Combined Platelet Rich Plasma (PRP) And Amniotic Membrane Graft (AMG) Versus Amniotic Membrane Graft (AMG) Alone For The Treatment Of Perforated Central Corneal Ulcer

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Abstract

Purpose: The aim of this work is to evaluate the safety and efficacy of combined use of topical platelet rich plasma (PRP) -PRP clot and eye drops- and amniotic membrane graft (AMG) in the management of corneal perforations and compare the results with AMG alone.

Methods: This is an interventional comparative prospective randomized clinical study. Thirty-six eyes with infectious perforated central corneal ulcer. These eyes were classified into two groups each of 18 eyes. In group (A), eyes were treated by AMG combined with PRP clot to seal the central corneal perforation followed by PRP eye drops 6 times per day. In group (B), eyes were treated by AMG alone.

Results: Most of the cases had anterior chamber formation within 24 hours; 94.4% and 66.7% in groups (A & B), respectively with statistically highly significant (P = .035), stability of the graft was better in group (A) without statistically significant difference (P = .03). Complete resolution of infectious perforated corneal ulcer was achieved in 18 eyes (100 %) and 14 (77.8%) in groups (A & B), respectively with statistically highly significant difference (P <0.001). Failure was not observed in any eyes (0 %) in group (A) and in 4 eyes (22.2%) in group (B) with statistically highly significant difference (P < .001).

Conclusions: PRP clot and eye drops can be used as an available and effective adjuvant therapy to AMG for treating infectious central corneal perforation. This enhances the sealing of corneal perforation and anterior chamber (AC) reformation and fasten the healing of infectious perforated corneal ulcer with better visual improvement and less complications.

Keywords: Platelets rich plasma ; corneal perforation ; central ; amniotic membrane graft.

Introduction

Many factors that cause corneal melting can lead to the potentially fatal complication of corneal perforation. Microbes are just one of the many causes **[1]**.

However, there are some significant issues that need to be taken into account when using amniotic membranes in the management of corneal perforations. These issues include the possibility of viral or bacterial contamination, the prolonged healing time in some cases, and the surgical challenge of fashioning the membrane to ensure proper seal **[2,3]**.

For that, the addition of another therapeutic approach is required, such as PRP, which may lessen some of the drawbacks of using amniotic membranes alone. PRP clots include an abundance of growth factors that speed up wound healing and have a platelet concentration that is roughly 20 times higher than that of blood. **[4]**.

PRP has been used for surface restoration following ocular perforation brought on by transplanting amniotic membrane. Although it necessitates adhering to rigorous sterility conditions, utilising sterile and disposable materials, and operating inside a laminar flow hood, the preparation of PRP in the two accessible formulations—eye drops and clot—is inexpensive and simple. **[5-6]**.

In this study we compare the safety and efficacy of combined PRP and AMG versus AMG alone for the treatment of perforated infectious central corneal ulcer.

Patients and Methods

This is a prospective comparative study conducted between October 2022 and February 2023 at the Ophthalmology Department, Zagazig University Hospital, Egypt. The Research Institute of Ophthalmology Institutional Review Board (IRB) approved the study protocol, which adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants before participation. Informed consent was obtained from each patient in which the aim and procedure were discussed to the patient. **This study was successfully submitted to CTRI (Clinical Trials Registeration of India) under the CTRI number CTRI/2023/04/051934 [Registered on: 24/04/2023] - Trial Registered Retrospectively CTRI Website URL - http://ctri.nic.in**

The study included adult patients more than 18 years of age (both sexes included) seeking for treatment of corneal perforation after infection, central perforation size between 2 and 4 mm. Exclusion criteria were intraocular infection, perforation with non-infective keratitis, large perforation >4 mm and very small perforation <2 mm and severe dry eye.

Identification of organisms was done by direct smears and cultures, which were sent to the laboratory for differentiation of organisms whether bacterial, viral, fungal, or acanthamoeba. The corneal perforation was evidenced by positive Seidel testing.

Clinical examination of the anterior chamber (AC) and eye evaluation by slit-lamp (SL) biomicroscopy and anterior segment optical coherence tomography (AS-OCT) before and after treatment were performed for measuring the stability of AMG. The same was repeated after one month. Evidence of intraocular infection was determined by indirect ophthalmoscopy of the diseased eye (if seen) or B-scan ultrasonography.

