The Egyptian Journal of Hospital Medicine (July 2021) Vol. 84, Page 2065-2070

Evaluating the Role of Autologous Platelet-Rich-Plasma in The Treatment of Dry Eye Disease

Doaa Hatem Farhan Emam*, Gamal Youssef El Mashad, Ayman Ahmed Alkawas, Sherif Mohammed Sharaf El Deen
Ophthalmology Department, Faculty of Medicine, Zagazig University, Egypt.
***Corresponding Author**: Doaa Hatem Farhan Emam, **Mobile:** 01004229389, **E-mail:** dodohatemfarhan@gmail.com

ABSTRACT
Background: Platelets are irregularly shaped, non-nucleated cytoplasmic bodies derived from fragmentation of megakaryocyte precursors. **Objective:** This study aimed to evaluate the role of autologous platelet-rich-plasma (PRP) as a monotherapy versus artificial tears (Hyaluronic acid) use for better management of dry eye disease. **Patients and Methods**: A prospective clinical randomized trial study was conducted on 62 patients with moderate to severe dry eye disease (Schirmer`s test outcomes of 5.5 mm or lower). Patients had been recruited from the Outpatient Clinics of Ophthalmology Department in Zagazig University Hospital during the period from February 2020 to January 2021. They were divided into two equal groups; group A was treated with PRP and group B was treated with artificial tears. **Results**: There was a significant decrease regarding ocular surface disease index (OSDI) in both groups after treatment, but the improvement was more significantly in PRP group compared to artificial tears group. There was a significant increase in tear break-up time (TBUT) in PRP group only after treatment; although, there was an increase in TBUT in artificial tears group but without statistical significance. There was a significant increase in BCVA in both groups after treatment, but the improvement was more significantly in PRP group compared to artificial tears group. **Conclusion**: PRP is an interesting alternative therapy in symptomatic dry eye.
Keywords: Dry Eye Disease (DED)**,** Platelet-Rich-Plasma (PRP), Tear Break-up Time (TBUT).

INTRODUCTION

Dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles[(1)]**.**

DED is classified into aqueous-deficient dry eye and evaporative dry eye. Aqueous-deficient dry eye has two major subtypes: Sjögren syndrome and non-Sjögren DED as lacrimal deficiency, lacrimal gland duct obstruction, reflex block and systemic drugs. Evaporative dry eye is subdivided into intrinsic causes as (meibomian oil deficiency, disorders of lid aperture and low blink rate) and extrinsic as (vitamin A deficiency, contact lens wear). Activities like watching television, extended computer use, and reading can trigger and/or aggravate dry eye symptoms[(2)]**.**

Lacrimal gland dysfunction is the most common cause of severe dry eye and is secondary to several factors, including acinar atrophy, lacrimal gland fibrosis and ductal obstruction, as well as lymphocyte infiltration with upregulation of proinflammatory cytokines leading to a severe reduction in lacrimal gland secretion[(3)]**.**

The standard treatment for dry eye is topical use of artificial tears, although the expected results are not satisfying and often ineffective. This has led to the use of other therapeutic methods based on blood derivatives. Autologous serum (AS) has been suggested to be a more efficient treatment for severe dry eye disease (DED) rather than preservative-free artificial molecules, with varying success rates[(4)]**.**

The use of platelet-rich plasma (PRP) has become a strategy in the management of several disorders of the ocular surface, including corneal ulcers [(5)] and persistent ocular epithelial defects[(6)]**.** Platelet rich plasma (PRP) and plasma rich in growth factors (PRGF) have also been reported as successful therapies for moderate to severe dry eye, with advantages over AS due to its higher concentration of anti-inflammatory, cytokines, growth factors and other platelet derivatives, that can stimulate the proliferation and regeneration of stem cells which could be with high benefit for the required ocular surface restoration in cases of moderate and severe dry eye[(7)]**.** PRP has an antiapoptotic effect on corneal stromal cells[(8)] and autologous platelets have been used for the management of macular holes[(9)]**.** Autologous platelets in the form of eye-drops have also been used in the management of several eye conditions as corneal ulcer depending on the desired effect and the purpose of the clinical intervention. In this way, PRP has great utility in the management of several ocular diseases[(10)]**.**

The aim of the present study was to evaluate the role of autologous PRP as a monotherapy versus artificial tears (hyaluronic acid) use for better management of dry eye disease.

