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Original Article

Autologous Platelet Rich Plasma versus Autologous Serum Eye Drops in Treatment of Severe Dry Eye Disease

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ABSTRACT

Background: Dry eye disease [DED] results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. Biological tear substitutes, a blood derivative, demonstrated good efficacy in improvement of symptoms and ocular surface staining reduction in patients with moderate-to-severe dry eye disease.

Aim of the work: To evaluate the efficacy of autologous serum [AS] eye drops [20%], platelet rich plasma [PRP] eye drops [20%], for treatment of severe DED and comparing their results with each other.

Patients and methods: A prospective comparative study included 120 eyes of 60 patients with severe DED aged 18 - 67 years. Patients divided into 2 groups according to treatment protocol: Group I: 60 eyes of 30 patients treated with autologous serum eye drops, Group II: 60 eyes of 30 patients treated with autologous platelet rich plasma eye drops. They underwent a comprehensive ophthalmic examination, evaluation of best corrected visual acuity [BCVA], tear break-up time [TBUT], Schirmer's test, corneal fluorescein staining [CFS; oxford grading scale], and level of Conjunctival hyperaemia [Efron grading scale] during 6 weeks of treatment. Conjunctival impression cytology [CIC] studied before and after treatment.

Results: Statistically significant larger improvement in BUT [p=0.001], Schirmer's test [p < 0.001] and significantly larger reduction of CFS [p < 0.001] in Group II compared to Group I at six weeks after treatment. Likewise, a significant improvement of Nelson grading of CIC [p =0.04] was found in Group II. Greater reduction of conjunctival hyperaemia but not statistically significant [p = 0.275], also the improvement in BCVA [log MAR], [p=0.242], in Group II compared to Group I at six weeks after treatment.

Conclusion: PRP eye drops induces a more significant improvement in different DED signs than AS in treatment of severe dry eye disease.

Keywords: Dry eye; Autologous serum; Platelet rich plasma; Tear break-up time; Schirmer's test; Corneal Fluorescein staining; Conjunctival hyperaemia; Conjunctival impression cytology.

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INTRODUCTION

Dry eye disease [DED] is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface [1]. DED can influence individual's ability to perform daily tasks and can negatively affect their quality of life [QoL] via many ways relating to decreased quality of vision and/or the development of psychological issues such as anxiety and depression [2].

Prevalence of DED is higher in older age and in women as compared to men. The prevalence was 11.3% among all adults aged above 50 years of age in a recent study and was 22.8% among women aged above 75 years [3].

Conventional treatments used to treat DED depend on its severity. For patients with a clinical diagnosis of mild dry eye; exacerbating exogenous factors should be addressed. As the severity of the dryness increases; aqueous enhancement using topical agents is convenient [4].

In patients with moderate-to-severe DED, initial treatments with modification of lifestyle and artificial tears can be unsuccessful [5].

Biological tear substitutes, a blood derivative, demonstrated a good efficacy in improvement of symptoms and ocular surface staining reduction in patients with moderate-to-severe dry eye disease [6]. Autologous serum eye drops afford improvement in both symptoms and objective signs of DED across multiple studies [7].

THE AIM OF THE WORK

The aim of this study is to evaluate the efficacy of using autologous serum, platelet rich plasma eye drops as a treatment of severe DED and comparing their results with each other.

PATIENTS AND METHODS

This prospective comparative study conducted at Al-Zahraa University Hospital [Cairo, Egypt] in the period from Oct. 2021 to March 2022, and included 120 eyes of 60 patients, with severe dry eye disease, and ineffective conventional therapy of artificial tears for months or even years.

Adult patients [mean age of 46.27 years], with severe DED according to the Dry Eye severity grading scheme proposed by the Dry Eye Workshop [DEWS] [1] and **Baudouin et al.** [8] were included in this study. This scheme considers the following parameters: ocular discomfort, visual symptoms, tear film break-up time [TBUT], Schirmer's test score [without anesthetic], conjunctival and corneal fluorescein staining [CFS], and conjunctival injection [1].

