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Autologous PRP Usage as a Mono Therapy for the Treatment of Moderate to Severe DED

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ABSTRACT

Background: Platelets have a lot of important functions such as repairing tissue damage, coagulation which prevents blood loss,and 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.This study aimed 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 randomized clinical trial was carried out at Ophthalmology Department inZagazig University Hospital during the period from February 2020 to January 2021, included 62 patients with moderate to severe dry eye disease(Schirmer`s test outcomes of 5.5 mm or lower). They were divided into two groups randomly 31 patients in each group. Autologous PRP had been prepared from fresh blood samples of group (A) patients with DED and used in the form of eye-drops while, artificial tears (Hyaluronic acid) were used in group (B). **Results**: 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. There is 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). **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.

Keywords:Dry Eye Disease (DED), Platelet-Rich Plasma (PRP), Plasma rich in growth factors (PRGF)

INTRODUCTION

The dry eye is a multifactorial disease of the tears and ocular surface, which is accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles, leading to hyperosmolarity that stimulates more inflammation, and another factor recognized in dry eye pathogenesis is oxidative stress[(1)].

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Dry Eye Disease (DED) is common in the general population, increasing with age, and is one of the most frequent occurrences in daily practice, with a substantial impact on the individual's quality of life[2].

Platelets play a main role in the wound healing process, since they have high concentrations of GFs and cytokines contained in their α-granules, such as PDGF, TGF-β, and platelet factor IV[3].

Platelets-based preparations are extremely versatile; exist under forms of variable solidity from liquid to gel; and have been largely used in regenerative medicine, orthopedic and maxillo-facial surgery in order to promote tissue healing through the delivery of several bioactive factors. Various preparations (namely PRP, PRGF, and platelet lysate) have quite recently been introduced as possible therapy of different ocular surface disorders. In platelet-derived eye drops, the autologous source still represents the most used product in DED patients, whereas allogeneic PRP, developed for other fields of application, is still at its beginning as an eye-drop treatment[4].

The main standard treatment for dry eye is topical administration of artificial tears, although the expected results are not optimal and often ineffective. This has led to the use of other therapeutic strategies based on hemoderivatives. Autologous serum (AS) has been suggested as a more adequate treatment for severe DED over preservative-free artificial molecules, with variable success rates[5,6].

Platelet rich plasma (PRP) and plasma rich in growth factors (PRGF) have also been reported as successful treatments for moderate to severe dry eye, presenting advantages over AS due to its richer concentration of growth factors, anti-inflammatory cytokines, and other platelet derivatives, which could be beneficial for the required ocular surface restoration in moderate to severe forms of dry eye[7].

Autologous PRP is a hemoderivative with a high concentration of platelets obtained through a relatively simple process, which requires minimal manipulation and no addition of any other particular substance[8]. A study of **Tandon et al.** [9]has shown that these components help in the proliferation, migration, and differentiation of corneal epithelial cells. 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

This was a prospective randomized clinical trial that was carried out at Zagazig University Hospital- Ophthalmology Department, Two groups were differentiated depending on the treatment applied: **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. **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.

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

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Ophthalmic examination:

1-The best corrected visual acuity: after refraction, BCVA was estimated using Landolt`s broken ring chart which was recorded as its decimal equivalent.

2-Slit-lamp biomicroscopy: The cornea was examined for evidence of corneal scars, corneal edema or keratic precipitates. The anterior chamber examined for depth, regularity, aqueous flare and cells. GoldmannApplanation Tonometry to record baseline intraocular pressure.

3-Fundus examination: Using indirect ophthalmoscopy and auxillary lenses (+78 D lenses) to examine retina to exclude possiple pathology e.g.; cystoid macular edema, retinal breaks, macular scars…etc.

4-The patients had been asked not to use any type of eye drops during the 6 weeks, 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 had been extracted, to a sterile glass tube containing 1ml of sodium citrate and gel. Autologous PRP had been extracted, whole blood had been centrifuged at 3500 RPM for 3 minutes, the supernatant PRP had been withdrawn to a sterile plastic eye dropper that had been used as eye drops**.** The bottle that had been used should be kept at +4–8 °C for one week. The patients had been asked not to touch tip by their hands or eyes.

