ORIGINAL ARTICLE

Antiseptic efficacy of topical diluted povidone iodine 2.5% and 1.25% on bacterial conjunctival load with intravitreal injection

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ABSTRACT

Background: *Intravitreal injections (IVIs) are considered one of the most important and minimally invasive procedures for treatment of many retinal diseases. Recently the instillation of povidone iodine to the surface of the eye is the only proven prophylactic measure that can lower the risk of endophthalmitis following intravitreal injection.* ***Objective:*** *The study aims to assess the antimicrobial efficacy of lower povidone iodine concentrations 2.5% and 1.25% in intravitreal injection.* ***Methodology:*** *This is a prospective interventional study that was conducted on 60 eyes of 45 patients with diabetic macular edema, retinal vein occlusion (RVO) and age related macular degeneration (AMD). Patients were divided into three groups, group one involved 20 eyes that received application of povidone iodine 5%, groups 2 and 3 received povidone iodine 2.5% and 1.25% respectively. Conjunctival swabs were taken before and after povidone iodine in the three groups, colony forming units (CFU) were calculated to detect the efficacy of povidone iodine in decreasing the bacterial load of the conjunctiva. The patient discomfort was recorded in a chart scale to evaluate pain.* ***Results:*** *The CFU was markedly decreased in the 3 groups with no detected statistical significance between all groups confirming the antiseptic efficacy of lower concentrations of povidone iodine. The lower concentration 1.25% showed more patient comfort and tolerability with lower score on pain chart scale.* ***Conclusion:*** *Lower concentrations of povidone iodine can achieve similar antiseptic effect compared to the standard 5% concentration with better tolerability.*

INTRODUCTION

Intravitreal injections (IVIs) are one of the most effective and minimally invasive procedures for management of many retinal diseases. The efficacy of such therapy is now well established in the treatment of exudative age-related macular degeneration, diabetic macular edema and edema associated with branch and central retinal vein occlusions. As the incidence of diabetes mellitus and cardiovascular diseases is expected to be elevated, the rate of IVIs will steadily continue to rise[1,2].

The incidence of endophthalmitis after anti-VEGF injection varies in the literature; one study reported low incidence of 1:6,450[3] and another one detected an incidence of 1:1200[4]. However, a group of 43 studies meta-analysis that included over 350,000 injections detected the rate at approximately1:1,800[5]. Unfortunately, about half of patients who develop post-injection endophthalmitis will not gain their primary level of best corrected visual acuity despite optimum treatment with intravitreal antibiotics, denoting the importance of prophylaxis against post injection endophthalmitis[3,5].

Up till now, no available data present to support that prophylactic use of antibiotics after intravitreal injection can reduce the incidence of endophthalmitis, many ophthalmologists continue to recommend the use of topical antibiotic eye drops prior to and after intravitreal injection. Recently, Bhavsar et al showed that the use of topical antibiotics did not reduce the endophthalmitis rate either when used before, in the same day of injection, or following intravitreal injections[6].

In another study, patients that received short-term repeated courses of topical antibiotic eye drops accompanying IVI showed increased incidence of infectious endophthalmitis by increasing antibiotic resistance of conjunctival flora[7]. This has been confirmed in several studies[8,9] that reported increased antibiotic resistance of conjunctival flora due to repeated use of fluoroquinolone drops.

Nowadays application of povidone iodine to the ocular surface is the only proven prophylactic measure that can decrease the incidence of post injection endophthalmitis[10,11]. However, the ideal concentration of PVI to use remains controversial, most of retina specialists use a concentration between 1.25% and 10%. On the other hand, the more bactericidal effect was

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obtained with reduced concentrations of PVI due to greater availability of free iodine[12,13].

This study evaluates the efficacy of lower concentrations of povidone iodine 2.5% and 1.25% as compared to the conventional 5% concentration in prophylaxis against endophthalmitis after intravitreal injection and reduction of mean CFU value of conjunctival flora together with relieved ocular discomfort assisted by comparative pain chart.

METHODOLOGY

Study design:

This is a prospective randomized interventional study that included 60 eyes of 45 patients attending Tanta University Eye Hospital in cooperation with Microbiology and Immunology Department, Faculty of Medicine, Tanta University from January 2020 till June 2020 after approval of the ethical committee of the Faculty of Medicine in Tanta University and under the rules of the 1964 Helsinki Declaration and its later amendment. All candidates explained the study objectives, methodology, duration, and possible risks before signing their informed consent for participation.