The study was approved by the ethical board of Al-Azhar University. Written informed consent was obtained from each participant after an explanation of the study protocol was provided.

Exclusion criteria

- Patients with positive hepatitis B or C virus, or human immunodeficiency virus serology.
- Patients with hemolyzed or lipaemic serum by visual inspection of the specimen before and after centrifugation.
- Contact lens wearers.
- Eyelid abnormality interfere with blinking.
- Antecedents of herpetic keratitis also corneas with neurotrophic ulcer or scar.

All patients instructed to stop topical treatment 48 hr before first visit, in the 1st visit they underwent a comprehensive ophthalmic examination, including measurement of best-corrected visual acuity [BCVA], level of conjunctival hyperemia grading, CFS, TBUT, Schirmer's test score, Fundus examination, conjunctival impression cytology [CIC].

The patients were divided randomly by simple randomization [even =group 1, odd =group 2] into two groups according to treatment protocol:

Group I: 30 patients [60 eyes] were treated with autologous serum eye drops.

Group II: 30 patients [60 eyes] were treated with autologous platelet rich plasma eye drops.

Once patients met the inclusion criteria, the eye drops bottles containing autologous serum and platelet rich plasma were provided for use 6 times/ day. Once treatment started, a second, third and fourth visit at 2, 4, 6 weeks were done. The same tests as in the baseline visit were performed at each visit, except CIC that was only performed in the last visit.

Conjunctival Impression Cytology [CIC]: Cellulose acetate filter paper [Millipore filter paper] was cut into small strips [5 mm × 5 mm]. With blunt forceps, the filter paper was applied on the anesthetized bulbar conjunctiva with Gentle pressure for 10 s and then removed with a peeling motion. The filter paper then immediately pressed cell side down onto a clear glass slide. Specimens were stained with hematoxylin and eosin after fixation with alcohol 95%. These slides then examined under light microscope with low-power and high-power magnifications. Staging was following the Nelson's grading system.

Autologous Serum Eye Drops Preparation: The blood was first drawn from the recipient and allowed to clot. After clotting, the sample was centrifuged by SW-12 centrifuge at 4000RPM for 10 minutes at room temperature [20-40] C to separate serum from basal components with no hemolysis. After centrifugation, the serum was transferred into a sterile tube and diluted with a sterile saline solution to a 20% concentration, the final preparation was divided into 5-mL bottles. The patients were instructed to store theses bottles at -20℃ until use maximum for 3 months. Bottles being in use were maintained under refrigerated conditions at 4℃ maximum for 7 days.

Autologous Platelet Rich Plasma Eye Drops Preparation: The blood was first drawn from the recipient, placed in five vacutainer tubes [2-mL] containing anticoagulant, citrate-dextrose solution, and centrifuged by Hermle Z326K High-Speed Centrifuge [HERMLE Labortech-nik GmbH] at 1200 rpm for 10 minutes. The upper two layers, the plasma and buffy coat layer, were separated and diluted to 20% with a sterile saline solution, the final preparation was divided into 5-mL bottles. Patients were instructed to store theses bottles at -20℃ until use maximum for 3 months. Bottles being in use were maintained under refrigerated conditions at 4℃ maximum for 7 days.

Statistical Analysis: All statistical tests were performed using the Statistical package for Social Science [SPSS 25]. Descriptive statistics included mean, standard deviation [SD] for numerical data and frequency and percentage of non-numerical data. Analytical statistics included student t test to compare numerical data between the two groups, paired t-test to compare numerical data for the same study group before and after treatment, chi-Square test to examine the relationship between two qualitative variables, linear by linear association test to compare between two qualitative variables at least one of them is ordinal, and marginal homogeneity test to compare a variable with multiple categories for the same study group before and after treatment. The confidence interval was set to be 95%, the p-value < 0.05 is significant.