Technique:

All patients was 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, 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 was 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 had been evaluated using the modified Oxford score**.**
- After the period of treatment (6 weeks) we recorded subjective symptoms (OSDI), the tear meniscus, Schirmer`s test, tear film break up time (TBUT), and the best corrected visual acuity (BCVA) was evaluated.

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

Statistical analysis

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Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences SPSS version 20.0 software for analysis. P value was set at < 0.05 for significant results &<0.001 for highly significant result.

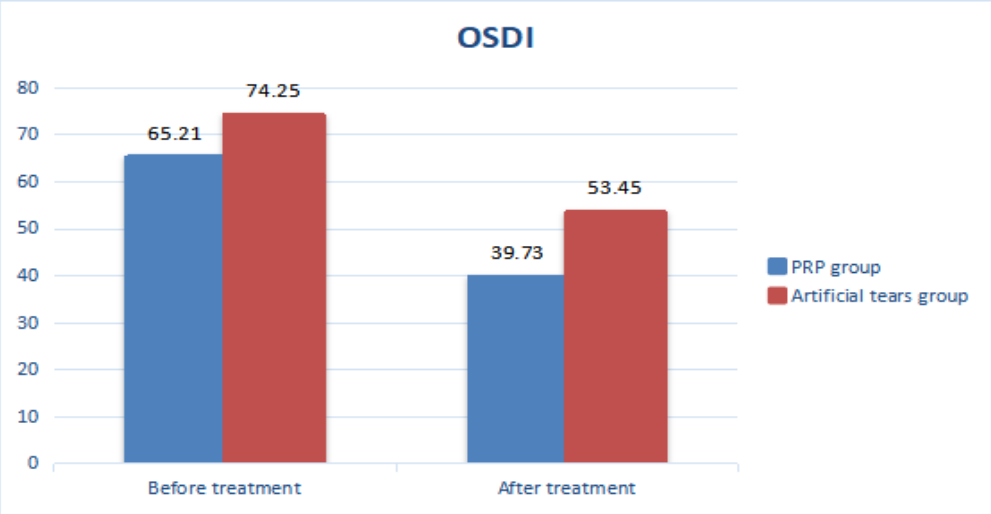
RESULTS

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. Females in group (A) were 58.1% and in group (B) were 64.5% while males in group (A) were 41.9% and in group (B) were 35.5%. There was no statistically significant difference found between the two studied groups **(Table 1).**

Table (1): Demographic distribution among the two studied groups

Variable		**Group (A)** (n=31pt\62eyes)	**Group (B)** (n=31pt\62eyes)	x^2	**p**
Age (years) **Mean ± SD** **Range**		61.40 ± 7.54 28 – 69	63.45 ± 8.09 27 - 72	1.03	0.306
Sex	**Male**	13 (41.9%)	11 (35.5%)	0.272	0.602
	Female	18 (58.1%)	20 (64.5%)		

