Preferred Practice

Autologous serum eye drops in dry eye disease: Preferred practice pattern guidelines

Jayesh Vazirani, Uma Sridhar[1], Nikhil Gokhale[2], Venkateswara Rao Doddigarla[3], Savitri Sharma[3], Sayan Basu[4]

Autologous serum eye drops provide lubrication and promote epithelial healing. They have been successfully used in the management of ocular surface disorders such as dry eye disease, persistent epithelial defects and neurotrophic keratopathy for many decades. A great deal of variation in the methods of preparation of autologous serum eye drops, the end concentration and the duration of use exists in published literature. In this review, simplified recommendations for preparation, transport, storage and use of autologous serum are described. Evidence for the use of this modality in aqueous deficient dry eye disease is summarized, along with expertise-based rationale.

Key words: Autologous serum, dry eye, ocular surface disease, persistent epithelial defect

Access this article online

Website: www.ijo.in

DOI: 10.4103/IJO.IJO_2756_22

Quick Response Code:

Aqueous deficient dry eye disease (ADDE) can be a chronic and potentially blinding condition that occurs due to lacrimal gland insufficiency, and the more severe forms occur in patients with underlying immunological disorders like Sjogren's syndrome.[1] The mainstay of topical therapy in ADDE, therefore, is the supplementation of the aqueous tear deficiency with artificial tear eye drops or lubricants.[1] Natural tears have several growth factors that are essential for maintaining a healthy ocular surface, and these are lacking in artificial tears. Therefore, artificial tears have limited benefit in the more severe forms of dry eye. These patients benefit from autologous serum eye drops that are prepared from the patient's own blood.[2] Serum is the liquid component of blood and has remarkable similarities with tears in terms of composition as well as biochemical properties.[2] The use of autologous serum eye drops in the management of ocular surface conditions such as dry eye disease and persistent corneal epithelial defects (PED) has been described for many decades, and favorable results in terms of both subjective and objective outcome measures have been reported.[3–5] Nonetheless, the use of this modality comes with its own set of limitations and challenges.

Unfortunately, in India there are only a few centers where autologous serum eye drops are regularly prepared and dispensed. This severely impacts the access to and sustainability of this therapy for patients who need it or can benefit from its use. In this review, we aim to provide a simplified guide to help ophthalmologists understand the clinical indications for the use of autologous serum eye drops as well as methods of preparation, dispensing, and storage.

Rationale and Mechanism of Action

The secretions of the lacrimal gland, which are an integral part of the aqueous component of the preocular tear film, support conjunctival and corneal epithelial cell proliferation, migration, and differentiation.[2] Additionally, proteins like fibronectin, complement factors, lactoferrin, and immunoglobulins are released into the tears from conjunctival vessels to help in ocular surface epithelial wound healing.[2,6,7] A lack of the epitheliotrophic factors in the tears due to lacrimal gland insufficiency can lead to epithelial defects which are extremely challenging to manage in ADDE.[8] In such situations, it is not enough to just lubricate the surface but to also provide epitheliotrophic factor supplementation.

Serum is very similar to unstimulated human tears in terms of pH (7.4) and osmolality (296-8 mOsm/kg H_2O), while containing similar or higher concentrations of growth factors like epidermal growth factor (EGF), transforming growth factor-b (TGF-b), Vitamin A, lysozyme, and fibronectin than in

Center for Excellence in Cornea and Ocular Surface Disorders, Excel Eye Care, Ahmedabad, Gujarat, [1]Department of Cornea and Cataract, ICARE Eye Hospital and Postgraduate Institute, Noida, Uttar Pradesh, [2]Cornea and Ocular Surface, Gokhale Eye Hospital, Mumbai, Maharashtra, [3]Jhaveri Microbiology Centre, L V Prasad Eye Institute, Hyderabad, Telangana, [4]The Cornea Institute, Kallam Anji Reddy Campus, LV Prasad Eye Institute, Hyderabad, Telangana, India

Correspondence to: Dr. Sayan Basu, Director and Prof. D Balasubramanian Chair of Eye Research, Prof. Brien Holden Eye Research Centre, LV Prasad Eye Institute, Hyderabad, Telangana, India. E-mail: sayanbasu@lvpei.org

Received: 20-Oct-2022 Revision: 09-Feb-2023
Accepted: 10-Feb-2023 Published: 05-Apr-2023

Cite this article as: Vazirani J, Sridhar U, Gokhale N, Doddigarla VR, Sharma S, Basu S. Autologous serum eye drops in dry eye disease: Preferred practice pattern guidelines. Indian J Ophthalmol 2023;71:1357-63.