RESULTS

Sixty patients with severe DED were included in this study, 17 were males [28.3%], and 43 were females [71.7%], age ranging from 18 to 67 years. There was no statistically significant difference regarding age and sex between the two studied groups [Table 1].

There was no statistically significant difference in baseline BCVA between the two studied groups. There was significant difference in baseline break up time test, Schirmer's test, Oxford staining score, and level of hyperemia [Efron grading scale] between both groups. Break up time test, in group 1 improved from 3.45 ± 0.86 sec. to 5.13 ± 0.83 after the treatment [p<0.001], and in group 2 from 4.02 ± 0.65 to 5.71 ± 0.95 [p <0.001]. The Schirmer's test value in group 1 improved from 3.73 ± 1.00 mm to 4.68 ± 1.01 after the treatment [p<0.001], and in group 2 from 4.00±1.15 to 6.33±1.13 [p <0.001]. After the treatment, a decrease in the CFS from 2.23 ± 0.46 to 2.02 ± 0.5 after the treatment [p<0.001], and in group 2 from 1.97 ± 0.45 to 0.65 ± 0.73 [p <0.001]. A decrease of level of hyperemia in group 1 from 1.82 ± 0.62 mm to 0.53 ± 0.5 after the treatment [p<0.001], and in group 2 from 2.13 ± 0.5 to 0.43 ± 0.5 [p <0.001]. All patients treated, received consecutive 6 weeks of treatment either AS [group I] or PRP [Group II]. By comparing the two groups, improvement in vision in group 2, with no significant difference between the two groups. More improvement in BUT [p=0.001], Schirmer's test [p < 0.001], significantly larger reduction of CFS [p < 0.001], in Group II compared to Group I, 6 weeks after treatment. Larger reduction conjunctival hyperemia but not statistically significant [p = 0.275] [Table 2].

A statistically significant improvement of Nelson grading of CIC after treatment for both groups [p < 0.001, p < 0.001, respectively], as shown in table 3 and figure 1. More improvement in Group II that was statistically significant [p=0.04].

Table [1]: Comparison between Group 1 and Group 2 according to Age and sex

		Group [1]	Group [2]	P –value
Age		50.53 ± 12.81	46.27 ± 12.93	0.204*
Sex	Male	9 [30.0%]	8 [26.7%]	0.774**
	Female	21 [70.0%]	22 [73.3%]	

* t-test; ** Chi-Square test

Table [2]: Mean baseline and mean change 6 weeks after treatment in group1 and group 2

Variable	Group [1] Mean± SD	Group [2] Mean± SD	p- value [Student's t-test]
BCVA [log MAR]			
Baseline	0.15 ±0.22	0.15±0.25	0.969
Change after Treatment	0.00	-0.05*	0.242
p- value [paired t-test]	1.00	0.001	
Break up time test			
Baseline	3.73 ± 0.86	4.02 ± 0.65	**0.044**
Change after Treatment	1.4*	1.69*	**0.001**
p- value [paired t-test]	<0.001	<0.001	
Schirmer's test			
Baseline	3.45±1.00	4.00±1.15	**0.006**
Change after Treatment	1.23*	2.33*	**<0.001**
p- value [paired t-test]	<0.001	<0.001	
Corneal fluorescein Staining			
Baseline	2.23 ± 0.46	1.97 ± 0.45	**0.002**
Change after Treatment	-0.21*	-1.32*	**<0.001**
p- value [paired t-test]	<0.001	<0.001	
Level of hyperemia			
Baseline	1.82 ± 0.62	2.13 ± 0.5	**0.002**
Change after Treatment	-1.29*	-1.7*	0.275
p- value [paired t-test]	<0.001	<0.001	

Data are expressed as mean ± standard deviation and analyzed by Student's t test to compare between the two study groups means. p-values in bold are statistically significant. *Demonstrated change in variables for the same study group [before and after treatment] that was statistically significant [P < 0.05], Paired t-test.

