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Subconjunctival injection of anti-VEGF agent bevacizumab as treatment in patients with dry eye disease

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Abstract: To assess the effect of subconjunctival injection of anti-VEGF bevacizumab in the management of dry eye disease in a tertiary care hospital. In this quasi-experimental trial 150 eyes of 75 patients were selected using non-probability consecutive sampling technique. Detailed clinical examination was performed, the ocular surface disease index (OSDI) questionnaire score, tear film break-up time (TBUT) and Schirmer test 2 were measured and compared pre and post injection. Six patients were excluded and sixty-six patients were included having the mean age was 65.3 (SD=±10.2) years, 50% were aged 66-83 years old, 65.2% were female. Pre injection OSDI score was 30.3 (SD=±2.79), whereas post injection it was 20.2 (SD=±3.01). Pre injection TBUT was 3.0 (SD=±0.30), whereas post injection it was 5.17 (SD=±0.40). Pre injection Schirmer 2 test was 7.97 (SD=±0.51), whereas post injection it was 10.5 (SD=±0.50). Ten patients suffered mild subconjunctival hemorrhage which resolved spontaneously. Three patients were lost to follow up. Subconjunctival injection of anti-VEGF agent bevacizumab can offer a modern and safe solution in patients suffering from dry eye disease nevertheless more trials with large number of patients and longer follow up durations are required for widespread adaptation.

Keywords: Bevacizumab, dry eye disease, ocular surface disease index, tear film break-up time, Schirmer test 2, ocular surface microenvironment.

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INTRODUCTION

Dry Eye Disease (DED) is more prevalent in Asia compared to Europe and North America signifying a role of racial and cultural influences. It is one of the most shared ocular condition distressing tens of millions of patients worldwide (Tsubota *et al*., 2020a).

A single global consensus regarding the definition and diagnosis of DED does not exist however practical discussions held among practitioners based on The Asia Dry Eye Society Report (Tsubota *et al*., 2017), TFOS DEWS II ® Definition and Classification Report (Craig *et al*., 2017) and The Dry Eye Syndrome Preferred Practice Pattern® of AAO (Akpek *et al*., 2019) proposed that it is a multifactorial illness, it is specified by a constant unstable and /or deficient tear film, it causes discomfort and /or visual loss and it usually occurs with inflammation, neurosensory irregularities and ocular surface epitheliopathies (Tsubota *et al*., 2020a).

Patients suffering from DED develop a chronic ocular surface inflammation associated with a hyperosmolar tear film (Jiang *et al*., 2015). These patients suffer from ocular irritation, pain, redness, blurred vision and epiphora due to an unbalanced tear film (Kamil, 2021). The corneal epithelium, bulbar conjunctiva, meibomian glands, main and accessory lacrimal glands, connecting nerve networks, immune & matrix cells, micro biome and hormones all interact in accord to form a strong Lacrimal Function Unit (LFU) and Ocular Surface Microenvironment (OSM). DED is known as the disruption of this homeostasis (Zhang *et al*., 2017).

One of the pathogenic factors causing inflammation in dry eye disease is lymphangiogenesis and neovascularization caused by Vascular Endothelial Growth Factor (VEGF) production. Bevacizumab, is a humanized monoclonal antibody that adheres to as well as blocks the biological activity of all the subtypes of VEGF (Eski *et al*., 2022). Studies have shown a drastic effect of eliminating symptoms of dry eye in patients by topical, intra meibomian gland and subconjunctival injection of bevacizumab (Jiang *et al*., 2015, Jiang *et al*., 2018, Kamil 2021, Kasetsuwan *et al*., 2020).

Our present study aims to treat the symptoms of dry eye disease by sharing the experience of injecting subconjunctival bevacizumab in patients of dry eye in a tertiary care hospital.