natural tears.[2] The presence of these epitheliotrophic factors is considered to be responsible for the therapeutic effect of autologous serum both *in vitro* in epithelial cell cultures and *in vivo* in clinical application.[5,9] Although Fox was the first to systematically describe the use of autologous serum eye drops for the treatment of dry eye disease in patients with keratoconjunctivitis sicca in 1984,[4] it is Tsubota who is largely credited for reintroducing and popularizing this therapy in the management of ocular surface disorders.[5]

Indications for Use

The use of autologous serum eye drops has been described in a variety of ocular surface conditions, most importantly dry eye disease, non-healing corneal epithelial defects, and neurotrophic keratopathy.[10–12] However, preparation of autologous serum eye drops requires relatively specialized equipment and facilities for maintenance of sterility. Transport and storage also have specific temperature requirements. These preconditions place practical limits on the use of this modality. Our recommendations on when to use autologous serum eye drops are summarized in Table 1. Illustrative examples are shown in Fig. 1. In view of the logistical challenges, autologous serum eye drops are not recommended as the first line of therapy for dry eye disease, especially evaporative dry eye disease due to meibomian gland dysfunction. It is the authors recommendation to reserve this modality for recalcitrant cases of severe ADDE when the patient is symptomatic despite maximum use of lubricants and anti-inflammatory medications. Typically, these are patients who present with either diffuse superficial punctate keratitis and severe ocular inflammation or with frank epithelial defect or sterile ulceration. There are some patients with drug or preservative toxicity (to usually anti-glaucoma medications, but also topical decongestants, and even lubricants) who do not tolerate any topical medication and can be managed only with autologous serum eye drops.

Preparation, Dispensing, and Storage

There is no universally accepted protocol for preparation of autologous serum eye drops. Studies have reported use of concentrations ranging from 20% to 100%.[13] Variables in the preparation process include tests carried out on patients, the time allowed for clotting of blood as well as the force and time used for centrifugation. Differences in the concentration as well as in the preparation process may have a bearing on the biochemical properties of the end product.[2]

Autologous serum eye drops can be prepared in a blood bank, eye bank, or laboratory having appropriate equipment and sterile work areas such as laminar flow hood. The process used by us for preparation of 20% autologous serum eye drops is shown in Table 2 and Fig. 2. Approximately 10 ml blood needs to be collected in plain vacutainer tubes without anticoagulant and allowed to clot for 30–60 minutes at room temperature. This is then centrifuged at 2500–3000 rpm, and the supernatant serum (amber color) is collected aseptically in a tube using a pipette, taking care to avoid aspirating red blood cells. Slightly hemolyzed blood may render light reddish color to the serum which is harmless. However, heavily lyzed blood cannot be used. The dilution and distribution of diluted serum in smaller vials (eye dropper vials) should be done in laminar flow hood under aseptic conditions. Any suitable culture medium such as blood agar, thioglycollate broth, brain heart infusion broth, etc., can be used for sterility check of the diluted serum. The