No fund by the university or any organization or entity was present in this study.

Participants:

Inclusion criteria included diagnosis of diabetic macular edema (DME), macular edema due to branch or central retinal vein occlusion (RVO), exudative age related macular degeneration (AMD) naïve patients were scheduled for injection of 0.5 mg ranibizumab into the vitreous with age more than 18 years old.

Patients with previous ocular surgery except cataract surgery, previous application of topical antibiotics or steroids and evidence of active intraocular inflammation or infection were not enrolled in the study.

After inclusion, the examined eyes were randomly assigned to three groups, group 1, 2, 3 that received topical application of povidone iodine 5%, 2.5% and 1.25% respectively for three minutes before intravitreal injection.

For pain assessment, a diary was provided for each patient to evaluate patient comfort in the 3 groups with recorded scale from 0 to 10 (0 = very comfortable; 1–3 = mild discomfort; 4–6 = moderate discomfort; 7–10 = severe discomfort). The highest value recorded for every patient throughout the study duration was considered for analysis purpose (figure 1).

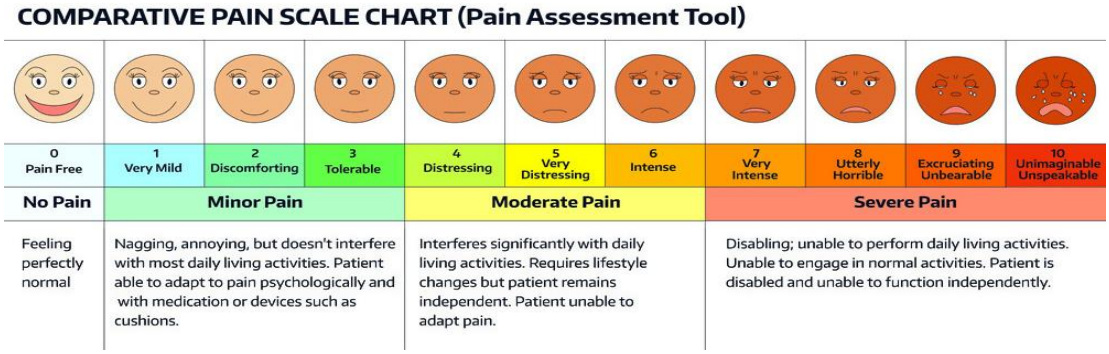

Fig. 1: Illustrates comparative pain scale chart to assess the patients discomfort score in the three groups.

Collection of the Conjunctival Sample:

Two conjunctival swabs from each eye in the three groups were taken before and 3 minutes after instillation of povidone iodine with different concentrations using sterile cotton swabs. The tip of the swab was applied to bulbar conjunctiva in the infero-temporal quadrant about 4 mm from the limbus (injection site) and was rotated over the conjunctiva. Care was taken to avoid contact with lashes, eyelids and skin. If it occurred, the patient was ruled out. Then the swabs were emulsified in 1ml of normal sterile saline from which the blood and chocolate agar plates were aseptically inoculated.

Injection procedure:

The intravitreal injection was performed under complete aseptic technique in the operating theatre with an operating microscope and the use of surgical mask.

The eye was prepared in a standard fashion using a drop of (Benoxinate hydrochloride 0.4%, Benox, Epico, Egypt) ophthalmic solution to the ocular surface for topical anaesthesia followed by topical application of 10% povidone iodine (Betadine) for periocular area, lids and eye lashes and povidone iodine (5% in group 1, 2.5% in group 2 and 1.25% in group 3) inside the conjunctival sac for three minutes before the intravitreal injection, and then sterile lid speculum was placed.

0.5 mg (0.05 ml) of ranibizumab (lucentis; Genenetch, South San Francisco, CA) was injected into the vitreous cavity in the inferotemporal quadrant of the globe using 30gauge needle 4 mm posterior to the limbus in phakic patients and 3.5 mm in pseudophakic patients.

