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Original Research Article

To study the role of platelet rich plasma as treatment in various etiologies of dry eye

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ARTICLE INFO ABSTRACT

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Purpose: To evaluate the effect of platelet rich plasma as treatment modality in various etiologies of dry eye.

Materials and Methods: A prospective study was conducted at a tertiary eye care hospital on 65 eyes of 40 patients for a duration of three years, who presented with the complaints of dry eye due to various etiologies. Platelet rich plasma (PRP) was made from the patient's serum and they were advised to be instilled in topical eyedrop form six times a day.

Results: The study included 29 men and 11 women. The mean age was 40yrs (34.45±15.17), range 10 - 60 years. Dry eye symptoms like burning sensation, itching, dryness, blurred vision and redness were studied and were found to be significantly reduced (p=0.0001)post treatment with PRP after one month. The improvement in symptoms was most likely due to indirect reduction of inflammation. Around 30.7% eyes had improvement in visual acuity of atleast one line. The Schirmer's test also showed improvement with Schirmer values before treatment being 5.34mm± 2.18 mm to 15.90 mm±7.75mm after treatment. Around 46.87% right eye and 39.9% left eye also had improvement with tear break up time more than 10 sec.

Conclusion: The use of PRP eye drops for the treatment of various etiologies of dry eye was shown to be effective in reducing the symptoms of dry eye without any significant complications.

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1. Introduction

Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, tear film instability with potential damage to ocular surface. It is accompanied by increased osmolarity of tear film and inflammation of ocular surface.¹ It can present with complications like vision deficit, scarring and corneal perforation.²

The underlying causative process of dry eye are generally irreversible and management is therefore structured around the control of symptoms and prevention of surface damage. Lubricating supplements are the medications most commonly used to treat dry eye. However severe dry eye

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1.1. Platelet rich plasma (PRP)

Autologous PRP was first used in 1987 in cardiac surgery.³ Since then PRP has been used in various specialties such as maxillofacial, cosmetic, spine, orthopedic, and pediatric as well for general wound healing.^{4,5} Recently, PRP has been investigated in the use of ophthalmic conditions including dry eye, chemical burns,⁶ post laser assisted in situ keratomileusis (LASIK) dry eye syndrome,⁷ dormant corneal ulcer,⁸ moderate to severe dry eve and also in patients who discontinued contact lens wear because of discomfort. PRP contains concentrated platelet along with the platelet growth factors which helpin healing. PRP is

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cases are usually unresponsive to conventional artificial tear drops.

different from autologous serum as it contains concentrated platelets along with growth factors whereas autologous serum contains components similar to natural tears but does not contain the formed elements of blood (Red blood cells, White blood cells or Platelets) nor the clotting proteins.

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Alio et al. in Spain conducted a study to evaluate the effect of platelet rich plasma in the treatment of ocular surface disorders.⁹ They found out that autologous E-PRP eye drop application improves regeneration of the ocular surface and relieves symptoms in patient in symptomatic dry eye and post LASIK ocular surface syndrome.

Similarly, a study on autologous platelet rich plasma eye drops in treatment of recurrent corneal erosions (RCE) was conducted by Jun Hun Lee et al.¹⁰ It was seen that 80% of the patients treated just with lubricating eye drops had major recurrences while 22.2% patients had major recurrences in the PRP treated group. Therefore, PRP eye drop usage was considered to be safe and it reduced recurrence rate without any significant complications.

Mohit Jain et al. conducted a randomized, prospective, comparative study in on topical autologous platelet rich plasma eyedrops for acute corneal chemical injury¹¹ on 20 patients. They concluded that addition of topical autologous PRP to standard treatment protocols help in rapid epithelization of ocular surface and achieve better corneal clarity.

Hence it seemed worthwhile to conduct this study to evaluate the effect of Platelet Rich Plasma on various conditions related to dry eye- chemical injury, recurrent corneal erosions, neurotropic ulcer.

2. Materials and Methods

Hospital based prospective study was done on 65 eyes of 40 patients who presented at Ophthalmology out patient department of tertiary eye hospital. The study included patients above 10 years and less than 60 years of age with symptoms of dry eye presenting with Schirmer test less than 15mm and tear break up time less than 10 seconds. Patients who were excluded from the study were those who refused for the study, those with abnormal platelet count, thrombocytopenia, severe metabolic and systemic disorders, acute and chronic infections, lactating and pregnant women, with history of glaucoma, previous ocular surgery, patient on regular use of antiglaucoma eyedrops or known tear interfering systemic drugs (hormone replacement and antihistaminics).