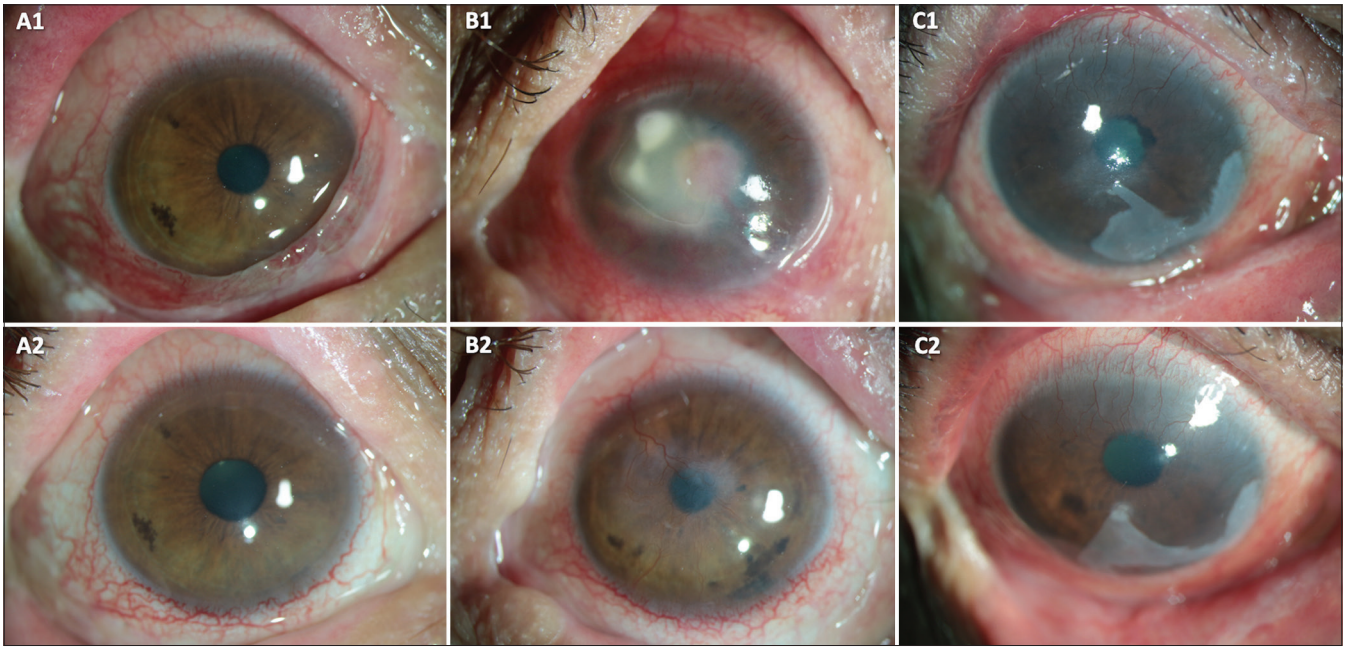

Figure 1: Before and after images of 20% autologous serum eye drops therapy. Panels A and B show the right and left eye of middle aged lady with Stevens–Johnson syndrome and aqueous deficiency dry eye disease who developed ocular surface inflammation despite undergoing mucous membrane grafting. There was resolution of conjunctival inflammation (a2) and corneal ulceration (b2) after 2 weeks of treatment. Panel C shows the right eye of a middle aged man with SJS, who had undergone MMG and was using scleral contact lenses on maximal medical therapy but still developed progressive corneal keratinization and anterior stromal scarring in the visual axis. After a month of autologous serum eye drops (c2), the keratinization started to regress with clearing of the visual axis

Figure 2: Illustrative collage of images describing the process of preparing 20% autologous serum eye drops. (a) Blood collected in sterile plain vacutainers and kept at room temperature (30 minutes) for clotting; (b and c) Centrifugation of the tubes for serum separation; (d) Well-separated serum seen in the upper part of the tubes with RBC compacted at the bottom; (e) Laminar flow hood with UV light exposure (30 minutes) to sterilize all items required for serum separation, dilution, and packaging; (f) Serum being pipetted out in a sterile tube; (g and h) Sterile normal saline being added to the serum for dilution; (i and j) Millipore syringe filter of 0.22 μm being fixed to a syringe containing the diluted serum; (k) 5 mL of sterile diluted serum delivered into sterile eye dropper vials (note: few drops of the diluted serum is placed in culture media at this stage); (l) Appropriate label fixed on the eye dropper vials with relevant details

Table 1: Suggested indications for the use of autologous serum eye drops

A	Recalcitrant symptoms/signs in severe aqueous deficiency dry eye disease despite maximum use of lubricants and anti-inflammatory medications
B	Slow or non-healing epithelium or recurrent epithelial breakdown following surgery in severe dry eye or ocular surface disease
	Ocular surface reconstruction with/without limbal transplantation
	Keratoprosthesis or keratoplasty surgery
	Tenon's patch graft
	Amniotic membrane application
C	Non-healing corneal epithelial defect in chronic ocular surface disease
	Post ocular surface burns
	Graft vs host disease
	Chronic cicatrizing conjunctivitis
	Exposure keratopathy
	Neurotrophic keratopathy
	Radiation keratopathy
D	Neuropathic ocular pain

volume of the saline diluent can be adjusted based on the serum volume available, keeping the final concentration at 20%.

An alternative technique used by one of our senior authors (NG) requires a centrifuge but uses the operation theatre premises as a clean room facility instead of a laminar flow hood. Blood is collected into four autoclaved sterile test tubes. After standing for 30 minutes at room temperature, the blood is agitated at the edges with a sterile cannula to disentangle the clot adherent to the glass and centrifuged at 1500 rpm for 5 minutes. Then the serum is aspirated using a sterile luer lock 10 cc syringe. The serum is then diluted in ringer lactate or 0.5% carboxy methyl cellulose eye drops with purite preservative.