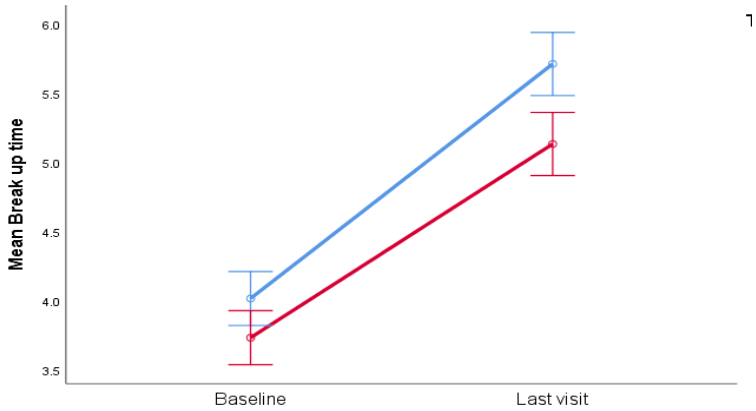

Figure [1]: Change in tear break-up time test, comparison between group 1 and group 2

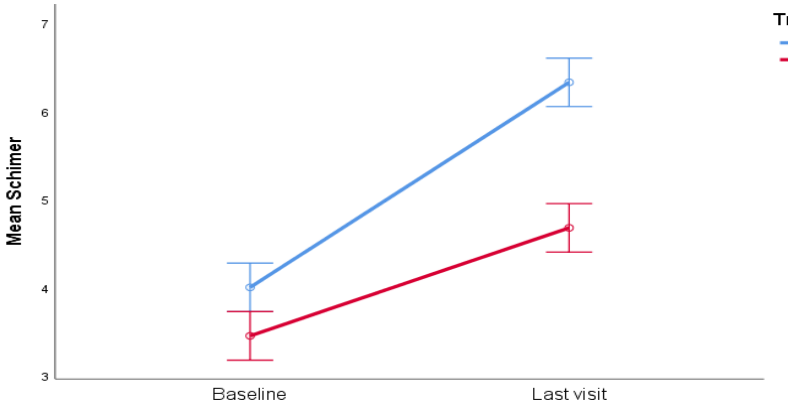

Figure [2]: Change in Schirmer's test, comparison between Group 1 and Group 2

Table [3]: Conjunctival impression cytology grading [baseline and 6 weeks after treatment] in group1 and group 2

Impression cytology	Group [1] No., %	Group [2] No., %	P value [Linear-by-linear association test]
Baseline			
Grade 0	0 [0.0%]	0 [0.0%]	1.00
Grade 1	9 [15%]	6 [10%]	
Grade 2	24 [40%]	30 [50%]	
Grade 3	27 [45%]	24 [40%]	
Change after Treatment			
Grade 0	1 [1.7%]	2 [3.3]	**0.04**
Grade 1	30 [50%]	40 [66.7]	
Grade 2	29 [48.3]	18 [30%]	
Grade 3	0 [0.0%]	0 [0.0%]	
P-value [Marginal homogeneity test]	**<0.001***	**<0.001***	

Data are expressed as number and percentage and analyzed by Linear-by-linear association test to compare between the two study groups. p-values in bold are statistically significant. *Demonstrated change in variables for the same study group [before and after treatment] that was statistically significant [P < 0.05], Marginal homogeneity test.

