	2	*Staphylococcus hominis*
7	14	*Staphylococcus epidermidis*	**22**	15 100	*Staphylococcus epidermidis* Diphtheroid rods
8	1 3	*Streptococcus parasanguis* Diphtheroid rods	**23**	5 100	*Staphylococcus epidermidis* Diphtheroid rods
9	None	No growth	**24**	None	No growth
10	1	*Staphylococcus epidermidis*	**25**	None	No growth
11	None	No growth	**26**	1 50	*Staphylococcus aureus* Diphtheroid rods
12	1	*Staphylococcus hominis*	**27**	3	Diphtheroid rods
13	1	*Staphylococcus hominis*	**28**	None	No growth
14	None	No growth	**29**	2	*Staphylococcus haemolyticus*
15	None	No growth	**30**	2	*Staphylococcus epidermidis*

to the patient to squeeze the lids, which would double the meibomian gland discharge. Possibly, a guarded lid-speculum could have an impact on results as it would keep the lid margin more covered. On the other hand, it should not be expected to cause less discomfort for the patient, preventing squeeze and resulting in less meibomian gland discharge.

In the current study, we found a slight, albeit not significant, difference of culture negative cases in favor of the manually assisted eyelid retraction technique (36.7% vs. 26.7%). We consider this is in accordance with the proposed benefit of the manually assisted retraction technique, resulting in less meibomian gland excretion.

In our group, male patients had significantly higher culture positivity. This surprising finding could be result of the relatively small sample size. No qualitative or quantitative gender impact could be found on conjunctival flora.[16] One might consider a hormonal influence as meibomian glands – consequently ocular surface flora – are mediated by sex steroids.[17] However, the study group comprises elderly patients with a median age of 66. Thus, the influence of sex hormones on the results should be minor, if any.

The median number of injections for each patient was 4 in both groups (p=0.248). However, the number of prior injections ranged from 1 to 14 in culture negative cases and 1 to 11 in culture positive cases. Hence, we believe repeated use of post-injection antibiotics or PI did not have an impact on microbial results.

In this current study, all samples were obtained from the inferior fornix prior to the application of topical anesthesia and inoculated in a maximum of 2-hours time. Although the lack of anesthesia was somewhat annoying for the patient, we believe it prevented accidental contamination. Also, topical anesthetic drugs are known to have antimicrobial effects, even little, which would interfere with the outcome.[18] Moreover, quick delivery to the laboratory additionally helped to obtain an actual outcome, as conjunctival flora was reported to be dynamic and subject to change over time.[2,19] Operation room conditions and face mask wearing staff also minimized the risk of contamination. Likewise,

all isolated bacteria were belonging to ocular surface flora, e.g. *staphylococci*, diphteroids; no colonies of exogenous bacteria such as *Pseudomonas*, was isolated. One might consider that, treating the lids with 10% PI would be problematic as the antiseptic might have come in contact with the conjunctive and influence the culture results. However, we believe such an effect can be neglected, because such an interference would diminish, even inhibit bacterial growth; thus resulting in all cases to be culture negative.

Perhaps, there are some issues of concern. First of all, one could consider that finger flora would interfere with the microbiological outcome in the assisted retraction technique. We believe this could be neglected, as the assisting nurse was also wearing sterile gloves. Secondly, even resistance to manual technique could be considered; however, we did not encounter asuch resistance, the lids were easily retracted as the patients felt convenience perhaps. As a last - but not least – concern, the effect of various ocular flora patterns in various retinal disorders on the microbiological outcome could be questioned. The high glucose levels in the diabetic patients, might induce bacterial growth in the skin, ocular surface, and the conjunctive.[20] Diabetic patients were reported significantly to have gram-negative bacterial colonization compared to non-diabetics.[21] Diabetic retinopathy was found to be associated with higher prevalence of pathogenic bacteria in the conjunctival flora.[22] In our population in Group 1, 12 of the 15 patients with diabetes revealed a positive microbiological outcome; whereas in Group 2, the culture positive rate in diabetics was 13 of 16. Thus, we believe this did not interfere with the outcome.

The main limitation of our study is the relatively small sample size. Moreover, microbiological investigation together with the biochemical analysis of the meibomian glands could give more precise data. On the other hand, its prospective and blinded nature is the main strength. To the best of our knowledge, this is the first study to compare the two IV injection techniques by means of microbiological evaluation. Up to date, studies on IV injections mainly focused on aseptic agents and antibiotic use. However, manually assisted lid retraction technique provides two advantages: diminishing the risk of post-injection endophthalmitis and increasing patient comfort.

In conclusion, assisted manual lid retraction is a promising technique for IV injections, with enhanced microbiological outcome. Further studies in larger series combining microbiological evaluation with meibomian gland biochemistry and even pain scoring would be beneficial.

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