Original Article

Epitheliotrophic Effect of Autologous Serum in Persistent Corneal Epithelial Defects

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Purpose: To evaluate the efficacy and complications of autologous serum in cases of persistent corneal epithelial defect (PED).

Material and Methods: Patients with PED unresponsive to conventional lubricant treatment were included in this clinical, prospective study which was conducted at IIMCT and Railways teaching Hospital. Patients were examined on day 1 and appropriate treatment including lubricant therapy was instituted. Subsequently, they were examined after 3 weeks and started on 20% autologous serum eye drops in cases of PED. All the other previously used lubricant therapy was stopped. Then, they were examined every 2 weeks for the next three visits. At each visit, in addition to routine eye examination some additional tests were performed i.e. Slitlamp examination for corneal epithelial defects, marginal tear strip evaluation, Schirmer's test without anesthesia, tear film breakup time, fluorescein and rose Bengal staining and corneal sensitivity.

The healing was defined as effective if occurred within 2 weeks of initiating serum therapy, partially effective if occurred within one month and ineffective if didn't occur within one month.

Results: A total of 17 eyes in 10 patients were studied. The local spectrum of indications was severe vernal limbitis with keratopathy (6 eyes, 35.3%), keratoconjunctivitis sicca (7 eyes, 41.1%), drug toxicity (1 eye, 5.9%), lime instillation (2 eyes, 11.8%) and trophic ulcer (1 eye, 5.9%).

Healing in 9 eyes occurred within 2 weeks and in 3 eyes within further 2 weeks. In 5 eyes, healing didn't occur within one month. All the patients were able to complete the follow-up of the study. Accordingly, healing was declared as effective in 9 out of 17 eyes (52.9%), partially effective in 3 out of 17 eyes (17.7%) and ineffective in 5 out of 17 eyes (29.4%). There were no adverse effects except for one red eye without discharge.

Response rate was excellent in all except those with severe keratoconjunctivitis sicca (KCS) and below-normal Schirmer's test value before serum therapy. The mean duration of PED before initiating serum therapy was compared between the effective and ineffective groups. Independent sample t-test was applied and the difference was found to be highly insignificant (*p* value 0.537).

Conclusion: Autologous serum was considered effective in majority of the cases with PED recalcitrant to conventional lubricant therapy due to the presence of essential factors. Healing depended more upon the etiology than the duration of

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PED. Due to scarcity of literature on serum therapy, further studies are required to establish the efficacy

Corneal epithelial surface may be damaged in a variety of conditions like KCS, mechanical and chemical trauma, corneal infections, neurotrophic keratopathy, corneal dystrophies and long-term topical steroid therapy. Moreover, there may be defective regeneration of healthy corneal epithelium in cases of limbal stem cell deficiency and vernal limbitis.

Various pharmacological lubricant preparations used to treat PED include hydroxypropylmethylcellulose (HPMC), polyvinyl alcohol, povidine and sodium hyaluronate. Recently, autologous serum[1,2] and umbilical cord serum therapy[3] have shown promising results in the treatment of PED. Surgical options for the management of dry eyes and PED include punctal occlusion, lateral and central tarsorrhaphy and amniotic membrane transplantation [4,5].

Serum is the fluid component of blood minus its clotting factors and cellular components. The natural tears have optical, mechanical, antimicrobial and nutritional properties. They contain epidermal growth factor (EGF), fibronectin and vitamin A which help in proliferation, migration and differentiation of corneal epithelial cells[6]. EGF may also help in reepithelialization due to its anti-apoptotic properties[7,8] . Serum also contains lysozyme, IgG and complement which may reduce the risk of infection. Autologous serum eye drops are non-allergenic with properties similar to the natural tears. They have also been used for cases of keratoconjunctivitis sicca[9,10]. Vitamin A is found in higher concentrations in serum as compared to tears. It may help in decreasing the squamous metaplasia in cases of KCS[11].