2.1. Methodology

A thorough history taking and physical examination was done. The symptoms of dry eye - decreased vision, burning sensation, foreign body sensation, redness, and photophobia were noted along with onset and progression. Severity of perceived symptoms was evaluated by their frequency using classical symptoms of dry eye e.g- dryness, itching, burning and redness.

Each symptom was graded from 1 to 4 with Grade 1: mild / episodic, Grade 2: moderate episodic or chronic, Grade 3: severe or constant and Grade 4: severe or disabling.

History related to usage of antiglaucoma medications or any other medication was noted. Patients were evaluated for systemic morbidities- diabetes, hypertension, cardiovascular disease. Local examination includedunaided visual acuity, best corrected visual acuity, automated refraction, Schirmer 1 (in mm) without anesthesia, tear break up time, slit lamp examination of anterior segment, staining of cornea with fluorescein strips.

2.2. Schirmer test

The patients underwent Schirmer test on the day of presentation and itwas subsequently repeated on follow up visits on day 7, day 15 and at 1month. The Schirmer values were studied before and after the application of PRP drops. The values were grouped into four grades.

Grade 1: variable or normal (15-30mm)

Grade 2: Schirmer<10 mm,

Grade 3: Schirmer <5mm and

Grade4: Schirmer≤ 2mm.

Tear break up time was studied after instilling fluorescein drops and noting first dry spot on slit lamp. In our study we graded the TBUT value in 4 grades-

Grade 1: variable or normal, Grade2: >5 sec to \leq 10sec, Grade 3: \leq 5 sec Grade 4: immediate

2.3. Preparation of platelet rich plasma (PRP)

Before venipuncture, we used povidone iodine 10% and alcohol swab to scrub the area in all directions from intended site of venipuncture three times. Blood sample was drawn in 10 ml syringe.9ml of whole blood was placed in 10ml vacutainer tubes containing anticoagulant-citratedextrose solution (ACD). Samples were gently agitated to mix the anticoagulant thoroughly with the whole blood and centrifugation done at 2000 rpm for 11 min. After centrifugation upper two layer of centrifuged blood, the plasma and buffy coat layer were separated in sterile manner. Around 1-2 ml plasma was collected as final autologous PRP product. The final preparation was stored in 5ml empty bottle of antibiotic eye drops to avoid any risk of infection and was stored in refrigerator at 4°C. Patients were instructed to store the vial in refrigerator in upright position.

Dosage: Patients were advised to instill the drop every 2 hourly i.e. six times a day for one month after taking

precautions.



Fig. 1: a: Centrifugation machine. **b:** Showing PRP tube containing Acid Citrate Dextrose (ACD Tube)

3. Results

The study included 65 eyes of 40 patients who fulfilled the inclusion criteria.

3.1. Demographic details

All patients were in the age group from 10 years to 60 years. Maximum number of patients i.e. around 20% of the study population were in the age group 40-50 years. However, the mean age of presentation was 34.45 ± 15.17 . Out of these 29 (72.50%) were males and 11 (27.50%) were females.24 cases (60%) were from urban background while 16 cases (40%) were from rural background. Left eye were involved more than right eye. According to occupation 18(45%) patients who presented were quarry workers others were farmers 02 (5%), factory workers 05 (12.50%), students 06(15%) and housewives 09(22.50%).

In our study 24(60%) cases were dry eye related to chemical injury. Other causes included post herpetic dry eye 02(5%), severe vernal keratoconjunctivitis 03(7.50%), rheumatoid arthritis 02(5%), recurrent corneal erosions 02(5%) and other idiopathic conditions 06(15%).

3.2. Effect of PRP on symptoms of dry eye

Patients were evaluated for signs and symptoms of dry eye which included itching, dryness, burning, redness and blurred vision. The symptoms were graded on scale of 1 to 4 with 1 being mild and 4 being the worst as described earlier.(Table 1) Patients were evaluated on day of presentation (day 0) then followed up at day 7, 15 and at 1 month after the instillation of PRP drops.

Each symptom was studied individually and comparison was made in the grade of symptoms before and after application of PRP.

As for itching and foreign body sensation, although all patients had improvement, 35% (14/40) had total absence of symptoms and 65% (26/40) had some improvement.For dryness 22.5% (9/40) had total absence of symptoms and 77.5% (31/40) had some improvement. Burning sensation

42.5% (17/40) had total absence of symptoms while 57.5% (23/40) had some improvement About 80% (32/40) had complete absence of symptoms of redness, while 20% (8/40) had some improvement. Regarding blurred vision 87.5% (35/40) had some improvement while 12.5% (5/40) had no improvement in this symptom.

