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Autologous platelet-rich plasma eye drop for moderate-to-severe bacterial corneal ulcers: Changes in interleukin-6 tear concentration and clinical outcomes

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Abstract:

PURPOSE: The objective of this study was to evaluate interleukin-6 (IL-6) tear concentration and clinical outcome in patients with moderate-to-severe bacterial corneal ulcers post autologous platelet-rich plasma (PRP) eye drop therapy.

MATERIALS AND METHODS: This was a pre–post designed study involving 21 moderate-severe corneal ulcer patients who got autologous PRP eye drop. Subjects were got autologous PRP eye drop as adjuvant therapy. Patients with moderate-to-severe infectious bacterial corneal ulcers were included in this study. Tear sampling was performed before therapy using sterile Schirmer paper from conjunctival inferior fornix. PRP therapy was performed for 7 days. Data recording and tear sampling were then performed at day 0 (pre-PRP), day 7 (D+7), and day 14 (D+14) after PRP therapy. Data recording included presence of pericorneal injection, blepharospasm, size of corneal defects, and hypopyon.

RESULTS: There was a decrease in IL-6 tear concentration by day 14 after PRP therapy ($P < 0.001$). IL-6 concentration at day 7 after therapy (7525.67 ± 7092 pg/mL) tended to be lower before therapy (10,599 ± 6158 pg/mL), but not statistically significant ($P = 0.156$). The size of corneal defects decreased significantly post PRP at day 7 ($P = 0.035$) and at day 14 ($P = 0.001$). There was a significant blepharospasm at day 7 ($P = 0.012$) and day 14 ($P < 0.001$). There was a significant pericorneal injection only at day 14 ($P = 0.002$). There was no significant decreased hypopyon.

CONCLUSION: There was a significant reduction in IL-6 tear concentration and clinical improvement in moderate-to-severe bacterial corneal ulcers which got autologous PRP eye drop as adjuvant therapy.

Keywords:

Corneal ulceration, interleukin-6, platelet-rich plasma

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Introduction

Corneal ulceration is a serious ocular infection that can cause severe visual loss, and it is also the most significant common cause of corneal blindness.[1,2] It begins with an epithelial defect to stromal degradation, and it becomes worse due to inadequate healing process.[3]

Platelet-rich plasma (PRP) has been demonstrated to be effective in promoting optimal healing of dormant corneal ulcers of different etiologies and corneal neurotrophic ulcer.[4,5] PRP harbors high concentrations of essential growth factors being used widely in ophthalmology to promotion of wound healing. Platelets can translocate rapidly to the wound site and enhance

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the physiological process for healing reaction via eye drops.[6,7] PRP contains more concentrated platelets than whole blood, and it also contains the growth factors, such as platelet-derived growth factors, transforming growth factor-β, platelet factor IV, vascular endothelial growth factor, and epithelial growth factor.[7,8]

Interleukin-6 (IL-6) is a potential mediator of intraocular inflammation and plays an important role in corneal infection and inflammation. In corneal ulceration, IL-6 contributes directly or indirectly by promoting the recruitment and activation of PMNs. IL-6 trans-signaling pathway plays a significant role in ocular surface inflammation.[9,10] IL-6 is a pro-inflammatory cytokine released by macrophages, as a result of injured keratocytes. In acute phase corneal ulceration, more keratocytes will be injured, so that more IL-6 are released by macrophages.[11]

The present study aimed to evaluate IL-6 tear concentration and clinical outcomes in patients with moderate-to-severe bacterial corneal ulcers post autologous PRP eye drop therapy.

Materials and Methods

Subject

This study used a pre–post design; 21 adult patients with moderate-to-severe infectious bacterial corneal ulcers were included in this study. Table 1 shows the grade of corneal ulcer.