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) **(Fig. 1).**



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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12429 - 12438
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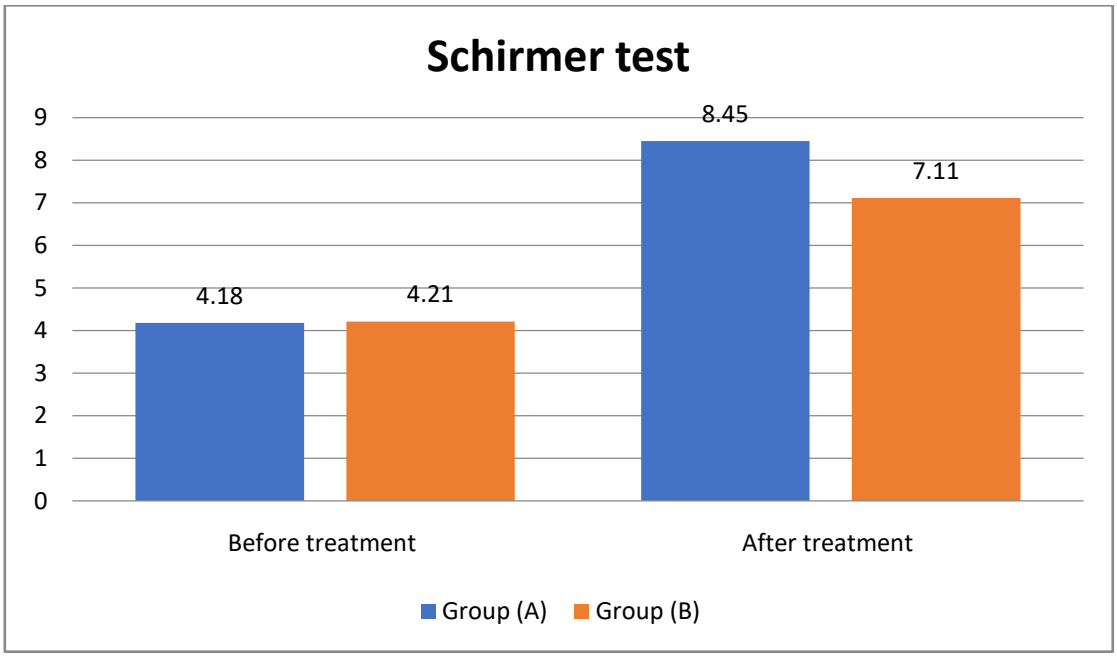
We compared Schirmer`s test statistically in group (A) before and after treatment and in group (B) before and after treatment then we compared Schirmer`s test between the two groups before and after treatment. There was a statistically significant increase regarding Schirmer`s test in group (A)from before to after treatment. There wasstatistically significant difference regarding Schirmer`s test in group (B) from before to after treatment. Meanwhile, there is no statistically significant difference in Schirmer`s test between the two studied groups [group (A) and (B)] before and after treatment **(Fig. 2)**.



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

We compared TBUT statistically in group (A) before and after treatment and in group (B) before and after treatment then we compared TBUT between the two groups before and after treatment. 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 is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment TBUT **(Fig. 3).**

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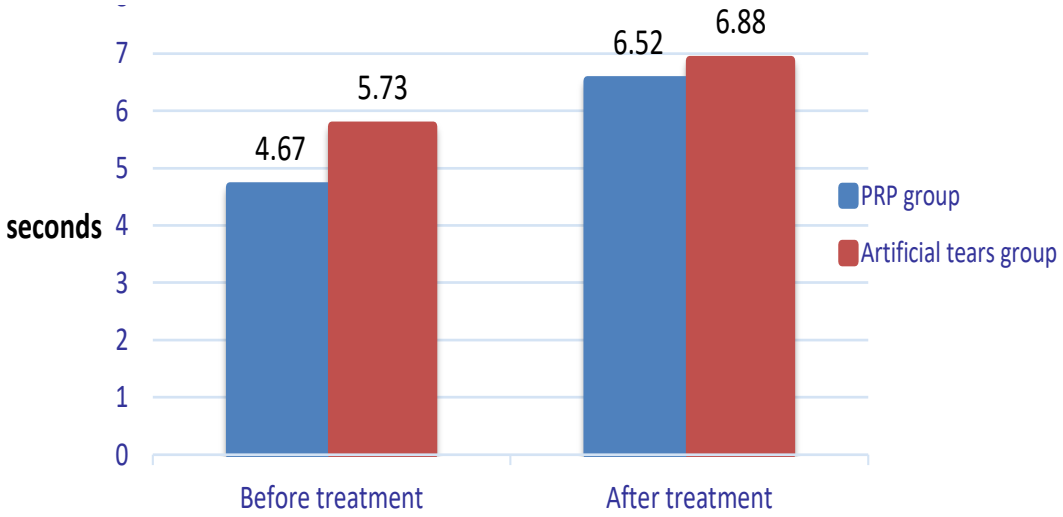
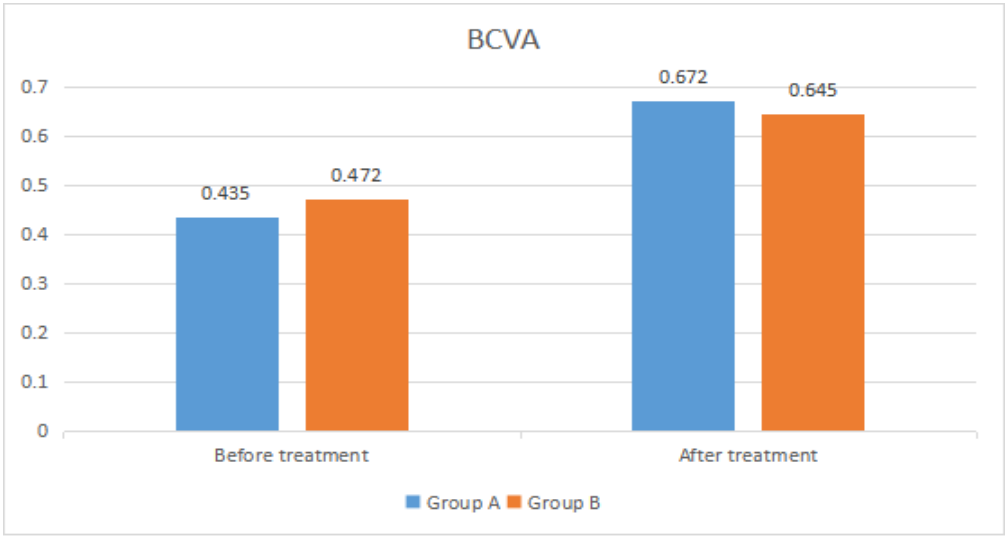