Poon et al conducted a clinical pilot study to compare the in-vitro toxicity of serum drops with unpreserved hypromellose (hydroxypropylmethylcellulose 0.3%) on corneal epithelial cell cultures[12]. Serum drops were found to have reduced toxicity compared with unpreserved hypromellose. The morphology and ATP levels of cultured cells exposed to serum were maintained better as compared to hypromellose. Both are found to be well established parameters of viability of the cells and used for evaluation of cellular toxicity[13,14].

The present study was conducted to evaluate the efficacy of autologous serum eye drops in cases of PED resistant to conventional lubricant therapy.

MATERIAL AND METHODS

All the patients with PED not responding to conventional lubricant dry eye therapy were included in this study. The study design was prospective. The case recording was done between Sept' 2004 and Aug' 2006.

At the initial visit, detailed history was taken, specifically asking for previous topical treatment and duration. Eye examination included visual acuity, slit-lamp examination for corneal epithelial defects, marginal tear strip evaluation, Schirmer's test without anesthesia, tear film breakup time (BUT), fluorescein and rose Bengal staining and corneal sensitivity. Appropriate lubricant treatment for dry eyes was instituted. The patients were subsequently examined after three weeks and started on autologous serum eye drops, if the corneal epithelial defects had not healed. At the same visit, previously used dry eye treatment was stopped to monitor the isolated effect of autologous serum.

The protocol for autologous serum eye drops preparation was strictly followed in each case. In collaboration with pathology department of the hospital, patients' blood was drawn under sterile conditions. It was centrifuged at 1500 revolutions per minute for five minutes[2]. Once the serum was separated, it was diluted with 0.9% normal saline to make a 20% preparation. This 20% preparation of serum was placed in multiple ultraviolet protected bottles under absolute sterile conditions. No preservative was added to the serum.

In order to ensure sterility, patients were instructed to place all the bottles in the freezer compartment at -4°C. The bottle to be used the next day was to be put in the refrigerator (lower) compartment at +4°C, one night earlier. The frequency of instillation was adjusted at 2, 6 or 8 hourly, depending upon the severity of PED. Each bottle was to be discarded at the end of the day, in order to avoid any contamination.

Then the patients were examined every two weeks for the next three visits. At each visit, the same protocol for eye examination was maintained as the initial one.

The healing was graded as effective, partially effective or ineffective. If healing of all epithelial lesions occurred within two weeks of initiating serum therapy, it was declared effective. Healing between 2-4 weeks was declared partially effective and no healing within one month meant ineffective treatment.

Mean duration of PED before serum therapy was compared between effective and ineffective groups and independent sample t-test applied.

RESULTS

This study comprised of 17 eyes belonging to 10 patients. All had persistent corneal epithelial defects in spite of previous lubricant therapy. There were 7 males and 3 females in the study. The ages ranged between 6 and 72 years (mean 34.2 years).

All the patients aged 30 years or above had PED secondary to severe dry eyes or topical drug toxicity. In the patients below 30 years of age, PED was seen as a result of severe vernal limbitis with keratopathy, lime burns and trophic ulcer (Table 1).

Table 1: Diagnosis in various age groups

Age (Yrs)	No. of eyes	Diagnosis
12	2	Vernal limbitis with keratopathy
72	1	KCS
58	1	Drug toxicity
70	2	KCS
22	2	Lime instillation
6	1	Trophic ulcer
55	2	KCS
6	2	Vernal limbitis with keratopathy
11	2	Vernal limbitis with keratopathy
30	2	KCS

The patients with KCS and trophic ulcer were already on artificial tears eye drops, with non-healing corneal epithelium, at the time of inclusion in the study. After a further 3 weeks trial of topical lubricant treatment and persistence of epithelial defects, all the eyes were put on autologous serum therapy.