PATIENTS AND METHODS

A prospective clinical randomized trial study was conducted on 62 patients with moderate to severe dry eye disease (Schirmer`s test outcomes of 5.5 mm or lower). Patients had been recruited from the Outpatient Clinics of Ophthalmology Department in Zagazig University Hospital during the period from February 2020 to January 2021.

Received:11 /4 /2021
Accepted:7 /6 /2021

They were divided into two groups randomly: **Group (A) [PRP group]:** Nine ml venous blood was taken under complete aseptic conditions, the extracted PRP was put in a sterile plastic bottle with eye dropper, the PRP was extracted weekly, and used as eye drops for 6 weeks 4 times daily in 31 patients, and **Group (B) [artificial tears group]:** artificial tears (hyaluronic acid) were used for treatment of dry eye disease in another 31 patients, for 6 weeks 4 times daily.

Ethical approval:

An informed consent form and any other written information to be given to patients were reviewed and approved by the Ethics Committee of the Zagazig University Hospital. The investigator explained to each patient the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks, benefits involved and any discomfort may be caused. This Work was performed according to the code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: Adult patients aged above 18 years old. Moderate to severe dry eye disease**:** Schirmer`s test outcomes of 5.5 mm or lower, presence of dry eye symptoms evaluated with the Ocular Surface Disease Index (OSDI) questionnaire (OSDI ≥ 13).

Exclusion Criteria: Eye lid disorders as facial palsy and ectropion. Conjunctival disorders as pterygium. Corneal ulcers. Previous cataract surgery. Topical ocular treatment, e.g. anti-glaucoma for one year. Previous refractive surgery.

All patients underwent full clinical history taking, ophthalmic examination included the best corrected visual acuity: after refraction. Best spectacle corrected visual acuity (BCVA) was estimated using Landolt`s broken ring chart, which was recorded as its decimal equivalent. The cornea was examined for evidence of corneal scars, corneal edema or keratic precipitates. The anterior chamber was examined for depth, regularity, aqueous flare and cells. Goldmann applanation tonometry to record baseline intraocular pressure. Using indirect ophthalmoscopy and auxiliary lenses (+78 D lenses) to examine retina to exclude possible pathology, e.g.; cystoid macular edema, retinal breaks, macular scars…etc.

The patients had been asked not to use any type of eye drops during the 6 weeks of treatment, and to stop the PRP and artificial tears at least 24 hours before the first and second assessment.

Preparation of PRP:

Group (A) [PRP group] had been subjected to PRP preparation with its precautions: using a 10 ml sterile plastic syringe with a wide pore needle, 9 ml of fresh blood were extracted, to a sterile glass tube containing 1 ml of sodium citrate and gel. Autologous PRP was extracted, whole blood was centrifuged at 3500 RPM for 3 minutes, and the supernatant PRP was withdrawn to a sterile plastic eye dropper that was used as eye drops. The bottle that was used was kept at +4–8 °C for one week. The patients were asked not to touch tip by their hands or eyes.

Technique:

All patients were subjected to a self-assessed questionnaire of Ocular Surface Disease Index (OSDI) at the beginning of application of PRP or artificial tears (Hyaluronic acid) and one day after completing the treatment (after 6 weeks). Slit lamp examination and evaluation of tear meniscus. Schirmer`s test without anesthesia using a filter paper strip inside the lower eyelid of the two eyes that was tested at the same time. The patient was asked to close his eyes gently for five minutes. After five minutes, the doctor removed the paper and measure how many millimeters moistened**.** Tear film break up time (TBUT) using fluorescein stain to the cornea and calculating the time between the last blink and the appearance of the first area of break up. Corneal fluorescein staining (CFS) was assessed by application of fluorescein strips to the lower eye lids then was examined by slit lamp blue filter and the corneal and conjunctival staining was evaluated using the modified Oxford score**.** After the period of treatment (6 weeks) we recorded subjective symptoms (OSDI), the tear meniscus, Schirmer test, tear film break up time (TBUT), and the best corrected visual acuity (BCVA) was evaluated.

Main Outcome Measures: Subjective symptoms were evaluated based on the ocular surface disease index (OSDI) self-administered questionnaire. Modified Oxford scale and corneal fluorescein staining (CFS) were used[(11)].

Follow-Up: Cases were followed up and data were recorded for the purpose of this study at the initial visit and at the end of the 6 weeks of treatment.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

The two studied groups were classified according to age and sex. The range of age in group (A) was (28-69) years and in group (B) was (27-72) years. Sex distribution in the 2 groups is shown in figure 1. There was no statistically significant difference found between the two studied groups.