MATERIALS AND METHODS

This quasi-experimental trial was conducted in the out-patient department (OPD) of The Layton Rahmatullah Benevolent Trust (LRBT) Tertiary Teaching Eye Hospital, Korangi, Karachi from February 2023 to July 2023. After obtaining approval from the Hospital Ethical Review Committee (LRBT/TTEH/ERC/4405/01), in this single center study, we used non-probability consecutive

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sampling technique and included a total of 150 eyes of 75 patients after taking informed consent and explanation of the nature and possibility of the adverse consequences of the procedure during the 6 months of study.

The following inclusion criteria were applied: patients aged 40-83 years, chronic symptoms of ocular irritation, pain, redness, blurred vision and epiphora in both eyes for >6 months, no history of ocular surgery or trauma in last 6 months, and ability for regular follow-ups. The study excluded patients with structural abnormalities like eyelid trichiasis, entropion and scarring, patients with pinguecula or pterygium, patients with any active allergy or inflammation at the ocular surface unrelated to dry eye, patients with intraocular inflammation or structural change in the iris or anterior chamber, patients with glaucoma or any vitreoretinal pathology, patients using any topical medication other than artificial tears, past history of ocular surgery or trauma in last 6 months.

Before the injection, a detailed clinical examination with the slit lamp biomicroscopy was performed and patient's age, gender, status of the lens, Best Corrected Visual Acuity (BCVA) and Intra Ocular Pressure (IOP) were recorded. The patient's symptoms were assessed using the Ocular Surface Diseases Index (OSDI) questionnaire. The Tear Film Break-Up Time (TBUT) was measured and the Schirmer test 2 was performed without using anesthesia. After baseline evaluation, in the operating room the patients received bilateral subconjunctival injection of bevacizumab (100ul, 2.5mg/0.1ml, Avastin). Before the injection, a drop of proparacaine was instilled in the conjunctival sac to serve as a topical anesthetic. All the injections were injected at the same site that is 3mm beneath the inferior limbus in the bulbar conjunctiva. A drop of moxifloxacin was applied immediately after the injection. All the patients were re-evaluated at 1week, 1 month, 3 months and 6 months after the injection. The changes in the OSDI questionnaire score, TBUT and Schirmer test 2 were recorded.

STATISTICAL ANALYSIS

The data was stored and analyzed using IBM-SPSS version 23.0; counts with percentages were reported for age group and gender. Mean and standard deviation of pre and post injection OSDI, TBUT and Schirmer 2 test were reported. Paired sample t-test was used to compare the mean difference for overall, age group and gender classification of gender. P-values less than 0.05 were considered statistically significant, tables were also used to give graphical presentation of study findings.

RESULTS

Table 1 reports the baseline characteristics of studied samples, in the present study there were sixty-six samples, mean age was 65.3 (SD=±10.2) years, 50% were aged 66 - 83 years old, 65.2% were female gender.

Table 2 reports the mean comparison of pre and post injection OSDI scores, results showed pre injection OSDI score of overall samples was 30.3 (SD=±2.79), of age 40-65 years old samples was 29.68 (SD=±2.69), of age 66-83 years old samples was 31.07 (SD=±2.76), of female samples was 30.58 (SD=±2.64) and of male samples was 29.99 (SD=±3.09), whereas post injection OSDI scores of overall samples was 20.2 (SD=±3.01), of age 40-65 years old samples was 19.11 (SD=±2.34), of age 66-83 years old samples was 21.49 (SD=±3.17), of female samples was 20.40 (SD=±2.97) and of male samples was 20.11 (SD=±3.16). Paired sample t-test showed a significant decrease in post injection OSDI scores with $p<0.001$ for overall, age group and gender classification.

Table 3 reports the mean comparison of pre and post injection TBUT, results showed pre injection TBUT of overall samples was 3.0 (SD=±0.30), of age 40-65 years old samples was 2.92 (SD=±0.31), of age 66-83 years old samples was 3.09 (SD=±0.27), of female samples was 2.99 (SD=±0.30) and of male samples was 3.04 (SD=±0.32), whereas post injection TBUT of overall samples was 5.17 (SD=±0.40), of age 40 – 65 years old samples was 5.05 (SD=±0.42), of age 66 – 83 years old samples was 5.29 (SD=±0.37), of female samples was 5.12 (SD=±0.38), and of male samples was 5.27 (SD=±0.45). Paired sample t-test showed a significant increase in post injection TBUT with $p<0.001$ for overall, age group and gender classification.