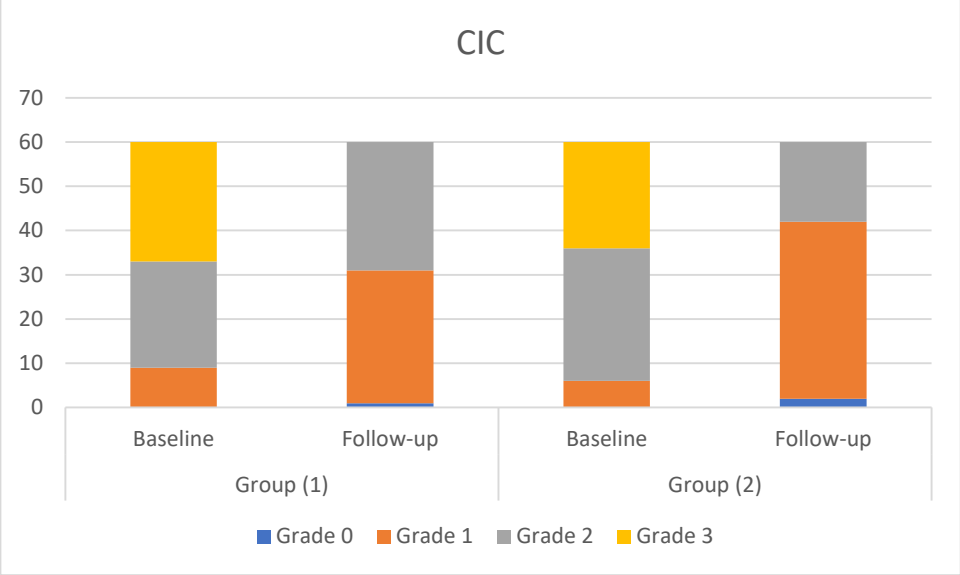

Figure [3]: Comparison between Group 1 and Group 2 according to impression cytology at baseline visit and the last visit after treatment

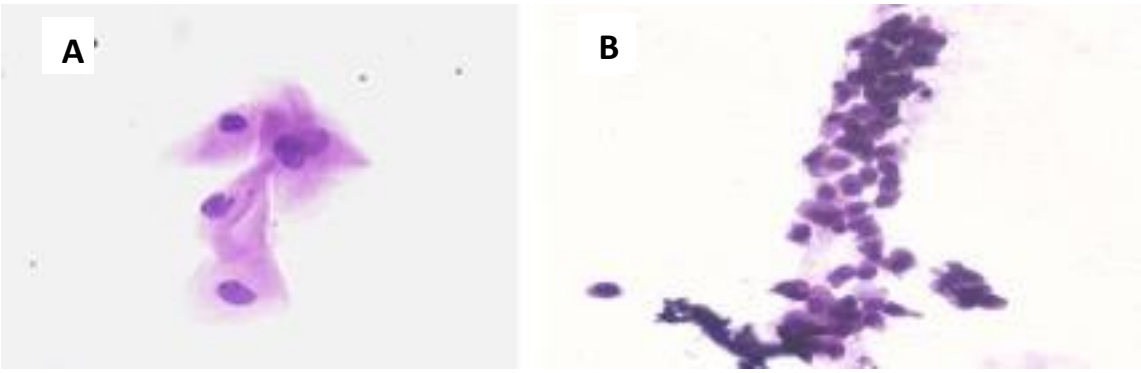

Figure [4]: Conjunctival impression cytology, left eye of 50-year-old female with sever DED [Schirmer's test 5mm, BUT 3 Sec., grade 2 conjunctival hyperemia, grade 2 corneal staining]. CIC showed improvement from Nelson grade III [A] to Nelson grade II [B] after treatment with AS