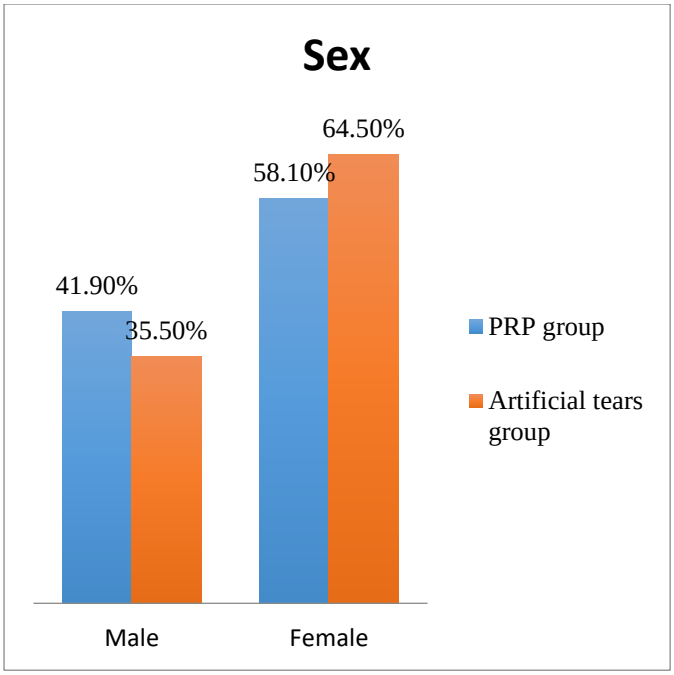

Fig. (1): Sex distribution between the two studied groups.

There was a statistically significant decrease regarding OSDI in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B) (Table 1).

Table (1): Symptoms assessment by OSDI before and after treatment between the two studied groups

		Group (A) (n=62 eyes)	Group (B) (n=62 eyes)	P
OSDI				
Before treatment Mean ± SD		65.21 ± 20.33	74.25 ± 16.55	0.059
After treatment Mean ± SD		39.73 ± 18.35	53.45 ± 19.86	**0.006**
Wilcoxon test	**P**	**0.01**	**0.02**	--

There was a statistically significant increase regarding Schirmer`s test in each group (A and B) from before to after treatment. Meanwhile, there was no statistically significant difference in Schirmer`s test between the two studied groups [group (A) and (B)] before and after treatment **(**Table 2).

Table (2): Schirmer`s test before and after treatment between the two studied groups

		Group (A) (n=62 eyes)	Group (B) (n=62 eyes)	P
Schirmer test				
Before treatment (mm) Mean ± SD		4.18 ± 1.27	4.21 ± 1.15	0.533
After treatment (mm) Mean ± SD		8.45 ± 1.23	7.11 ± 1.34	<0.001
Wilcoxon test	**P**	**0.032**	0.049	--

There was a statistically significant increase in TBUT in group (A) only from before to after treatment. Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment TBUT (Table 3).

Table (3): Assessment of tear film break up time (TBUT) before and after treatment between the two studied groups

		Group (A) (n=62 eyes)	Group (B) (n=62 eyes)	P
TBUT				
Before treatment (seconds) Mean ± SD		4.67 ± 3.28	5.73 ± 2.65	0.167
After treatment (seconds) Mean ± SD		6.52 ± 2.13	6.88 ± 2.74	0.567
Wilcoxon test	**P**	**0.011**	0.098	--

There was a statistically significant increase in BCVA in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B). Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment BCVA (Table 4).

Table (4): Assessment of best corrected visual acuity before and after treatment between the two studied groups

		Group A (n=62 eyes)	Group B (n=62 eyes)	P
BCVA				
Before treatment Mean ± SD		0.435 ± 0.182	0.472 ± 0.175	0.418
After treatment Mean ± SD		0.672 ± 0.217	0.645 ± 0.184	0.599
Wilcoxon test	**p**	**0.01**	**0.03**	--

There was a statistically significant decrease in CFS in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B). Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment CFS (Table 5).

Table (5): Assessment of Corneal fluorescein staining (CFS) before and after treatment between the two studied groups

		Group (A) (n=62 eyes)	Group (B) (n=62 eyes)	p
CFS				
Before treatment Mean ± SD		2.24 ± 0.852	2.16 ± 0.946	0.728
After treatment Mean ± SD		0.583 ± 0.361	0.761 ± 0.452	0.092
Wilcoxon test	***P***	**0.01**	**0.02**	--

DISCUSSION

In agreement with our findings, a randomized clinical trial of **Alio *et al.*[7]** in which three hundred and sixty-eight patients with DED were included. Seventy-one patients were men (19.3%) and 297 patients were women (80.7%) with ages ranging from 18 to 77 years (mean 50.1 ± 15.8) and 18 to 89 years (mean 56.1 ± 16.6), respectively.