Hence, on comparing symptoms before and after treatment at one month, it was seen that there was significant improvement (p=0.0001) in the symptoms of dry eye after application of topical PRP.

Before treatment most of the symptoms were in grade 3 and after 1 month there was symptomatic improvement and mean grade ranged between $1.60\pm0.54 - 2.12\pm0.03$ that is, symptoms improved from Grade 3 to Grade 1.(Table 2)

3.3. Improvement in best corrected visual acuity

At the time of presentation 23.33% (14) eyes had visual acuity less than 6/60 and 61.5% (40) eyes had visual acuity in the range of 6/60 - 6/24. Hence, most of the patient presented with visual acuity 6/60 - 6/24 at day 0. Vision between 6/18 - 6/12 was seen in 9 eyes, 6/9 vision in 2 eyes, while none of the patient had visual acuity of 6/6 on Day 0 at presentation.(Table 3)

At the end of 1 month 26 eyes (40 %) did not show any improvement in visual acuity. Around 20 eyes (30.7%) showed 1 line improvement in visual acuity. (11 Right eyes (34.38%) and 9 Left eyes (27.27%) showed one line improvement). 11 (16.92%) eyes showed 2 line improvement in vision whereas 8 eyes (12.30%) showed improvement in more than 2 lines. (Table 4)

3.4. Schirmer test value

Schirmer test was done using Schirmer strip and wetting of the paper was noted and graded on scale of 1- 4 with 1 being normal and 4 being <2mm.

Most of the patients presented with grade 3(<5mm)Schirmer level in both eyes before starting PRP treatment, that is around 23 right eye and 15 left eye had Schirmer values less than 5 mm before administration of PRP which subsequently showed improvement and it was seen that after one month of treatment with PRP, 46.15% (16 right and 14 left eye out of 65 eyes) had Schirmer values of grade 1 > 20mm. (Table 5)

Considering the value in millimeters, we found an improvement in mean Schirmer test from $5.34\text{mm}\pm2.18$ in Right eye before treatment to $15.90\text{mm}\pm7.75$ after treatment, while in left eye the readings were 5.87 ± 3.06 to 15.72 ± 8.36 . (Table 5). On comparing Schirmer test value for both eyes at 0 day and at 1 month using student t test. There was significant improvement in Schirmer test value (p<0.0001) in both right and left eye.

Grade	Itching (No.of patients)		g (No.of Dryness (No.of patients)		Burning (No.of patients)		Redness (No.of patients)		Blurred Vn (No.of patients)	
	Before RX	1mth after RX	Before RX	1mth after RX	Before RX	1mth after RX	Before RX	1mth after RX	Before RX	1mth after RX
1	01	14	00	09	01	17	15	32	00	00
2	09	23	05	26	09	22	19	07	16	35
3	23	03	27	05	25	01	03	01	24	05
4	07	00	08	00	04	00	03	00	00	00
Total	40	40	40	40	40	40	40	40	40	40
Mean±SI	2 .90±0.70	1.72+0.59	3.07 ± 0.57	1.90=0.59	2.82 ± 0.63	1.60=0.54	1.85 ± 0.86	1.22=0.47	2.82 ± 0.63	2.12+0.33

Table 1: Symptoms before and 1 month after treatment with PRP

Table 2: Statistical analysis of symptoms using t test. Symptoms were compared before and after treatment at 1 month and it was seen there was significant improvement (p=0.0001) in the symptoms of dry eye after application of topical PRP.

Days	Itchingavg .of grades	Drynessavg. of grades	Burningavg. of grades	RednessAvg. of grades	Blurred VNavg. of grades
0 days	2.90 ± 0.70	3.07 ± 0.57	2.82 ± 0.63	1.85 ± 0.86	2.82 ± 0.63
7 days	2.60 ± 0.59	2.77 ± 0.47	2.42 ± 0.63	1.65 ± 0.76	2.55 ± 0.50
15 days	2.17 ± 0.50	2.25 ± 0.49	2.05 ± 0.55	1.32 ± 0.52	2.37±0.49
1 months	1.72±0.59	1.90 ± 0.59	1.60 ± 0.54	1.22 ± 0.47	2.12±0.33
0 day vs7 day s(p value)	0.04	0.01	0.005	0.218	0.036
0 day vs 15 days (p value)	<0.0001	<0.0001	<0.0001	0.0008	0.0006
0 day vs 1 month(p value)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 3: Visual acuity at 0 day, 15th day and 1 month after treatment with PRP.