(Fig. 3): Assessment of TBUT before and after treatment between the two studied groups

There is 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 is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment BCVA **(Fig. 4).**



(Fig.4): Assessment of best corrected visual acuity before and after treatment among the two studied groups

There is 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 is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment CFS **(Fig. 5).**

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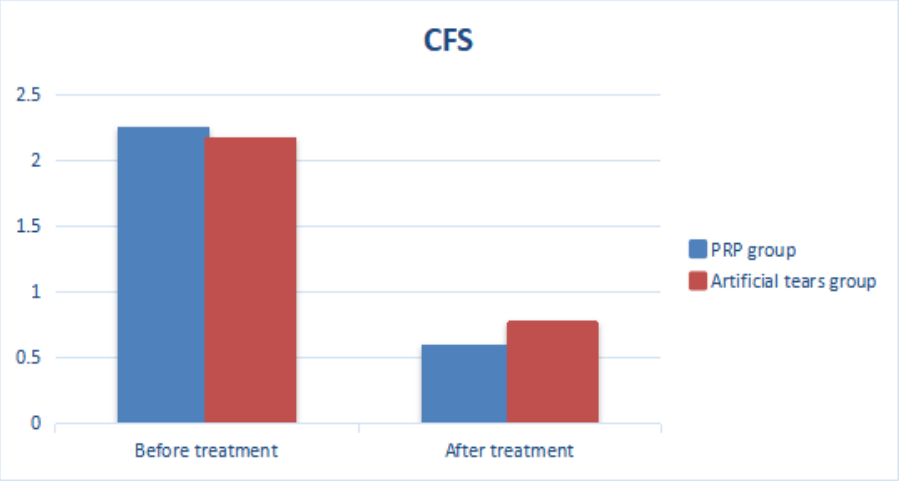

(Fig. 5): Assessment of Corneal fluorescein staining (CFS) before and after treatment between the two studied groups

DISCUSSION

In agreement with our findings, a randomized clinical trial of **Alio et al.** [10]in which three hundred and sixty-eight patients with DED were included in this study. 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.

Furthermore, the study of **Alioet al.** [11]was conducted on one hundred and fifty-six eyes of 80 myopic patients, Sixty-two patients were female (77.5%), and 18 patients were male (22.5%), with ages ranging from 22 to 82 years (mean 43.7 ± 12.3).

In the present study, we evaluated the symptoms assessment by OSDI before and after treatment between the two studied group, 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)**.**

In **García-Conca et al.** [12], a reduction in the OSDI score was found with both type of treatments (PRP group treated with PRP, and SH group treated with artificial tears of a hypotonic aqueous solution), although the magnitude of this change was significantly higher with the use of PRP. Therefore, a more significant level of control of symptoms seems to be achievable with PRP. Furthermore, the improvement in symptoms was more significant during the first 2 weeks of treatment. The reason for this may be that the regenerative effect of PRP is more active in the first days of treatment due to the biological stability of the platelets and growth factors, which is affected by changes over time as well as by their way of conservation.