Patients with infectious corneal ulcer were given PRP eye drop therapy six times a day for 7 days as adjunctive treatment, simultaneous with the bacterial corneal ulcer protocol treatment. The inclusion criteria in this study were all patients diagnosed with moderate-to-severe bacterial corneal ulcers, willing to do PRP therapy, willing to follow the research, and signed the research informed consent sheet. Exclusion criteria in this study included patients who after PRP therapy refused to provide tear specimens as well as patients whose tear specimens were damaged or lost in the transport, and patients who failed to follow the monitoring schedule. Figure 1 shows the study protocol. The treatment protocol was given at the beginning before the corneal swab result revealed and based only clinical appearance of corneal bacterial infection. The patients were then excluded from the analysis if later the swab showed negative result (56.6% Gram-positive cocci). PRP eye drop preparation was described in the Supplementary Material.

Data collection

Tear sampling was performed before PRP treatment, 7 days after (D+7), and 14 days (D+14) after PRP therapy, using sterile Schirmer paper, extracted from conjunctival inferior fornix. The primary outcome was IL-6 concentration in tears, obtained from ELISA results done at the Laboratory of Molecular Biology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia. Clinical outcomes were size of corneal defects, hypopyon, blepharospasm (using Jankovic Spasm Grading[12]), and pericorneal injection (Efron and McMonnies Conjunctival Hyperemia Grading[13]). The study followed the tenets of the Declaration of Helsinki. The Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito General Hospital, approved the study protocol (Approval Number: KE/FK/1241/EC/2019). After detailed explanation, informed consent was obtained from each patient prior to examination.

Statistical analysis

Changes of IL-6 concentration (pre-PRP, D+7, and D+14); the differences of the corneal defect and hypopyon were analyzed by the Wilcoxon signed-rank test. Clinical proportions of hyperemia and blepharospasm were analyzed by Chi-square test.

Results

The study involving 21 eyes in 21 patients and the average age was 49.80 ± 12.48 years old. Table 2 shows the subjects' characteristics.

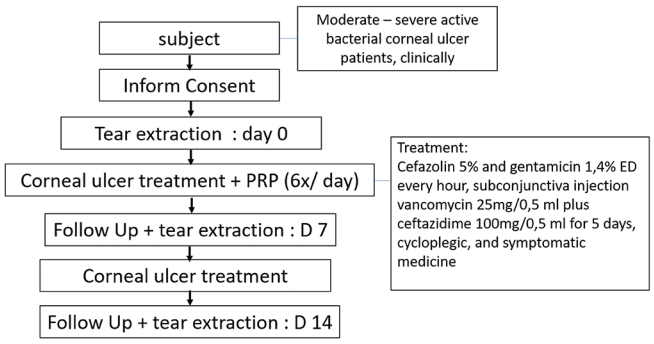

Figure 1: Study protocol

Table 1: Grade of corneal ulcer

Factor	Mild	Moderate	Severe
Location	Nonaxial	Central or peripheral	Central or peripheral
Area (mm)	2	2-6	>6
Depth	≤1/3 anterior stromal infiltration	1/3-2/3 anterior stromal infiltration	>2/3 stromal infiltration
Anterior segment inflammation	Mild	Moderate or severe; exudate, fibrin	Severe; hypopyon

There was a significant decrease in tear IL-6 concentration pre and D+14 after PRP therapy, with differences 8430 ± 6274 pg/mL (P = 0.000). The difference between D+7 and D+14 after PRP therapy is 5356 ± 6526 (P = 0.001). There was no statistically significant decrease in IL-6 concentration pre and D+7 after PRP therapy, with differences 3073 ± 9543 pg/mL [P = 0.156; Table 3].

The mean size of corneal defects before and after PRP therapy also significantly reduced; pre PRP defect size was 25.22 ± 11.37 mm^2, D+7 post PRP was 19.25 ± 11.15 mm^2, and D+14 post PRP was 14.53 ± 9.66 mm^2. Figure 2 shows the improvement of corneal defect size after treatment. The difference between pre and D+7 post PRP is 14.53 ± 9.66 mm^2 (P = 0.035), pre and D+14 post PRP is 10.69 ± 7.48 mm^2 (P = 0.001), and D+7 and D+14 post PRP is 4.71 ± 6.11 mm^2 (P = 0.028). The mean hypopyon before and after PRP therapy in this study was not found to change significantly [Table 4].