Medical treatment:

Both groups were treated by topical antimicrobial therapy according to culture and sensitivity tests after identification of the causative micro-organisms. Oral doxycycline once daily to prevent further corneal melting to inhibit corneal collagenase was used together with oral vitamin C to enhance collagen synthesis.

Preparation of AMG: Fresh and sterile amniotic membrane screened for HIV, hepatitis B, hepatitis C and syphilis was used.

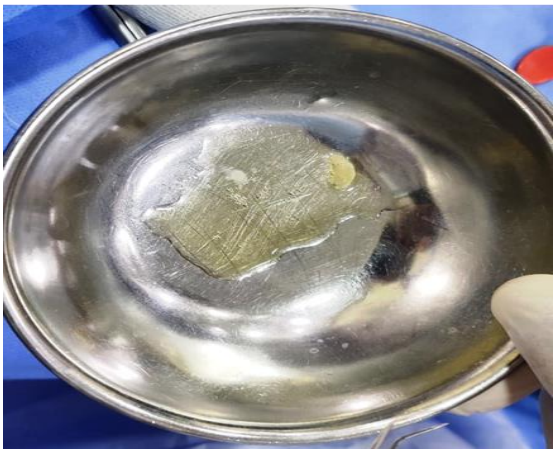
Preparation of autologous platelet-rich plasma (PRP): 60 ml blood was drawn from each patient and sent to the university laboratory for the preparation of both the PRP clot and eye drops on the same day. The collected sample was divided into two tubes with the addition of sodium citrate to prevent coagulation. The centrifugation was done for about 10 min at 5˚ C, after the plasma separation the lower part was used and divided into two parts to create the clot, and the eye drops. The part for the clot is placed in the tissue culture plates under sterile conditions and after adding 10% calcium chloride the plates were incubated for 30 min at 37 ˚C. The PRP clots were transferred directly for use in the surgical operation room while the part used for eye drops were stored in the freezer until used.

Interventional Treatment (figure 1):

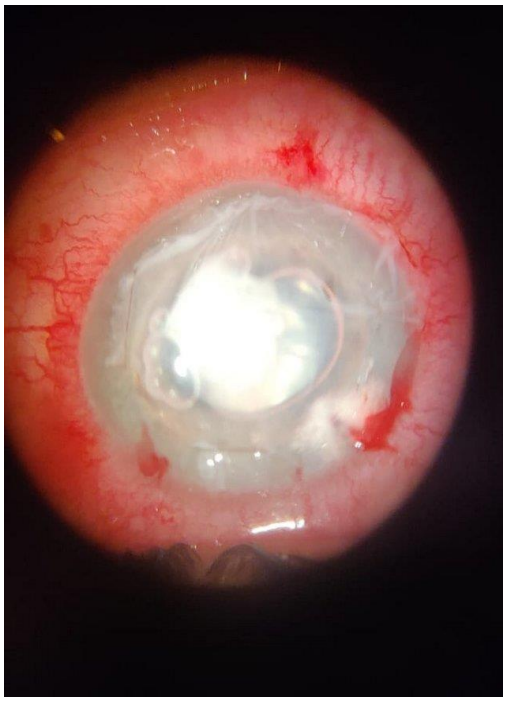
All surgeries were done under complete aseptic conditions. After surface anesthesia in the form of benoxinate hydrochloride 0.4% (Benox 0.4 %) eye drops, debridement of the corneal epithelium was done surrounding the defect. Cleaning of the wound was done to remove any necrotic tissue, to ensure clean edges of the perforation wound and the debrided tissue was sent for culture and sensitivity. Viscoelastic material was used for AC formation. The fresh sterile amniotic membrane was fashioned in a circular shape with a diameter at least 2 mm larger than the diameter of the corneal perforation and placed with its epithelial side up. Then 10-0 nylon sutures were used to suture the amniotic membrane to the underlying corneal tissue in the lower half of the cornea. For group (A), the PRP clot was introduced under the