Table 4 reports the mean comparison of pre and post injection Schirmer 2 test, results showed pre injection schemer 2 test of overall samples was 7.97 (SD=±0.51), of age 40-65 years old samples was 8.0 (SD=±0.5), of age 66-83 years old samples was 8.0 (SD=±0.5), of female samples was 8.0 (SD=±0.5), and of male samples was 8.0 (SD=±0.6), whereas post injection Schirmer 2 test of overall samples was 10.5 (SD=±0.50), of age 40-65 years old samples was 10.1 (SD=±0.5), of age 66-83 years old samples was 10.1 (SD=±0.4), of female samples was 10.1 (SD=±0.4) and of male samples was 10.0 (SD=±0.6). Paired sample t-test showed a significant increase in post injection schemer 2 test with $p<0.001$ for overall, age group and gender classification.

DISCUSSION

The most common reasons for patients visiting eye clinics are symptoms related to episodic flares in chronic dry eyes. The ocular surface of these patients is continually damaged via a vicious cycle of inflammatory cascade with frequent exacerbation of symptoms through stresses such as drafty environment, contact lens wear, medicinal eye drops, allergies or post cataract surgery (Perez *et al*., 2020).

Table 1: Baseline Characteristics of Studied Samples (n=66)

Characteristics		n	%
Age Group	40 - 65 years	33	50.0
	66 - 83 years	33	50.0
	Mean ±SD	65.3	±10.2
Gender	Female	43	65.2
	Male	23	34.8

Table 2: Pre and Post Injection OSDI Scores

Parameters		Pre-Injection OSDI Score		Post Injection OSDI Score		p-value
		Mean	SD	Mean	SD	
Overall	N=66	30.3	2.79	20.2	3.01	<0.01*
Age Group	40 - 65 years (n=33)	29.68	2.69	19.11	2.34	<0.01*
	66 - 83 years (n=33)	31.07	2.76	21.49	3.17	<0.01*
Gender	Female (n=43)	30.58	2.64	20.40	2.97	<0.01*
	Male (n=23)	29.99	3.09	20.11	3.16	<0.01*

*p<0.05 was considered statistically significant using paired sample t-test

Table 3: Pre and Post Injection TBUT

Parameters		Pre Injection TBUT		Post Injection TBUT		p-value
		Mean	SD	Mean	SD	
Overall	N=66	3.0	0.30	5.17	0.40	<0.01*
Age Group	40 - 65 years (n=33)	2.92	0.31	5.05	0.42	<0.01*
	66 - 83 years (n=33)	3.09	0.27	5.29	0.37	<0.01*
Gender	Female (n=43)	2.99	0.30	5.12	0.38	<0.01*
	Male (n=23)	3.04	0.32	5.27	0.45	<0.01*

*p<0.05 was considered statistically significant using paired sample t-test

Table 4: Pre and Post Injection Schirmer 2 test

Parameters		Pre-Injection Schirmer 2 test		Post Injection Schirmer 2 test		p-value
		Mean	SD	Mean	SD	
Overall	N=66	7.97	0.51	10.5	0.50	<0.01*
Age Group	40 - 65 years (n=33)	8.0	0.5	10.1	0.5	<0.01*
	66 - 83 years (n=33)	8.0	0.5	10.0	0.5	<0.01*
Gender	Female (n=43)	8.0	0.5	10.1	0.4	<0.01*
	Male (n=23)	8.0	0.6	10.0	0.6	<0.01*

*p<0.05 was considered statistically significant using paired sample t-test

Asthenopia is a common computer associated eye problem and is experienced by 55-81% of screen users but in recent times, a progressively common risk factor for dry eyes has emerged which is digital screen use (smartphones, tablets, laptops and computers). The accepted hypothesis regarding their effect is altered blinking dynamics, reduction in both blink rate and blink completeness, tear film instability, meibomian gland and goblet cell dysfunction, ocular surface exposure and corneal phototoxicity by peak emission wavelengths in modern LEDs causing dry eyes during extended periods of digital screen use. The American Academy of Ophthalmology and American Optometric Association recommends 15 minutes rest every 2 hours or the "20-20-20 Rule" which instructs the user to focus an object 20 feet away for 20 seconds after every 20 minutes of computer viewing. (Al-Mohtaseb *et al*., 2021, Mehra and Galor, 2020).