Visual aquity	0	Day	15	Day	1 Month		
visual acuity	R.E.	L.E.	R.E.	L.E.	R.E.	L.E.	
Less than 6/60	6	8	3	3	1	1	
6/60-6/24	21	19	21	22	22	23	
6/18-6/12	4	5	6	7	4	8	
6/9	1	1	2	0	4	0	
6/6	0	0	0	1	1	1	
Total	32	33	32	33	32	33	

Table 4: Visual acuity at the end of 1 month

	Visual acuity at 1 Month							
	R.E.	Percentage	L.E.	Percentage				
No improvement	11	34.38	15	45.45				
1line improvement	11	34.38	9	27.27				
2line improvement	5	15.63	6	18.18				
> 2line improvement	5	15.63	3	9.09				
Total	32	100.00	33	100.00				

3.5. Tear break up time

4. Discussion

Regarding tear break up time, most of the patients before application of PRP had test values <5 sec. Around 71% (23/32) right eye and 72.72% (24/33) left eye had values less than 5 seconds. After treatment with PRP 46.87% (15/32) right eyes and 39.39% (13/33) left eyes had and improvement in the test value.(Table 6)

Dry eye treatment is difficult due to multifactorial nature of this condition. A number of treatment modalities can be used depending upon the etiopathogenic factor and severity of disease: artificial lubricants, topical steroids, topical immunosuppressants, mucolytics and secretagogues, as the most common.¹² However, the limitation is that an ideal substitute for tear does not exist.

Schirmer test		Before treatment [0 day]		7 days		15 days		1 month	
Grade		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD
1 Variable or	R/E	0	-	0	-	6	23.00 ± 5.62	16	22.75 ± 4.48
normal	L/E	0		0		7	24.14 ± 4.6	14	26.64 ± 4.45
2(< 10)	R/E	07	8.85±1.06	08	9.00 ± 1.06	16	9.62 ± 0.81	13	10.00 ± 00
$2 (\leq 1011111)$	L/E	13	9.30 ± 0.94	12	9.25 ± 0.97	19	9.63±0.76	17	9.64 ± 0.79
2(<5mm)	R/E	23	4.73 ± 0.45	22	4.72 ± 0.63	10	4.8 ± 0.63	03	5.00 ± 00
$S(\leq \text{JIIIII})$	L/E	15	4.33 ± 0.72	19	4.42 ± 0.69	07	5.00 ± 00	02	5.00 ± 00
1 (< 2mm)	R/E	02	1.5 ± 0.71	2	1.5 ± 0.71	0	0	0	0
$4 (\leq 2 \operatorname{IIIII})$	L/E	05	1.6 ± 0.55	02	1.5 ± 0.71	0	0	0	0
Total	R/E	32	5.34 ± 2.18	32	5.59 ± 2.27	32	10.62 ± 6.83	32	15.90 ± 7.75
10(a)	L/E	33	5.87±3.06	33	6.00 ± 2.72	33	11.72±7.11	33	15.72±8.36

Table 5: Schirmer values for right and left eyes

Table (5:	TBUT	values	for	right	and	left	eyes
					· · · ·			

	TBUT								
Grade	1 Variable or normal		2 (≤ 10 sec.)		3 (≤5 sec.)				
4 immediate									
	R.E	L.E	R.E	L.E	R.E	L.E	R.E	L.E	
0 days	01	02	06	12	23	24	02	05	
7 days	01	02	10	17	19	13	02	01	
15 days	06	06	16	20	10	07	00	00	
1 months	15	13	14	18	03	02	00	00	
0 day vs 7 days	NA	NA	0.386	0.321	0.429	0.01	NA	0.199	
0 day vs 15 days	0.109	0.257	0.01	0.08	0.002	< 0.0001	0.472	0.062	
0 day vs 1 month	0.0002	0.003	0.059	0.216	< 0.0001	< 0.0001	0.472	0.062	

The revolution in treatment of dry eye was the autologous serum and recently, the PRP.

PRP is defined as a portion of the plasma fraction having a platelet concentration above baseline. Lee et al.,¹³ in his study reported that platelet count of PRP group was approximately 4.25 times higher than that of the whole blood group using a manufacturing process. With PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area. The seven known growth factors in PRP are: PDGFs aa, bb, and ab, TGF- β 1 and - β 2, vascular endothelial growth factor, and epithelial growth factor. But in our study we used centrifugation method to prepare PRP and found the platelet concentration to be two to three folds over total blood values.

PRP is a major source of growth factors, with more advantages than serum. Studies shows that it has more properties for stimulating corneal reepithelization and stimulates it better than human tear.¹⁴ It is preservative free biological product, with benefit of being obtained from patient's own blood. When all sterile procedures are followed and guaranteed, the risk of infection and contamination of the bottle and ocular surface is minimum. In the present study, we obtained preliminary results concerning the efficacy of PRP in various etiologies of dry eye.