amniotic membrane just over the perforation and then the amniotic membrane was sutured in the upper, nasal, and temporal cornea by 3 interrupted 10-0 nylon sutures. Interrupted sutures were done till membrane stabilization and proper fixation of the membrane. Then washing of the viscoelastic material, injecting air bubble into the AC and a bandage contact lens was applied. In group (A), postoperative PRP eye drops was used 6 times daily alongside the standard postoperative medical treatment such as broad spectrum topical antimicrobial therapy for different types of infectious keratitis such as fourth generation fluoroquinolones (e.g. gatifloxacin and moxifloxacin) as monotherapy for bacterial keratitis, voriconazole 1% or natamycin 5 % and itraconazole 1% for fungal keratitis and topical anti-amoebic agents (e.g. polyhexamethylenebiguanide 0.02 % or hexamidine 0.1 % and aminoglycosides) for acanthamoeba keratitis together with mydriatic-cycloplegics. In group (B), AMG only was used without PRP clots or eye drops.

Central infectious 4 mm perforated corneal ulcer



Preparation of PRP clot



PRP clot was put underneath the AMG , then AMG was sutured in all quadrants.

Fig. (1): Steps of AMG and PRP clot.

Postoperative follow-up evaluation:

Follow-up visits of the patients were done 1 and 3 days postoperatively, then on weekly basis for 4 weeks after which the AMG and sutures were removed. Patients were evaluated for sealing of perforation both clinically using slit lamp examination and negative Seidel testing and by AS-OCT after 4 weeks of surgery. AC formation and maintenance, stability of AMG by clinical examination and AS-OCT, resolution of infectious keratitis, intraocular pressure (IOP), uncorrected and best corrected visual acuity (UCVA & BCVA) were assessed before and after the procedures. Adverse events, including failure of closure of perforation, dislocation of AMG, and Infection, were reported at the final follow-up.

Statistics

The collected data were coded, entered, presented and analyzed by computer using a data base software program, Statistical Package for Social Science (SPSS) version 20. Mean ± SD, chi-square and t-test were used for determination of significance (P value). $P < 0.05$ is considered significant.

Results

The study constituted 36 eyes of 36 patients of infectious central corneal perforation. They were classified into two treatment groups each of 18 eyes. Group (A) was treated by PRP clot and AMG, while group (B) was treated by AMG only. Patients' age and patients' characteristics are represented in **table (1).**

Regarding the etiology, most of the corneal ulcers were due to bacterial keratitis (BK) (66.67 %), followed by fungal keratitis (27.78 %), while acanthamoeba (2.7 %) and viral (2.7 %) were the least as shown in **table (2)**. The most common risk factor for infectious keratitis was eye trauma in 10 patients (27.8%), then ocular surface diseases, e.g., dry eye syndrome in 8 eyes (22.2%), prolonged topical steroid treatment in 7 eyes (19.4%), contact lens in 6 eyes (16.7%) and trichiasis in 5 eyes (13.9%), as shown in **table (2).**

Statistically significant (p-value = 0.035) increased percentage of AC formation (after 1 day) was found in group A (17 patients, 94.4%) when compared to group B (12 eyes, 66.7%). No statistically significant difference (p-value = 0.310) was detected between group A and group B as regards stability of AMG as it was present in 18 eyes (100%) of group A and 17 eyes (94.4%) of group B. Statistically significant (p-value = 0.033) increased percentage of complete resolution (after 1 month) in group A (18 patients, 100%) was found when compared to group B (14 patients, 77.8%). Statistically significant (p-value = 0.033) difference in the percentage of failure after 1 month was detected in group A (0%) when compared with group B (22.2 %) as shown in **table (3).**

Post-treatment complications were lower in group (A) than group (B). Secondary infection was reported in one eye (5.6%) in each group, also, suture loosening in 1 eye (5.6%) in each group and incomplete healing of perforation was found in 3 eyes (16.7%) in each group. On the other hand, graft instability was found in one eye (5.6%) in group (B), and persistent corneal opacity was found in 1 eye (5.6%) in group (A) and in 4 eyes (22.2%) in group (B) as shown in **table (4).**

Visual acuity improved gradually after one month postoperatively, however, the improvement was faster in group (A) as shown in **figure (2)**. Also, the drop of IOP due to perforation improved and elevated gradually and became steady after one month in both groups as shown in **figure (3).**

Table (1): Patients' characteristics of the study population.