In another study of **García-Conca *et al.*[12]**, which was conducted on total of 84 patients with dry eye included in blind single-center prospective comparative randomized study, two groups were differentiated depending on the treatment applied: PRP group, including 44 patients treated with PRP, and SH (sodium hyaluronate) group, including 39 patients treated with artificial tears of a hypotonic aqueous solution. A total of 168 eyes of 84 patients with an age ranging from 36 to 90 years old were included in the study (mean: 64.0; standard deviation, SD: 11.2; median: 67.0 years). The sample comprised 81 females (96.4%) and 3 men (3.6%). Sjögren syndrome was the most frequent risk factor associated to these patients (41% in PRP group/31% in SH group).

In the present study, we evaluated the symptoms by OSDI before and after treatment between the two studied groups, there was a statistically significant decrease regarding OSDI in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B)**.** This is in comparison with the study of **Alio *et al.*[7]** in which the OSDI scores before the PRP treatment were 42.9 ± 26.7 in the evaporative dry eye disease (EDED) patients, and 69.6 ± 30.6 in the aqueous deficient DED (ADDED) patients. Both groups decreased significantly after the PRP treatment to 18.1 ± 17.0 and 43.2±14.5, respectively. These differences were statistically significant ($p< 0.05$).

Our results are supported by the study of **Ribeiro *et al.*[13]** where they evaluated each symptom individually. Treatment with PRP in this study showed improvement in symptoms of dryness, itching, redness, and burning in all patients with statistical significance. Considering crusting or mucus, it was present in only two patients, and both had absence of this complaint after PRP; blurred vision was present in 9 patients out of 12, and 7 had improvement of this symptom after treatment. Those two variables were not statistically significant. None of the patients got worse, except in the blurred vision report, as one patient developed diabetic retinopathy during the follow up.

Some clinical studies have already demonstrated the superiority of AS (autologous serum) versus artificial tears in terms of improvement of symptoms (OSDI), but with no statistically significant differences in corneal staining or tear film break-up time (TF-BUT)[14]. Likewise, **Celebi *et al.*[15]** also found statistically significant differences between AS and artificial tear in OSDI and TF-BUT, but not in Schirmer`s test result, and corneal and conjunctival staining. In comparison with AS, PRP provides a greater concentration of platelets and their release of growth factors.

In the present study, there was a statistically significant increase regarding Schirmer`s test in group (A) from before to after treatment (Mean ± SD: 4.18 ± 1.27, 8.45 ± 1.23 respectively). There was statistically significant difference regarding Schirmer`s test in group (B) from before to after treatment (Mean ± SD: 4.21 ± 1.15, 7.11±1.34 respectively). Meanwhile, there was no statistically significant difference in Schirmer`s test between the two studied groups before

and after treatment. In comparison with the study of **Ribeiro *et al.*** [13] which found an improvement in Schirmer`s test from 6.75 mm±3.66 before treatment to 8.96 mm ± 4.56 after, while **López-Plandolit *et al.*** [8] found a Schirmer`s test of 4.67 ±5.14 before and 6.91 ± 6.36 after treatment; a little disparity possibly due to the different etiology of dry eye between the studies. In cases of dry eye overall, literature shows that Schirmer`s and other objective tests do not establish a correlation with ocular damage or subjective symptoms[16].

On the other hand, as regard tear film break up time (TBUT) before treatment and after treatment; we demonstrated that there was a statistically significant increase in TBUT in group (A) only from before to after treatment. There was no statistically significant difference regarding TBUT in group (B) from before to after treatment. Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment TBUT.

García-Conca *et al.* [12] reported that changes in BUT were minimal and not statistically significant in most of cases. Indeed, TF-BUT was within the range of normality in all cases at baseline. Therefore, they could not establish the effect of PRP compared to SH on tear film stability as patients without alteration of this factor were selected for the study. Other studies comparing AS versus artificial tear did not find significant changes in TF-BUT[15,17,18].

In our results, we found that there was a statistically significant increase in BCVA in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B).

Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment BCVA**.** In accordance with our findings, the study of **Alio *et al.*** [18] reported that mean decimal BCVA of all subjects improved from 0.75 ± 0.3 at baseline to 0.82 ± 0.2 after the treatment with PRP. These values were not statistically significant. One hundred and six patients (28.8%) experienced an improvement of one or more lines of vision. No improvement in vision was observed in 246 (66.9%) patients, while 16 (4.3%) patients lost one line of vision. In the study of **Ribeiro *et al.*** [13], number of lines improvement in the right eye: 41.66% (5/12) had improvement of 1 or more lines, 8.33% (1/12) had reduction of at least one line and 50% (6/12) had no alteration in right eye BSCVA. Means logarithm of the minimum angle of resolution (log MAR) before the treatment was 0.39 ±0.32, and after treatment was 0.31±0.35 (p= 0.02). Number of lines improvement in the left eye: 41.67% (5/12) had improvement of 1 or more lines and 58.33% (7/12) had no alteration in left eye BSCVA. While in the study of **Alio *et al.*** [19], the mean decimal corrected distance visual acuity (CDVA) improved from 0.77±0.25 (range 0.1 to 1.0) at baseline to 0.89±0.17 (range 0.2 to 1.0) after treatment with PRP, which represents a statistically significant improvement in log MAR CDVA from 0.14±0.19 to 0.06±0.12 ($p <$ 0.001). The number of eyes presenting decimal CDVA≥0.8 increased from 101 (64.8%) to 124 (79.7%) before and after treatment, respectively. When considering eyes with decimal CDVA≤0.9 before treatment (and therefore with some potential for visual improvement), 74 (71.4%) eyes improved at least 1 line of vision, 25 (24.5%) had no change, and 4 eyes (4.1%) lost 1 line of vision. All 53 eyes who had decimal CDVA = 1.0 at baseline maintained the same visual acuity. Visual improvement was significantly higher in eyes with worse CDVA at baseline.

On the other hand, the present study revealed that there was a statistically significant decrease in CFS in both groups from before to after treatment but the improvement was more significant in group (A) compared to group (B). Meanwhile, there was no statistically significant difference between the two studied groups regarding before and after treatment CFS**.** All cases enrolled in the study of **Alio *et al.*** [7] initially showed superficial punctate keratopathy, with modified Oxford scale scores ranging from 0.5 to 4. After the treatment, a decrease in the area with fluorescein staining was observed in 280 (76.1%) patients. A decrease of at least 1 point in the modified Oxford scale score was indicative of improvement. Seventy-eight (21.2%) cases did not show any changes, while ten cases (2.7%), all women who had received one round of PRP, showed worsening in the extension of punctate keratopathy. The mean CFS score before treatment was 1.43 ± 0.94 and after the PRP was 0.24 ± 0.43 in the EDED patients. In the ADDED patients, the scores were 1.70 ± 0.90 and 0.39 ± 0.67, before and after, respectively. Both groups decreased significantly after the PRP treatment ($p <$ 0.05).

Therefore, autologous PRP eye drops seems to be an attractive alternative option for the treatment of both evaporative and aqueous deficient DED. Significant advantages are cost effectiveness, ease of preparation and the autologous origin. The effectiveness of PRP is determined by the presence of platelets, growth factors and cytokines which are involved in proliferation, migration and differentiation of corneal epithelial cells, thus helping to maintain a proper condition of the ocular surface. Moreover, autologous PRP has been used successfully in other ocular surface disorders such as post-LASIK ocular surface syndrome, persistent epithelial defects, alkali burn, dormant ulcers, and corneal surface reconstructions in corneal perforations[20]**.**

Limitations of this study are the small sample size that may lead to compromised statistical analysis. In addition, there may exist other etiologic factors involved in our cases: hormonal factors, use of medication that also could cause dry eye and the possibility that non-diagnosed diseases could contribute for this condition.

CONCLUSION

Monotherapy with autologous PRP eye drops has shown to be a very good option for the treatment of moderate to severe DED. It can therefore be concluded that PRP is an interesting alternative therapy in symptomatic dry eye. In clinical practice, it is important because it may prevent complications of dry eye and improve patient well-being. Significant advantages are ease of preparation, absence of preservatives, its autologous origin, safety, and minimal or no intolerance.

Based on our findings, we recommend further studies on large geographical scale and on larger sample size to emphasize our conclusion. Further investigations to assess the role of this therapeutic approach in the treatment of DED versus commercial artificial tear eye drops or other hemoderivatives are necessary to determine the best approach in the management of moderate to severe forms of this frequent ocular surface disease.

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