Post operative care:

The eye was patched for several hours and the patients were examined the next day and the third day after injection to exclude any complication like elevation of the IOP, endophthalmitis, retinal break, retinal detachment and vitreous haemorrhage.

Follow up period was 4weeks interval following the first injection.

Microbiological Determinations:

By using a pipette,100 µl of the saline suspension was used for aseptic inoculation of each agar plate (blood and chocolate agar plate) within one hour of collection and at each time at which swabs were taken (before and after application of povidone iodine) by using spread plating technique with sterile glass rod (spreader) which was used for spreading the sample over the plates surface together with rotating the plates[14]. Blood agar plates were incubated anaerobically and chocolate agar plates were incubated in 5% CO2 at 37°C for three days.

After three days, all the plates were examined macroscopically to detect any colony present. Then identification of the colonies was done by routine bacteriological examination and counted to detect the bacterial number in 1 ml (CFU/ml). As each plate was inoculated with 100 µl of the saline suspension, the colonies number was multiplied by 10 to detect colony forming unit per ml and the mean of the counted colony in the two plates represented the count of each time of taking the swabs. The limit for positive cases was 10 CFU/ml and any case <10 CFU/ml was considered culture negative[15].

Statistical analysis:

Statistical presentation and analysis of the study was performed, using the mean, standard deviation, student t- test, Chi-square, Linear Correlation Coefficient, and the analysis of variance [ANOVA] tests by (Statistical Package for Social Science; SPSS, Chicago, IL, USA). The unpaired Student T-test was used to compare between two groups in quantitative data. Chi-square indicates independent row and column variables, without indicating strength or direction of the relationship. Pearson Correlation was applied to detect the correlation between two quantitative variables in one group. Student t test and Paired t test were applied to compare 2 continuous parametric variables (2 independent groups and 2 dependent groups), respectively. P-value ≤ 0.05 is considered significant.

RESULTS

Base line demographic data including age, gender and cause of injection was illustrated in table (1). The mostly injected patient group was DME followed by RVO and AMD.

Table 1: Demographic data and diagnosis of all patients

		N	%
Age	**Range**	33 – 65	
	Mean ± SD	48.70 ± 7.21	
Gender	**Male**	30	66.66
	Female	15	33.33
Diagnosis	**DME**	32	53.3
	RVO	15	25
	AMD	13	21.7

DME: Diabetic macular edema RVO: Retinal vein occlusion
AMD: Age related macular degeneration

Thirty-three culture-positive cases were detected (tables 2,3), also the most frequently detected organisms were coagulase negative staphylococci (CONS) representing 66.7% followed by coagulase positive staphylococci, alpha hemolytic streptococci and bacillus species representing 18.2%, 9.1% and 6.1% respectively (figure 2).

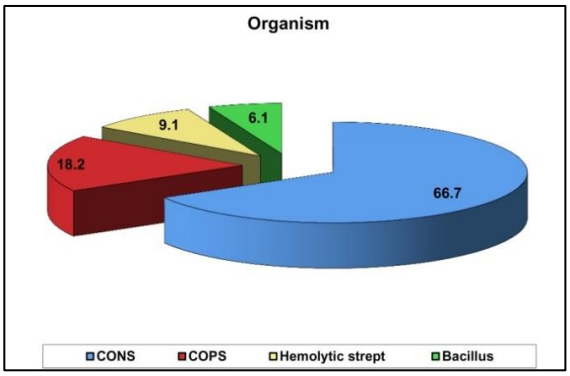

Fig. 2: Illustrates types of microorganisms detected in the study cases, the most common is CON (coagulase negative staph) followed by COP (coagulase positive staph), hemolytic streptococci and bacillus.

Table 2: Number of culture positive eyes in the studied patients

		N	%
Culture	**Negative**	27	45
	Positive	33	55

Table 3: Number of culture positive eyes in each group

Culture	**Group 1**		**Group 2**		**Group 3**		**Total**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Positive	12	60	10	50	11	55	33	55
Negative	8	40	10	50	9	45	27	45
Total	20	100	20	100	20	100	20	100

Regarding CFU in all groups, CFU was markedly decreased after instillation of povidone iodine in all groups with p value= 0.001*, with no statistically recorded significance between the three groups (table 4, figure 3).