	Total		Group (A)		Group (B)		Significance	
	No.	%	No.	%	No.	%	χ^2	P value
Patients	36	100	18	50.0	18	50.0	0.000	1.000
Males	16	44.4	8	44.4	8	44.4	0.000	1.000
Females	20	55.6	10	55.6	10	55.6	0.000	1.000
Urban	12	33.3	7	38.9	5	27.8	4.186	0.000*
Rural	24	66.7	11	61.1	13	72.2		
Educated	15	41.7	7	38.9	8	44.4	0.953	0.052
Ignorant	21	58.3	11	61.1	10	55.6		
VA > 6/60	4	11.1	2	11.1	2	11.1	0.000	1.000
VA ≤ 6.60	32	88.9	16	88.9	16	88.9	0.000	1.000
	Mean ± SD		Mean ± SD		Mean ± SD		t	P value
Age (years)	43.4 ± 4.82		41.9 ± 4.31		44.3 ± 5.02		0.035	0.462
Ulcer size (mm): • Mean ± SD • Range	3.15 ± 0.82 2.00 – 4.00		3.14 ± 0.76 2.03 – 3.97		3.16 ± 0.85 2.00 – 4.00		0.128	0.539

χ^2: Chi square test, t: unpaired t-test. *p <0.05 = statistically significant, VA: visual acuity.

Table (2): Etiology and risk factors of infectious keratitis in the study patients.

Causative microrganisms	Species	No.	%	OR	95% CI
Bacterial (24)	*Streptococci* *Staphylococci* *Pseudomonas aeruginosa*	10 8 6	27.8 22.2 16.7	5.37 4.24 3.51	2.89 – 9.64 1.95 – 6.81 1.17 – 5.34
Fungal (10)	*Fusarium solani* *Aspergillus flavus* *Penicillium*	5 4 1	13.9 11.1 2.78	3.36 2.75 3.18	1.36 – 5.14 1.12 – 4.96 1.27 – 5.11
Parasitic (1)	*Acanthamoeba*	1	2.78	3.21	1.75 – 5.58
Viral (1)	*Herpes Simplex*	1	2.78	3.15	1.22 – 4.97
Total	8	36	100		
Risk Factors					
• Direct trauma		10	27.8	13.8	8.58 – 21.14
• Contact lens wearers		6	16.7	6.95	2.96 – 9.75
• Trichiasis		5	13.9	5.65	2.48 – 7.85
• Abuse of steroid eye drops		7	19.4	8.86	6.17 – 12.18
• Ocular surface disease		8	22.2	9.57	7.14 – 11.97

N: number, OR: Odds Ratio, CI: Confidence interval.

Table (3): Outcome of treatment of infectious corneal ulcer of the study population.

Outcome	Group (A)		Group (B)		Significance	
	N (18)	%	N (18)	%	χ^2	P value
AC formation (after 1 day)	17	94.4	12	66.7	4.4	0.035*
Stability of AMG	18	100	17	94.4	1.02	0.310
Complete resolution after 1 month	18	100	14	77.8	4.5	0.033*
Failure after 1 month	0	0	4	22.2	4.5	0.033*

χ^2 = Chi square test, *p <0.05 = statistically significant. AMG: Amniotic membrane graft.

Table (4): Complications of treatment of corneal perforation among study groups

Outcome	Group (A)		Group (B)		Significance	
	N (18)	%	N (18)	%	χ^2	P value
Secondary infection	1	5.6	1	5.6	0.000	1.000
Suture loosening	1	5.6	1	5.6	0.000	1.000
Graft instability	0	0.00	1	5.6	1.635	0.002*
Incomplete healing	3	16.7	3	16.7	0.000	1.000`
Persistent corneal opacity	1	5.6	4	22.2	19.56	0.000*

χ^2 = Chi square test, *p <0.001 = statistically highly significant.