There was statistically significant decrease in pericorneal injection change pre and D+7 PRP (P = 0.002) also in D+7 and D+14 PRP (P = 0.005). The pericorneal changes in pre and D+7 post PRP were not found to change significantly. Blepharospasm changes were significant in pre and D+7 post PRP (P = 0.012), pre and D+1 post PRP (P = 0.000), and D+7 and D+14 post PRP (P = 0.004). Table 5 shows the pericorneal injection changes.

Table 2: Characteristics of the study subjects

Characteristics	*n* (%)
Age (year old)	
Mean±SD	49.80±12.48
Range (minimum-maximum)	30-69
Sex	
Male	17 (80.95)
Female	4 (19.05)
Size of corneal defect (mm^2)	
Mean±SD	25.22±11.37
Range (minimum-maximum)	4.40-43.55
Visual acuity (logMAR)	
Mean±SD	2.39±0.30
Range (minimum-maximum)	1.80-2.80
Pericorneal injection	
Grade 1	0
Grade 2	0
Grade 3	12 (57.14)
Grade 4	9 (42.86)
Hypopyon (mm)	
Mean±SD	1.08±0.85
Range (minimum-maximum)	0.00-2.50
Blepharospasm	
Grade 1	1 (4.76)
Grade 2	5 (23.81)
Grade 3	14 (66.67)
Grade 4	1 (4.76)

SD=Standard deviation

Discussion

A significant decrease in tear IL-6 concentration was found after PRP therapy followed by decreased clinical signs of inflammation (blepharospasm and pericorneal injection). PRP has been used to treat some corneal diseases, and it could be a novel treatment option for chronic ocular surface disease or some refractory corneal defects.[7,8] In a report by Kim *et al.*,[7] 11 patients with persistent corneal epithelial defects underwent PRP treatment achieved complete re-epithelialization with no additional treatment with the mean epithelial healing time 10.09 ± 2.49 days (range 2–30 days). In another study, Wróbel-Dudzińska et al. reported that the PRP was efficient in treatment of neurotrophic keratopathy. There was an 80% complete healing of the ulceration at the end of the study.

This study investigated IL-6 tear concentration in patients with moderate-to-severe bacterial corneal ulcers who underwent PRP as adjuvant treatment. As we can figure, no previous studies have reported it. This was similar to our previous study showing the IL-6 decrease after corneal cross-linking (CXL) treatment.[14] The exact mechanisms underlying these IL-6 changes remain

Table 3: Changes in interleukin-6 tear concentration after platelet-rich plasma treatment

IL-6 (pg/ml)	Pre-PRP (A)	D+7 PRP (B)	D+14 PRP (C)	pAB (Δ AB)	pAC (Δ AC)	pBC (Δ BC)
Mean±SD	10599±6158	7525±7095	2168±2166	0.156 (3073±9543)	0.000* (8430±6274)	0.001* (5356±6526)
Range	2219-22,613	225-22,613	88-7191			

*P<0.05. IL-6=Interleukin-6, PRP=Platelet-rich plasma, Δ=Difference

Table 4: Changes in average size of corneal defects and hypopyon

Variable	Pre-PRP (A)	D+7 PRP (B)	D+14 PRP (C)	pAB (Δ AB)	pAC (Δ AC)	pBC (Δ BC)
Corneal defect (mm^2)						
Mean±SD	25.22±11.37	19.25±11.15	14.53±9.66	0.035* (14.53±9.66)	0.001* (10.69±7.48)	0.028* (4.71±6.11)
Range	4.40-43.44	4.40-39.69	1.00-28.42			
Hypopyon (mm)						
Mean±SD	1.08±0.84	0.82±1.05	0.68±1.00	0.207 (0.26±0.65)	0.103 (0.40±0.74)	0.192 (0.14±0.09)
Range	0-2.5	0-2.5	0-2.5			