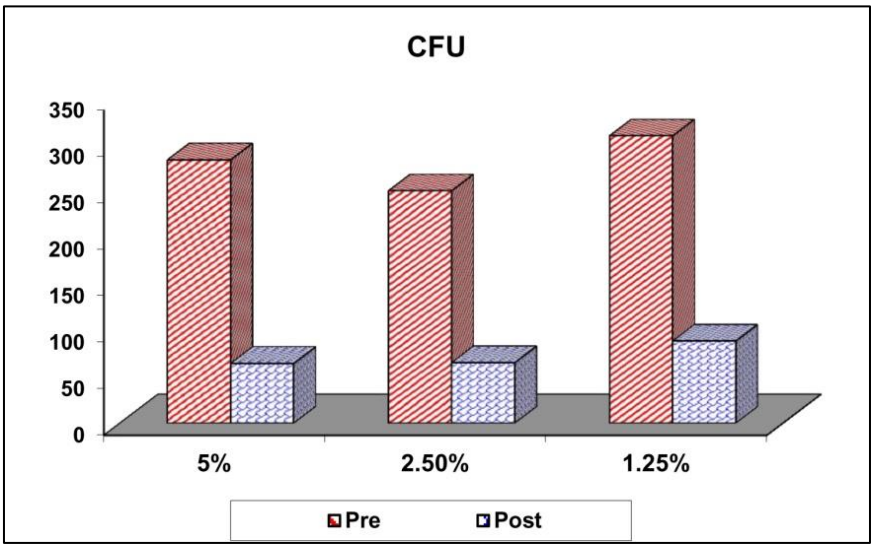

Fig. 3: Illustrates CFU (colony forming unit) before and after povidone iodine in the three groups.

Table (4): Number of CFU (colony forming unit) in each group before and after instillation of povidone iodine

CFU		Group 5%	Group 2.5%	Group 1.25%	F. test	P. value
Pre	**Range**	100 – 400	100 – 400	100 – 400	0.669	0.5619
	Mean ± SD	283.33 ± 119.34	250.00 ± 126.93	309.09 ± 104.45		
Post	**Range**	20 – 100	20 – 100	40 – 200	1.581	0.222
	Mean ± SD	64.17 ± 31.75	65.00 ± 33.42	88.18 ± 42.15		
	T. test	6.148	4.457	6.505		
	P. value	0.001*	0.001*	0.001*		

The patient discomfort score was markedly reduced with povidone iodine 1.25% group if compared to 5, 2.5% with p value= 0.001*; hence, the lowest concentration in group 3 achieved the least discomfort and ocular irritation in injected patients (table 5, figure 4). No endophthalmitis cases were recorded after injection.

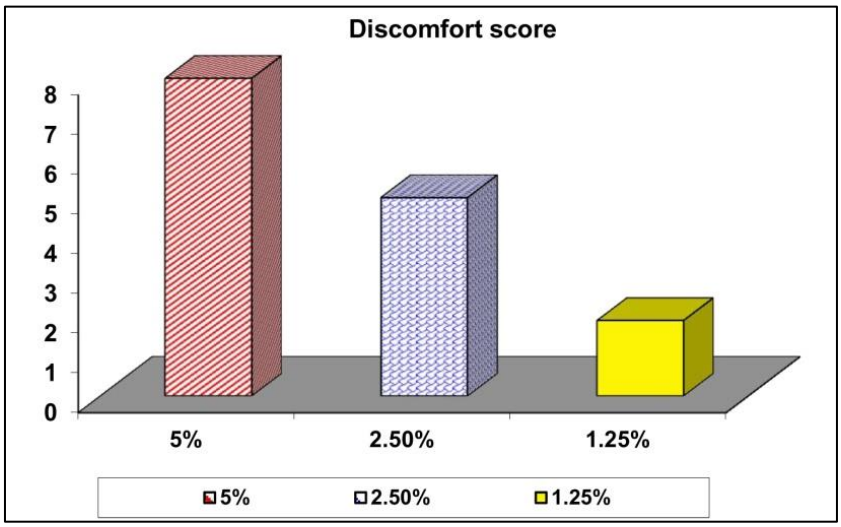

Fig. 4: Illustrates patient discomfort score in the three groups after instillation of povidone iodine.