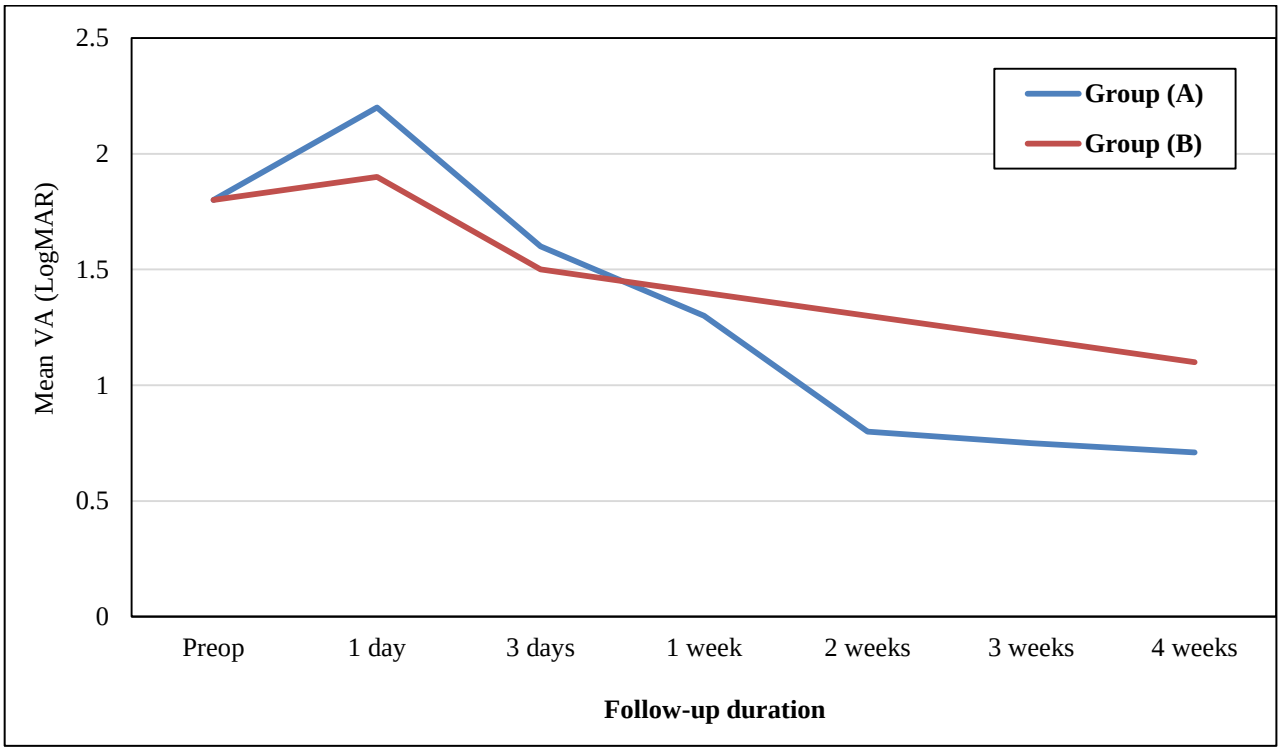


Fig. (2): Visual acuity improvement during the follow-up period.

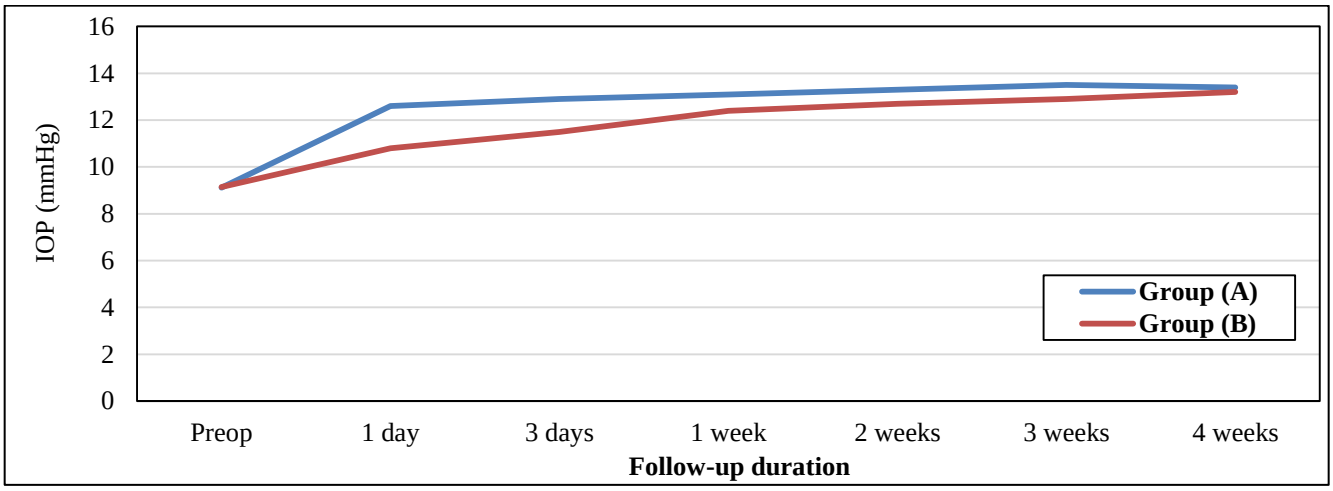


Fig. (3): Intraocular pressure (applanation tonometer) during the follow-up period