In nine eyes, total healing of corneal epithelium was seen within two weeks. Another three eyes healed in the following two weeks. Five eyes didn't heal within one month. No patient skipped follow up visit. Therefore, healing was declared effective in 9 (52.9%), partially effective in 3 (17.7%) and ineffective in 5 (29.4%) out of 17 eyes, Table 2 and Fig. I. No adverse effects were noted except for one red eye without discharge, seen three days after initiation of serum therapy.

Table 2: Percentage of healing pattern

Healing pattern	No. of eyes n (%)
Effective	9 (52.9)
Partially effective	3 (17.7)
Ineffective	5 (29.4)

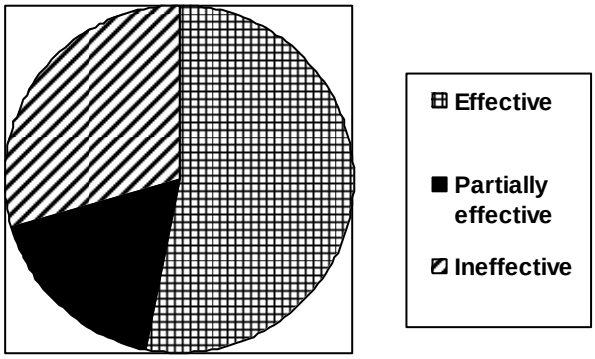

Fig. I: Healing pattern percentage

Results were better with good Schirmer's test value. However, two eyes with total absence of tears (Schirmer's value 0) also showed effective results. All the eyes with ineffective healing had below-normal Schirmer's test value before serum therapy (Table 3). Response rate was excellent in all except those with KCS (Table 4).

The mean duration of PED before initiating serum therapy was 106.2 days. In the effective group, it was 94.8 ± 67.1 days, while in the ineffective group, it was 117.6 ± 57.4 days. Independent sample t-test was applied between the effective and ineffective groups and the difference in the mean duration of PED before initiating serum therapy was found to be highly insignificant (p value 0.537).

DISCUSSION

Corneal epithelium may be damaged in a variety of clinical situations. Some of these which may result in non-healing epithelial defects include KCS, neurotrophic keratitis, exposure keratopathy, limbal stem cell failure, post-infectious corneal ulcers, topical drug toxicity, alkali burns, corneal dystrophies and diabetes mellitus[15].

Table 3: Summary of individual cases

No.	No. of eyes	Duration of PED (days)	Diagnosis	Schirmer's test value in mm		Treatment (Serum eye drops)	Healing Time (days)	Effectivity
				Right	Left			
1	2	98	Vernal limbitis	26	29	QID	10 Both eyes	Effective
2	1	112	KCS	0	-	QID	14 R eye	Effective
3	1	14	Drug toxicity	-	0	TDS	7 L eye	Effective
4	2	28	KCS	0	0	QID	28 R eye 60 L eye	R-Partially effective L-Ineffective
5	2	14	Lime instillation	25	25	2 hourly	2 Both eyes	Effective
6	1	168	Neurotrophic keratopathy	21	-	QID	3 R eye	Effective
7	2	168	KCS	7	0	2 hourly	None	Ineffective
8	2	168	Vernal limbitis	30	35	2 hourly	12 Both eyes	Effective
9	2	168	Vernal limbitis	30	34	2 hourly	21 Both eyes	Partially effective
10	2	112	KCS	0	0	2 hourly	None	Ineffective

R = Right, L = Left

Table 4. Response rate in different pathological conditions

Diagnosis	No. of eyes	Effective and partially effective/Total
Vernal limbitis with keratopathy	6	6/6
Severe KCS	7	2/7
Drug toxicity	1	1/1
Lime instillation	2	2/2
Neurotrophic keratopathy	1	1/1

Apart from persistent ocular discomfort, PED pose a potential threat to vision. Management of PED includes removal of etiological factors combined with promotion of epithelial healing. Any lid abnormality which may be responsible for PED such as chronic blepharitis, entropion, trichiasis or lagophthalmos should be corrected. Various preparations of ocular lubricants in the form of eye drops or gels are prescribed to promote epithelial healing.