In this study, most of the patient were in the age group of 30-40 years with mean age (34.45 ± 15.17). Males

formed 72.5% of study whereas females formed 27.50 % of the study population. Of the various causes of dry eye maximum cases presented with chemical injury (60%) As the population presented with dry eye were young, males who were main bread earners in the family, thus representing the burden on daily wages of workers which has a great implication on their economic life.

The results about symptoms indicate that PRP is efficacious in moderate to severe dry eye patients. Treatment with PRP in present study showed improvement in symptoms of dryness, itching, redness and burning in all patients with statistical significance.

Marina et al. in their research on the effect of PRP in dry eye in 12 patients, evaluated the symptoms of dry eye (itching, redness, dryness, blurred vision, crusting, burning.) individually and noticed improvement in itching, dryness, redness symptoms with statistical significance (p= 0.002) after instillation of PRP. Patients mostly presented with grade 3 symptoms and improvement was seen in all cases. In their study two variables i.e. crusting and blurring of vision were not statistically significant.

Alio et al. also found in their study that 89% of the cases had a significant improvement in symptoms and no patient got worse either.

While in our study improvement was seen in all symptoms and was statistically significant(p=0.0001). The improvement in symptoms most likely occurred because



Fig. 2: a: Day 0 -case presenting with chemical injury (alkali burn) related dry eye showing complete limbal and epithelial defect; **b:** Fluorescein staining of the same patient with chemical injury; **c:** Post PRP treatment healing started with decrease in limbal ischemia on day 7; **d:** Shows healing of wound. Limbal ischemia reduced and cornea started to heal from periphery to center

of an indirect reduction of inflammation, by decreasing tear osmolarity anddilution of proinflammatory factors in the ocular surface and because of inhibitors of inflammation, such as interleukin -1 receptor antagonist and inhibitors of metalloproteinase^{15,16} and other important growth factors, which are known to participate in corneal re epithelialization.

Considering improvement in visual acuity we found that around 61.65% had improvement in best corrected visual acuity of atleast one line. (34% showing improvement in R/E whereas 27% showing improvement in L/E).

Marina et al. had improvement in BCVA of at least one line in around 50 % of the cases. Alio et al. observed that 28% had improvement of atleast one line. This disparity could be due to different inclusion and exclusion criteria.

In our study we found Schirmer value in right eye before treatment (0 day) had mean value $5.34\text{mm}\pm2.18$ and at the end of 1 month mean Schirmer value was 15.90 mm ±7.75 . Schirmer test mean value for left eye at 0 day was $5.87\text{mm}\pm3.06$ and at 1 month mean value was

15.72mm±8.36.

Marina et al. in their study found an improvement in Schirmer from 6.75mm + 3.66 before treatment to 8.96mm \pm 4.56. This difference is due to various etiologies of dry eye included in our study. In cases of dry eye overall, literature shows that Schirmer and other objective tests do not establish a correlation with ocular damage or subjective symptoms.^{17,18}

Regarding tear break up time, most of the patients before application of PRP had test values <5 sec. Around 71% (23/32) right eye and 42.24% (14/33) left eye had values < 5 sec. After treatment with PRP 46.87% (15/32) right eyes and 39.39% (13/33) left eyes had an improvement in the test value i.e. they had tear break up time more than 10 sec. This result was very similar to Alio et al., where 50 % of the patient improved their BUT and the other 50% remained without changes.

The platelet rich plasma preparation was prepared under complete aseptic conditions. Patients were instructed to use the bottle with clean hands and not to touch the nozzle with hands while instilling the drops. The left over in the bottles were sent for microbiological evaluation and there was no significant growth of organisms noticed. No significant complaints were noticed, only 2 out of 40 patients complained of redness after its application.

5. Conclusion

The revolution in treatment in dry eye was autologous serum and recently, Platelet rich plasma (PRP). Autologous PRP is a preservative free biological product containing high levels of platelets and growth factors with healing enhancing properties. The study is first of its kind in establishing the results of PRP in various etiologies of dry eye. The study showed significant improvement in the symptoms of moderate to severe dry eye (dryness, itching, burning, redness). Study did not show any adverse effect of the preparation on dry eye or further worsening of symptoms of dry eye. The overall results show that PRP improved signs and symptoms in majority of patients, with good tolerance seen. It is therefore concluded that PRP therapy is safe and effective in treating moderate to severe dry eye. More clinical trials are required to create specific guidelines regarding the concentration and treatment protocols.

6. Source of Funding

None.

7. Conflict of Interest

None.

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