Discussion

Depending on the size of the perforation, numerous treatment methods were tested, such as the use of artificial tissue adhesives, synthetic or natural AMG, or the closure of the perforation with corneal or conjunctival flaps. The goal of the surgical technique is to seal the corneal perforation in order to avoid complications and to make it possible to cure the infection and hypotony that are connected with it. With the use of these modalities, the disease can be stabilised, leading to superior anatomical and optical results. **[7]**. Although corneal glue, whether made of cyanoacrylate or fibrin tissue, may be the first line of treatment for small perforations and is susceptible to bacterial infection, larger corneal defects may require the use of corneal tissue, which does not promote healing but rather creates a mechanical seal around the defect. **[5]**.

Arnalich et al. [8] concluded that PRP is a reliable and effective surgical coadjuvant to promote corneal wound healing in severe corneal ulcers and corneal perforations and it may be associated with other ocular surface reconstruction procedures.

We chose central perforations of 2 to 4 mm^3 for this study as mentioned previously **[1]** that perforations of up to 3 mm^3 that could be safely managed with fibrin glue and AMG, as these techniques allowed rapid reconstruction of the corneal surface, thus allowing keratoplasty to be performed under more favorable conditions. **[1,9,10]**

Statistically significant (p-value = 0.035) increased percentage of AC formation (after 1 day) was found in group A (17 patients, 94.4%) when compared to group B (12 eyes, 66.7%). No statistically significant difference (p-value = 0.310) was detected between group A and group B as regards stability of AMG as it was present in 18 eyes (100%) of group A and 17 eyes (94.4%) of group B. Statistically significant (p-value = 0.033) increased percentage of complete resolution (after 1 month) in group A (18 patients, 100%) was found when compared to group B (14 patients, 77.8%). Statistically significant (p-value = 0.033) difference in the percentage of failure after 1 month was detected in group A (0%) when compared with group B (22.2 %) as shown in **table (3).**

Abdelghaby et al. [5] used the same techniques, had 100% of cases with complete resolution 4 weeks after treatment without any failures in both groups. However, after 6 months, 3/10 of their patients underwent penetrating keratoplasty (PKP), to recover corneal clarity and visual potential with satisfactory visual outcomes and the rest waiting for corneal grafts. Similar studies used different combinations were reported previously. **Hick et al. [11]** study included 33 cases of corneal perforation in 14 patients. They concluded the perforations of up to 3 mm^3 that could be safely managed with fibrin glue and AMG. **Kotb and Elsayed [10]** successfully treated perforations of 20 eyes up to 3.4 mm^3 by SMILE lenticule graft, and **Tawfeek et al. [1]** used the same technique and both had successful results. They reported that complete closure was achieved in mean of 5.19±1.01 weeks in AMG.

López-Plandolit et al. [12] used plasma rich in growth factors for persistent corneal epithelial defects treatment reaching quite good results: epithelial defects healed in 17 of 20 cases. The main problem of this

hemo-derivative application is the preparation based on especially designed devices and double centrifugation technique with platelet activation.

Visual acuities improved gradually after one month postoperatively, however, the improvement was faster in group (A) as shown in figure (2). Also, the drop of IOP due to perforation improved and elevated gradually and became steady after one month in both groups as shown in figure (3).

Tawfeek et al. [1] reported that the improvement of BCVA was achieved in all cases of both groups; however, the SMILE lenticule group showed less improvement in BCVA than AMG with PRP group with non-significant difference.

In agreement with our results, **Abdelghaby et al. [5]** found improvement of IOP which became stable in all cases after one week.

Regarding complications, they were lower in group (A) than group (B). Secondary infection in one eye (5.6%) in each group, also, suture loosening in 1 eye (5.6%) in each group and incomplete healing of perforation was found in 3 eyes (16.7%) in each group. On the other hand, graft instability was found in one eye (5.6%) in group (B), and persistent corneal opacity was found in 1 eye (5.6%) in group (A) and in 4 eyes (22.2%) in group (B).