Non-healing lesions require additional strategies in the form of conservation of existing tears (punctal occlusion, tarsorhaphy), prevention of mechanical trauma (bandage contact lens, tarsorhaphy) and promotion of epithelial healing by using autologous serum eye drops and amniotic membrane[4,5] or limbal stem cell trasplantation[16,17]. Seitz et al[18] have recommended the use of autologous serum for PED secondary to neurotrophic keratopathy. Similarly, Das et al[19] have recommended the use of autologous serum for delayed epithelial healing in eyes with lattice corneal dystrophy, undergoing phototherapeutic keratectomy.

In our study, there were 7 males and 3 females. The mean age was 34.2 years. The factors responsible for PED were KCS, severe vernal limbitis with keratopathy, topical drug toxicity, alkali burns and neurotrophic keratopathy. The commonest cause of PED in older age group was KCS. The patients with dry eyes and neurotrophic keratopathy were already on artificial tears treatment at the time of presentation. The mean duration of PED before initiating serum

treatment was 106.2 days. In the effective and ineffective groups, it was 94.9 and 117.6 days respectively. Independent sample t-test depicted the difference to be highly insignificant (0.537).

In 9 eyes out of 17, PED healed within 2 weeks of initiating serum therapy and the treatment was declared effective. Out of these, 2 had a Schirmer's test value of 0 before serum therapy. Five eyes didn't heal even after one month and were included in the ineffective group. Four out of these had a pre-serum Schirmer's test value of 0. Results tend to be poorer in cases with total absence of tears.

All the cases healed within one month except those with KCS. Only two eyes with KCS healed within one month out of a total of 7 (Table 4).

In the study of autologous serum for PED by AL Young et al[2], there were ten patients with a gender ratio of 7M : 3F which resembled our study. The mean age was 36.8 years (range 17-73). Treatment was effective in 6 eyes out of 10(60%) as compared to 52.9% in our study. Case series of Tsubota et al[20] depicted healing of 43.8% within 2 weeks. In AL Young series, 2 eyes out of 10 (20%) did not heal after one month while another 20% defaulted follow-up. In our study, the treatment was in-effective (poor healing after one month) in 29.4% of eyes and there were no defaulters. No adverse effects were reported in their study as compared to a single case of red eye in ours.

There was no significant difference in the mean duration of PED before initiating serum therapy in effective and ineffective groups. However, healing depended more upon the etiology in our study (Table 4). Patients with KCS exhibited poorer healing. AL Young et al[2] observed that delayed onset of autologous serum treatment might be associated with tendency of poorer healing.

In the prospective study of Poon et al[12], success was defined as closure of epithelial defects beyond one month. Seven eyes out of 15 healed within one month (46.7%) as compared to 70.6% in ours. A concentration of 50 or 100% serum was used in their study. In our cases, 20% serum eye drops were used. In their study, three patients developed microbial infections that required cessation of serum therapy.

Alvarado Valero et al[21] studied the effect of 20% autologous serum on PED and squamous metaplasia in 17 eyes. The epithelial defects healed within 2 weeks in 6 eyes (35.2%). This figure was much less than our study (52.9%) with the same concentration of serum. The duration of PED before serum therapy was 36 days. Using impression cytology technique, they observed involution of squamous metaplasia in 6 of 7 eyes, 28 days after initiation of serum eye drops.

de Souza et al[22] studied autologous serum therapy for PED in 70 eyes. Out of these, 45 had corneal epithelial defects secondary to penetrating keratoplasty. A complete corneal re-epithelialization was achieved in 57 of 70 eyes (81%) after 3 to 45 (mean 15±12) days. They also concluded that eyes with accompanying deep stromal defects were not good candidates.