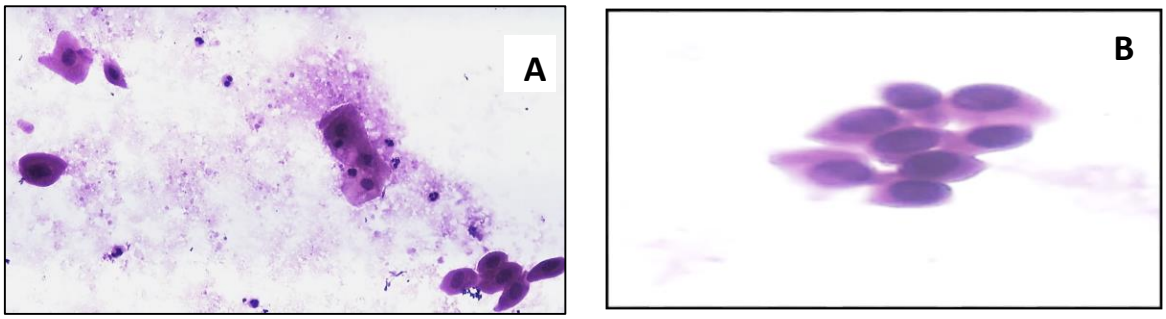

Figure [5]: Conjunctival impression cytology, right eye of 30-year-old female with sever DED [Schirmer's test 4mm, BUT 4Sec., grade 3 conjunctival hyperemia, grade 3 corneal staining]. CIC showed improvement from Nelson grade III [A] to Nelson grade I [B] after treatment with PRP

DISCUSSION

Artificial tears are the most common option in treatment of DED [9], AS drops has shown to be effective in treatment of dry eye with Sjögren's syndrome [10]. Other studies revealed that although superiority of AS over artificial tears in improvement of symptoms, but the differences in corneal staining and TBUT was not statistically significant [11].

In group I, our results showed that clinical variables measured [TBUT, Schirmer's test, level of hyperemia, CFS] were significantly improved after treatment, and these findings were similar to those found in other studies [12-14]. On the other hand, **Yoon *et al.*** [15] found non-significant improvement of Schirmer's test [p = 0.16] despite the significant improvement of TBUT [p= 0.01] after treatment with AS eye drops, and this is in agreement with **Jirsova *et al.*** [16] who found no statistically significant improvement of CFS [p = 0.10] and TBUT [p = 0.974], while the improvement of Schirmer's test was statistically significant [p < 0.01] after treatment with AS eye drops for 3 months in 17 patients with severe DED, which was explained by the marked worsening of CFS in 3 patients after treatment.

Regarding CIC in group I, our result showed a statistically significant improvement after treatment [p <0.001]; The degree of metaplasia lowered by one level in 37 eyes [61.6%]; 20 eyes [33%] improved from G3 to G2, 7 eyes [11%] improved from G3 to G1, 16 eyes [27%] improved from G2 to G1 and 1 eye improved from G1 to G0. A similar study with a statistically significant improvement of CIC after 1 month of treatment with AS eye drops, the grade of metaplasia in 7 patients [29.2%] lowered by one level; 4 patients with G4 metaplasia improved to G3, and three of the six patients with G3 improved to G2 [14]. Also, **López-García *et al.*** [13] found a statistically significant improvement of CIC [p =0.00], as the grade 2–3 improved by a mean between 1 and 2 grades after the treatment with AS eye drops for 2 months. **Jirsova *et al.*** [16] found similar results [p <0.05] after 3 months of treatment with AS eye drops.

Regarding visual outcome in group I, our results showed no change of BCVA after treatment [p =1.00]. These findings were similar to those found in other studies [13, 16] who reported that no statistically significant improvement of BCVA after treatment with AS eye drops.

PRP provides more concentration of platelets and growth factors. In a study by **Alio *et al.*** [17], an improvement in symptoms of patients with DED treated with PRP, also, improvement in conjunctival hyperemia, CFS, and CIC.

In group II, our results showed that clinical variables measured [TBUT, Schirmer's test, level of hyperaemia, CFS] were significantly improved after treatment. TBUT improved in 42% of patients by 2 sec and more and CFS improved in 22 patients [73%] and the level of conjunctival hyperemia improved to normal in 60% of patients. These findings were similar to those found by **García-Conca *et al.*** [18] who reported a statistically significant improvement in Schirmer's test [p <0.001], TBUT [p=0.003] after treatment with PRP eye drops for 1 month in 44 patients with hyposecretory DED. Also, **Alio *et al.*** [19] found a statistically significant improvement in Schirmer's test [p <0.05] and CFS [p <0.05], after 6 weeks of treatment. **Merayo-Lloves *et al.*** [20] showed similar performance with a statistically significant improvement of Schirmer's test [p = 0.05].