Abdelghaby et al. [5] reported no complications in their series; there was no evidence of corneal infection or intraocular inflammation, patients were compliant of mild symptoms in 1st postoperative week like foreign body sensation and lacrimation, resolved by removal of sutures.

On the other hand, **Tawfeek et al. [1]** reported few complications observed in both groups in 12/40 eyes. Graft sliding in 2 eyes (10%) in AMG with PRP group. Loose stitching was reported in one eye (5%) in the same group and leak was noticed in 1 eye (5%), which required re-suturing, shallow AC was observed in one eye (5%) and corneal opacity in 2 eyes (10%) of AMG with PRP group.

Theoretically, the adjuvant use of PRP with AMG would enhance the regenerative effect of these interventions, by release of growth factors that promote wound healing and decrease inflammation **[8]**.

Alió et al. [13] was the first to present a series of cases with perforated eyes or high probability of perforation due to deep chronic corneal ulcers treated with AMT combined with a clot of autologous PRP. Surgery consisted of wound debridement, excision and removal of devitalized tissue with a posterior application of AM to the wound site with the epithelial side up. A clot of autologous PRP was inserted beneath the AM to seal the imminent or existing corneal perforation with an increase in the therapeutic effect of the AM as we prescribed. Initial outcome measures were the decrease in size or depth of the corneal ulcer and improvement in BCVA by 57% with decreased inflammation within 2 weeks. They revealed that the healing effect of the PRP in combination with AM was higher than using AM alone. The prolonged synthesis and release of growth factors by the PRP clot provides additional long acting that would increase the benefit of PRP over autologous serum **[8,14]**.

It has been proven that platelet-rich plasma contains cytokines (e.g., PF4 and CD4OL) as well as growth factors such as PDGF (platelet-derived growth factor), TGF- (transforming growth factor-) ß1 and ß2, IGF- (insulin-like growth factor-) 1, VEGF, EGF, FGF-2, and IGF **[15,16]**. Most of its efficiency PRP owes to PDGF factor which is the first growth factor to appear in a wound, stimulating revascularization, collagen synthesis, and regeneration. Its role in healing process is to increase the number of repair cells, stimulate angiogenesis, support the development of new blood vessels, and activate macrophages responsible for cleaning the wound **[17]**.

Lee et al. [18] reported that the mean frequency of recurrence of corneal erosion was 0.06 ± 0.08 per month in the PRP eye drops treated group and 0.39 ± 0.24 per month in the conventional treatment group ($p = 0.003$).

PRP is effective in other forms of corneal ulcer perforation such as neurotrophic ulcer. **Palioura et al. [17]** concluded that the lack of preservatives, autologous quality, relative ease of its preparation, safety, and beneficial effects makes PRP a promising therapeutic tool for future regenerative medicine. Even though recombinant synthetic products are available for neurotrophic ulcer treatment, those high-priced goods contain only a single growth factor.

Conclusion:
A combination of AMG and PRP clot and eye drops was proven to be an effective and safe primary treatment for infectious central corneal perforation with few complications. We recommend using PRP as a coadjuvant to AMG for treating medium-sized corneal perforations post-infectious keatitis , however, more studies are recommended in large scale number for more evaluation of the outcome and complications.

Declarations:

-**Ethics approval and consent to participate**: This study was approved by the research ethical committee and the Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University,Egypt and met the ethical code of the World Medical Association for human experimentation, as stated in the Helsinki Declaration. Written informed consent was obtained from all study participants. For the non-educated participants, informed consent to participate was taken from these participants' parents/legal guardians.
-**Consent to publish:** Not applicable.
-**Availability of data and materials:** The deta-sets generated during and analysed during the current study are not available due to the protection of data security (the original data contains a lot of specifically demographic characteristics information and will be used again in the future follow-up study) but are available from the corresponding author on reasonable request.
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-Competing interests: The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.
-Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [***Dr. Mohammed M. Mahdy Tawfeek***], [***Dr. Mahmoud Yaseen Saber Atia***]. The first draft of the manuscript was written by ***[Prof. Dr. Sameh Mosaad Abd Al Raoof Fouda***] and [***Prof. Dr. Ezzat Abdelrahman Ibrahim Shahien***], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

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