*P<0.05. PRP=Platelet-rich plasma, Δ=Difference, SD=Standard deviation

Table 5: Changes in pericorneal injection and blepharospasm

Variable, *n* (%)	Pre-PRP (A)	D+7 PRP (B)	D+14 PRP (C)	pAB	pAC	pBC
Pericorneal injection						
Grade 1	0	0	0	0.096	0.002*	0.005*
Grade 2	0	3 (14.29)	9 (42.86)			
Grade 3	12 (57.14)	11 (52.38)	7 (33.33)			
Grade 4	9 (42.86)	7 (33.33)	5 (23.81)			
Blepharospasm						
Grade 1	1 (4.76)	2 (9.52)	7 (33.33)	0.012*	0.000*	0.004*
Grade 2	5 (23.81)	13 (61.90)	11 (52.38)			
Grade 3	14 (66.67)	5 (23.81)	3 (14.29)			
Grade 4	9 (4.76)	1 (4.77)	0			

*P<0.05. *n* (%)=Sample size (percentage), PRP=Platelet-rich plasma

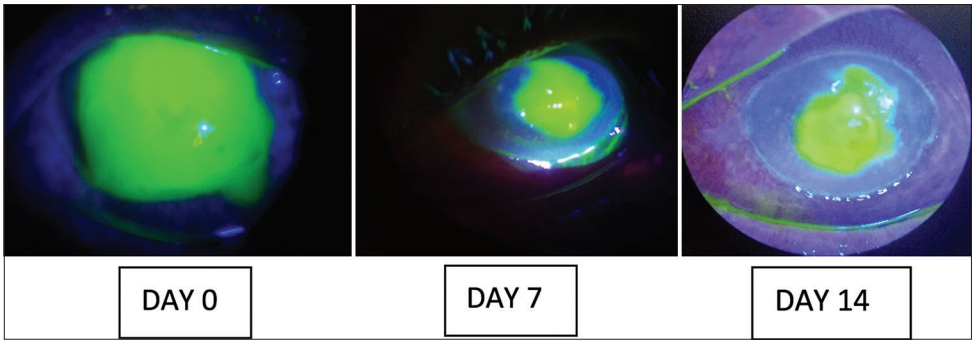

Figure 2: The improvement of corneal defect size after treatment

unknown, and this condition is also parallel with the clinical outcome improvement. However, corneal defect healing depends on limbal epithelial stem cell (LESC) activity and YAP activation promotes the activation and expansion of LESCs[15] which might be affected by inflammation process (represented by IL-6 in this study). From another study, we might conclude that the platelets secreted growth factors and active metabolites that might exert positive effects in clinical situations that require rapid healing and tissue regeneration.[7] IL-6 as a pro-inflammatory cytokine released by macrophages, as a result of injured keratocytes, and the healing of cornea could decrease the IL-6 release.

Conclusion

A decrease in IL-6 tear concentration was found after PRP therapy in patients with moderate-to-severe bacterial corneal ulcers. It was followed by decreased clinical signs of inflammation (blepharospasm and pericorneal injection). Therefore, autologous PRP is beneficial for corneal ulcers treatment, and a large multicenter clinical trial is needed for further study.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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Supplementary Material

Platelet-rich plasma eye drop preparation

a. Label the 10-ml syringe and write down the patient code
b. Insert citrate buffer as an anticlotting agent 1.4 ml into a 10 ml syringe
c. Take blood from the cubital vein up to 10 ml, then mix well
d. Divide the blood into 2 syringes with a volume of 5 ml
e. Insert the syringe into the centrifuge, rotated at 1200 rpm (150 g) for 10 min
f. Take a syringe from the centrifuge, you will see 3 layers, namely at the top it is a liquid plasma, in the middle there is a white area (buffy coat), and at the bottom there are red blood cells
g. Transfer the plasma and buffy coat by connecting the syringe filled with centrifuged blood to the empty syringe in a vertical position over the syringe filled with centrifuged blood
h. The syringe containing the buffy coat and plasma was centrifuged at 3500 rpm (1275 g) for 10 min to obtain platelets, then 2 layers formed in the form of plasma and platelet deposits on the bottom
i. Half of the plasma at the top was removed, then the plasma and platelets were pipetting to make it homogeneous
j. The platelet deposit contains the concentration of platelets in 1.5 cc of plasma fluid. That is called platelet-rich plasma (PRP)
k. PRP is put in an eye drop bottle
l. Stored in the refrigerator at 4°C.