Table 5: Patient discomfort score in all patients

Discomfort score	**Group 1**	**Group 2**	**Group 3**
Range	6 – 10	4 – 6	1 – 3
Mean ± SD	8.00±1.45	5.00±0.86	1.90±0.85
F. test	**56.428**		
p. value	**0.001***		
Group1,2	**Group 1,3**		**Group2.3**
0.001*	0.001*		0.001*

DISCUSSION

Povidone iodine is the most popular antiseptic agent used for pre-operative preparation in eye surgery, the recommended concentration being approved by the guidelines of the European society of cataract and refractive surgeons (ESCRS) and the American Academy of Ophthalmology (AAO) is 5%[16,17]. Prophylaxis against infection has become now a crucial step in intravitreal injection since the rate of intravitreal injection has markedly increased approaching 20 million approximately in 2016 [18,19]. It has been well documented that the rate of endophthalmitis following intravitreal injection did not decrease after topical antibiotic prophylaxis, but in contrast, it has been accompanied with a higher incidence of endophthalmitis[20], and antibiotic resistance[8].

The main microorganism isolated from conjunctival swab in culture-positive cases in the present study was the coagulase negative staphylococci (CONS) including the most popular *Staphylococcus epidermidis* followed by *Staph aureus*, alpha hemolytic streptococci and finally bacillus species that represent the least common microorganism, this was coincident with other study done by Reibaldi et al[21] that reported similar microorganism prevalence, besides, one more study done by Zhu et al[22] detected positive culture in 44.2% of cases with also prevalence of *Staph epidermidis* that agrees with our results.

Many studies recommended the use of povidone iodine 5% for antimicrobial prophylaxis; Stem et al. did the first one, who recorded nearly similar rate of endophthalmitis between povidone iodine 5 and 10%[23], another study performed by Hosseini H et al reported non inferiority of povidone iodine 5% for 15 minutes and 10% for 5 minutes for antimicrobial prophylaxis against endophthalmitis[24]. Multiple studies evaluated the antimicrobial effectiveness of more diluted PI solution[22,25,26,27]. This study evaluated the bacterial load of the cojunctiva with the use of different povidone iodine concentrations as most of bacterial inoculation occurs at the time of intravitreal injection following needle contact with conjunctival surface[28].

Regarding our study, the CFU was markedly reduced after instillation of all povidone iodine concentrations in all groups with recorded statistical significance (p value 0.001*), in other words all povidone iodine concentrations achieved approximately similar antimicrobial efficacy with no recorded statistical significance.

Other studies documented that very low concentration of povidone iodine 0.6% can result in similar efficacy to povidone iodine 5% with faster antimicrobial activity[29]. Increased availability of free iodine which is the active antimicrobial agent enhances the bactericidal efficacy of 0.6% PI compared to higher concentrations[12].

Although, this study was not to designed to detect a difference in endophthalmitis rates between the three groups, no endophthalmitis cases were recorded, this was quite similar to another study done on 0.6% povidone iodine that detected zero endophthalmitis cases[21]. In contrast, some studies reported very low endophthalmitis rates with povidone iodine and this is probably due to the small sample size in our study[22,27]. The purpose of this study was to assess whether the treatment with lower povidone iodine concentrations (2.5% and 1.25%) was effective in decreasing the bacterial load of the conjunctiva before the intravitreal injection.

For pain assessment, a scale chart was used to evaluate the patient discomfort which is graded as a score from 0 to 10, according to table (5) in results section, the lowest score was detected with the lowest concentration of povidone iodine 1.25% followed by higher concentration 2.5%, the highest concentration 5% showed the highest score, this indicates more patient tolerability and comfort with lower povidone iodine concentrations, this is supported by another study which reported the less povidone iodine concentrations have a better tolerability compared to 5% PI solution[30].

CONCLUSION

In conclusion, this study proved the efficacy of the treatment with lower povidone iodine concentrations 2.5%,1.25% in decreasing the bacterial load of the conjunctiva with excellent safety profile and more patient comfort and satisfaction.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Ethics approval and consent to participate: The research was approved by the Ethical Committee of the Faculty of medicine, Tanta University, Egypt. Written consent was obtained from all participants.

Competing interests: The authors declare that they have no competing interests.

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