Tears contain nourishing, antimicrobial, mechanical and optical properties[6]. Serum contains essential components in comparable concentrations to tears. These include EGF (epidermal growth factor), TGFβ (transforming growth factor β), PDGF-AB (platelet derived growth factor), neuropeptides (Substance P), insulin-like growth factor, fibronectin and vitamin A. The growth factors, fibronectin and vitamins support proliferation, migration and differentiation of corneal and conjunctival epithelium. The TGF-β family are fibrogenic cytokines responsible for fibroblast activation in wound healing[23]. The natural substitutes used as tears are superior to artificial tears because their pH, osmolality and biomechanical properties resemble natural tears. Secondly, they contain essential nutrients, like growth factors and vitamins. Thirdly, bacteriostatic components are present such as IgG, lysozyme and complement. Fourthly, there are no preservatives in natural substitutes[24]. Nistor and Nistor[25] observed improvement in fluorescein and rose Bengal scores, non-allergenicity and non-immunogenicity with the use of autologous serum for PED. Autologous serum can induce faster epithelial healing than artificial tears, which leads to a decrease in keratocyte apoptosis and migration of fibroblasts and myofibroblasts in the wound site, a decrease in migration of inflammatory cells and consequently, inhibition of cytokine release[26]. This could help in improving the long-term refractive results in eyes undergoing LASIK. The cellular morphology and ATP levels are better preserved in the cells exposed to serum drops as compared to unpreserved hypromellose[12].

In the study of Schrader et al[27] combination of serum eye drops and hydrogel bandage contact lenses were used in cases of PED, which had not responded well to previous amniotic membrane transplantation or keratoplasty. Five of six eyes healed after a treatment period of 14.2± 8.9 days. In one eye, the PED reduced in size and took 90 days to resolve

completely. Random control clinical trial between autologous serum and umbilical cord serum for PED has shown that umbilical cord serum leads to faster healing of PED compared to autologous serum[3].

Liu et al[24] observed that using a longer clotting time resulted in an increased concentration of all the epitheliotrophic factors in the serum. Moreover, the density of epithelial microvilli was greater when a longer clotting time was adopted. They concluded that clotting time, centrifugation and diluents have a significant impact on the composition and epitheliotrophic effects of serum. A clotting time of ≥120 min, a sharp centrifugation (3000xg for 15 min) and dilution with BSS (balanced salt solution) enhance the ability of serum to help proliferation, migration and differentiation of corneal epithelial cells.

Sauer et al[28] have concluded that if serum drops are applied by trained personnel, the absence of contamination can be ensured up to the fourth day. The additional application of prophylactic antibiotic drops can help avoid infection even if refrigerated non-preserved serum is used up to 7 days. We ensured sterility by preparing the serum under absolute sterile conditions, storing the serum bottles in the freezer at -4°C, shifting the bottle to be used into the refrigerator compartment at 4-8°C and discarding that bottle by the end of the day.

CONCLUSION

We consider autologous serum to be effective in majority of cases with PED recalcitrant to conventional lubricant therapy due to the presence of essential factors. Healing depended more upon the etiology than the duration of PED before serum therapy, in our study.

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Picture Quiz Answer

Bietti's Corneoretinal Dystrophy

Bietti's Corneoretinal Dystrophy, also called Crystalline Retinopathy, is a congenital disease with autosomal recessive type of inheritance involving the cornea and retina. The patients present usually during their third decade with mild to moderate decrease in visual acuity and some visual field loss. Night vision is markedly affected too.

On examination, vision can range from 6/6 to hand motion. Color vision is abnormal suggesting tritan deficiency. The cornea has sparkling crystals mostly at the peripheries. The fundus also has small shiny crystals all over posterior pole, which has RPE atrophy. Electron microscope crystal analysis reveals ultra structural resemblance to lipids within fibroblasts. Fluorescein angiography shows transmitted hyperfluorescence in the area of crystals. Adjacent areas with no crystals show confluent loss of RPE and choriocapillaris. ERG is normal with white light but abnormal with blue light. EOG is usually subnormal. Visual fields are affected only in advanced stages, the earliest defects being pericentral scotomas.

Unfortunately, like many other retinal conditions, there is no known treatment up till now.