Lopez-Plandolit et al. [7]found in their study a 43.75% substantial improvement plus a 31.25% moderate improvement in SDEQ 3 (modified score questionnaire for dry eye).It is difficult to compare these results, however, because of the different methodology used by these authors to evaluate patient symptom: **Alioet al.**[10]evaluated the overall ocular discomfort, and **Lopez-Plandolit et al.** [7]analyzed each symptom by the questionnaire score, but both studies found improvement in most of the symptoms like we did.

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In this 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 and p value: 0.032). 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 andp value: 0.049). Meanwhile, there is no statistically significant difference in Schirmer`s test between the two studied groups [group (A) and (B)] before and after treatment (p value: 0.533, 0.058 respectively).

Moreover, **Alióet al.**[10]reported that the Schirmer's test value in the evaporative DED patients improved from 9.5 ± 3.6 mm to 13.8 ± 8.7 after the treatment ($p< 0.05$), and in the ADDED patients from 4.7 ± 2.7 to 6.4 ± 2.4 ($p< 0.05$).

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 (Mean ± SD: 4.67 ± 3.28, 6.52 ± 2.13 respectively andp value: 0.011). There was no statistically significant difference regarding TBUT in group (B) from before to after treatment (Mean ± SD: 5.73 ± 2.65, 6.88 ± 2.74 respectively andp value:0.098). Meanwhile, there is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment TBUT (p value: 0.167, 0.567 respectively)**.**

In contrast, significant improvements in TF-BUT were reported by **Avila.**[13]in a group of subjects in which PRP injection was performed close to the lacrimal gland as well as in the clinical trial of **Kojima et al.** [14]comparing AS and artificial tear.

In our results, we found that there is 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), (Mean ± SD in group (A) from before to after treatment was 0.435 ± 0.182, 0.672 ± 0.217 respectively and p value: 0.01), while(Mean ± SD in group (B) from before to after treatment was 0.472 ± 0.175, 0.645 ± 0.184 respectively and p value: 0.03). Meanwhile, there is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment BCVA, (Mean ± SD in group (A & B) before treatment was 0.435 ± 0.182, 0.472 ± 0.175 respectively and p value: 0.418, while (Mean ± SD in group (A & B) after treatment was 0.672 ± 0.217, 0.645 ± 0.184 respectively and p value: 0.599)**.**

In the study of**Ribeiroet al.** [15], 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 log MAR before the treatment was log MAR 0.39 ±0.32, and after treatment was log MAR 0.31±0.35 (p= 0.02). Number of lines improvement in the left eye: 41.66% (5/ 12) had improvement of 1 or more lines and 58.33% (7/12) had no alteration in left eye BSCVA.

The present study revealed that there is 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) (Mean ± SD in group (A) from before to after treatment was 2.24 ± 0.852, 0.583 ± 0.361 respectively and p value: 0.01, while (Mean ± SD in group (B) from before to after treatment was 2.16 ± 0.946, 0.761 ± 0.452 respectively and p value: 0.02).

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Meanwhile, there is no statistically significant difference between the two studied groups [group (A) and (B)] regarding before and after treatment CFS, (Mean ± SD in group (A & B) before treatment was 2.24 ± 0.852, 2.16 ± 0.946 respectively and p value: 0.728, while (Mean ± SD in group (A & B) after treatment was 0.583 ± 0.361, 0.761 ± 0.452 respectively and p value: 0.092)**.**

This result is consistent with that reported by**Alióet al.**(10) who described an improvement in corneal staining by 72% in dry eyes treated with PRP.

Kojima et al. (14)also described a significant improvement in patients treated with autologous serum platelets compared to those treated with a saline solution.

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.Further randomized clinical trials and internationally recognized harmonized guidelines are still needed to provide better evidence, to improve quality of the final products, and to lead to a more widespread use of these therapies in daily ophthalmological practice.

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