The central machinery of DED is the unsteadiness in the tear film and the concept of categorizing it according to the layers of tear film is denoted as The Tear Film Oriented Diagnosis (TFOD) (Kojima *et al*., 2020). Recently three types of dry eyes have been recognized: 1) increased evaporation, 2) aqueous deficient and 3) decreased wettability, these coincide with the problem related to each layer affected: 1) Lipid, 2) aqueous/ secretory mucin and 3) membrane-associated mucin (Tsubota *et al*., 2020b). All three types have overlapping contributory factors like tear instability, increased tear film osmolarity, inflammation and ocular surface damage (Jiang *et al*., 2018).

The vital function of a strong OSM is to preserve a uniform tear film for the protection of the even optical surface, epithelial cell well-being and ocular relief. It also provides defense from environmental and microbial abuses (Zhang *et al*., 2017). The inflammation in DED is the result of increase release of inflammatory cytokines, such as interleukin (IL)-1α, IL-1β, tumor necrosis factor α (TNFα) and vascular endothelial growth factor (VEGF). VEGF, a hemangiogenic and lymphangiogenic agent, acts as a pro-inflammatory cytokine and encourages neovascularization, which in turn harvests more pro-inflammatory cytokines, creating a vicious cycle. Hence, a step towards treatment involves removing one of the factors in this cycle (Kasetsuwan *et al*., 2020).

Normal adult vasculature is not affected by VEGF. Anti-VEGF treatment combats tumors that use VEGF as a surviving factor without harming the normal healthy vessels. Its anti-angiogenesis and anti-lymphangiogenesis properties have been use in the treatment of systemic malignancies most notably colorectal carcinomas. When used intravitreally by eye care providers, it acts by inhibiting angiogenic processes.(Neves da Silva *et al*., 2022) Despite not being approved by the FDA for intravitreal use, the most widely used off-label biologic in Anti-VEGF class is Bevacizumab also known as Avastin® is a recombinant humanized monoclonal IgG1 antibody that can couple to all isoforms of VEGF-A, it binds and defuses VEGFs and stops their interaction with endothelial cell receptors (Mahmood *et al*., 2023).

Intravitreal bevacizumab has been applied in the management of several ocular diseases such as wet age-related macular degeneration with choroidal neovascularization, neovascular glaucoma, retinal vascular occlusions and diabetic retinopathy. Recently topical preparations of bevacizumab (eye drops, subconjunctival or intrastromal or intra meibomian gland injection) for the treatment of corneal, conjunctival and eyelid telangiectasia and vascularization has become popular (Baradaran-Rafii *et al*., 2021). In a recent study, management of primary pterygium with intralesional bevacizumab injection relieved symptoms with minimum complications (Mahmood *et al*., 2023). Previous studies report that intra meibomian gland injection of bevacizumab is safe and effective and can decrease lid margin telangiectasia by 42% and improve the symptoms and clinical signs of dry eyes and blepharitis (Tantipat *et al*., 2022). The core limitation of our study was a missing control group, a small sample size of just 75 patients, and a short follow up period of 6 months. More studies are required with bigger sample size and lengthier follow up periods to evaluate the safety and efficiency of this treatment.

CONCLUSION

Our present study suggests that subconjunctival injection of anti-VEGF agent Bevacizumab can offer a modern and safe solution in patients suffering from dry eyes however more controlled trials, randomized studies and clinical researches having longer follow up periods and greater cohorts are essential to ideally judge the safety and efficacy of bevacizumab in treatment of dry eye disease.

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