Regarding CIC in group II, our result showed a statistically significant improvement after treatment [p <0.001]; The grading at baseline was as following; 24 eyes [40%] with G3, 30 eyes [50%] G2, 6 eyes [10%] G1 after treatment improved to G2 in 18 eyes [30%], G1 in 40 eyes [66.7%], G0 in 2 eyes [3.3%]. **Alio *et al*.** [17] also reported a statistically significant improvement in CIC [p = 0.05] In contrast to our results, **López-Plandolit *et al*.** [13] who reported no statistically significant improvement in the degree of squamous metaplasia in 16 patients with moderate/severe DED, 3 months after treatment in spite improvement of symptoms and signs of DED. This may be because they included individuals with poor response to conventional treatments in their study [unpreserved artificial tears, topical corticosteroid, punctal plugs, lid hygiene, and/or systemic tetracycline] prior to involving in the study.

Regarding visual outcome in group II, our results showed a statistically significant improvement in BCVA after treatment [p <0.001]. These results were similar to the study done by **García-Conca *et al*.** [18] who found a statistically significant improvement in BCVA [p-value < 0.001] in 44 patients with hypo-secretory DED treated with PRP eye drops for 1 month. **Alio *et al*.** [19] found an improved of BCVA at least 1 line in 28.8% of 368 patients with moderate to severe DED 6 weeks after treatment, the improvement in vision was significantly correlated with the improvement in the CFS score [p = 0.04, r = 0.61]. Another study by **Merayo-Lloves *et al*.** [20] also reported a statistically significant improvement in BCVA in 27.4% of 156 patients, [p < 0.05].

Comparison between group I and group II showed a significant higher improvement in group II in TBUT [P = 0.001], Schirmer's test [p < 0.001] and CFS [P < 0.001] after treatment. Larger reduction conjunctival hyperemia but not significant [P = 0.275]. **Metheetrairut *et al*.** [21] reported that TBUT, Schirmer test and CFS score were slightly improved, but not significantly, 1 month after the use of PRP and AS. When they compared between the two groups; the changes in Schirmer's test were significantly higher for those treated with PRP than those treated with AS eye drops [p =0.036]. They compared the concentrations of four epitheliotrophic factors present in both AS and PRP, and found that the concentrations of epidermal growth factor [EGF], fibronectin, and transforming growth factor-beta1 [TGF-β1] were higher in PRP than in AS. On the other hand, the concentrations of platelet-derived growth factor-AB [PDGF-AB] were significantly higher in AS than in PRP and these factors did not decrease in concentrations in all storage conditions.

Regarding changes in CIC there was significant higher improvement in the degree of squamous metaplasia in group II compared to group I after treatment [p =0.04]. In 61.6% of group I [37 eyes]; the degree of metaplasia lowered by one level and more, while in 88.33% of group II [52 eyes]; the degree of metaplasia lowered by one level and more.

Regarding BCVA; although PRP eye drop significantly improved BCVA after treatment, the difference between our study groups was not statistically significant [p = 0.242]. Baseline BCVA in group I was [0 - 0.8 Log MAR] [6/36 – 6/6] and was [0 - 0.8 Log MAR] [6/36 – 6/6] after treatment, while in group II was [0 – 1.3 Log MAR] [4/60 – 6/6] and was [0 – 1.0 Log MAR] [5/60 – 6/6] after treatment. **Metheetrairut *et al*.** [21] found that PRP might be superior to AS as significant improvement of BCVA in PRP group, but the difference between the two groups was not statistically significant.

Conclusions: PRP eye drops induces a more significant improvement in different dry eye signs than AS in treatment of severe dry eye disease.

Conflict of interest: None to be declared.

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