Recommendations on Usage

As outlined above, 10 mL of blood collected by the phlebotomist yields about 5 mL of serum, which is then diluted in 20 mL of 0.9% normal saline to yield 25 mL of 20% autologous serum. The diluted serum is then divided and dispensed in five 5 mL eyedropper bottles for the patient to use. Since the eye drops do not contain preservatives, there are certain precautions that need to be observed. The eye drops are dispensed with a frozen gel pack and need to be refrigerated. During transportation, the bottles can either be kept in a plastic packet or a thermocol (expanded polystyrene) box with the frozen gel pack. At home or in the hospital, if the patient is admitted, two vials need to be kept in the door of the fridge, which is usually at 4°C, while the other vials are placed in the freezer (at -20°C). The patient or the attendants can mark one of the vials kept in the door and use it, keeping the second one on standby. Once the marked vial has been utilized, the second vial is marked and used, while another vial is retrieved from the freezer and kept at 4°C to be thawed. Since the patients will need to use the drops while commuting to the hospital, it is best if they can carry the bottle in a clean insulated lunch box or similar

Table 2: Method of preparation

A	Checklist of materials required	
	Patient's blood collection vacutainer (Blood collection tube plain)	5 Tubes (15 ml blood)
	Tarsons sterile tubes (50 ml)	2 Tubes
	5 ml sterile dropper vials	5 vials
	Sterile normal saline	20 ml
	Millipore 0.22 µm filter unit (Millex-GV, Cat, #SLGV025LS)	2
	10 ml syringe	2
B	Method of preparation	
	Centrifuge the clotted blood and separate 5 ml of serum. More than 5 ml serum will require a corresponding increase in volume of normal saline keeping the final concentration at 20%.	
	Take 20 ml of sterile normal saline in 50 ml Tarsons tube and add 5 ml of serum.	
	Mix well by shaking/vortexing	
	Withdraw the diluted serum in a syringe, affix a sterile filter unit and dispense 5 ml each in sterile dropper vials	
	Label the vials:	
	Patient's name and ID	
	20% autologous serum	
	Date of manufacturing	
	Keep at 4° C -when using, keep remaining bottles in freezer	
C	Quality Check	
	Place the last 2-3 drops on chocolate agar	
	Incubate for 48 hours at 37° C	
	Observe for growth (In case of growth inform the patient to stop usage and discard the vials) No growth indicates sterility of the product.	

temperature-controlled container with the frozen gel packs. It is also recommended that once dispensed, the eye drops should be used up completely by 2–3 weeks, as storing unpreserved eye drops beyond 3–4 weeks is not safe and increases the chances of contamination. Patients should also be warned not to use the eye drops if the original clear fluid turns turbid or if there is a change in color (amber/pink) or smell (odorless). It is therefore best that the eye drops are used very frequently, every hourly or half-hourly, like fortified antibiotic eye drops, rather than at a lower frequency. This has two advantages, firstly faster and better symptomatic relief and more efficient utilization of the eye drops without having to discard unused bottles after two weeks.

Outcomes

Evidence-based Recommendations

Multiple case reports, case series, and some randomized controlled clinical trials have described the outcomes with use of autologous serum eye drops [Table 3].[4,5,13-30] An updated systematic review published in the Cochrane database assessed the efficacy and safety of autologous serum eye drops when used in treatment of adults with dry eye disease.[14] The review identified five randomized control trials with 92 participants but assessed the certainty of evidence as low or very low. The authors concluded that there may be some benefit in using autologous serum eye drops over artificial tears for dry eye disease in the short term, but they found no evidence of an effect beyond two weeks of use.[14] Another recent systematic review and meta-analysis on the same topic included results from seven randomized controlled trials with 267 subjects. The analysis of the pooled data indicated that autologous serum outperformed artificial tears in terms of improvement in subjective symptoms as measured by the ocular surface disease index scores, as well as objective measures such as tear film breakup time and rose bengal staining scores in patients with dry eye.[15]

An Ophthalmic Technology Assessment report by the American Academy of Ophthalmology assessed the safety and efficacy of autologous serum eye drops when used for severe dry eye as well as persistent corneal epithelial defects.[13] The authors included 13 studies in their analysis, each of which had one month or more of follow-up and included 20 or more patients treated for severe dry eye disease or 15 or more patients treated for non-healing epithelial defect. Eight of these studies were rated as level II evidence, while five were rated as level III. Ten studies evaluated the use of autologous serum eye drops in severe dry eye disease, of which six reported a statistically significant improvement in subjective symptom scores. Statistically significant improvement in at least one objective clinical measure was shown in eight of these studies. All four studies that evaluated the use of autologous serum eye drops for the treatment of persistent corneal epithelial defects reported substantial improvement in the defects. Most studies have not reported any significant adverse effects with use of autologous serum eye drops. One study reported microbial growth from a bottle of autologous serum that matched the bacterium (*Serratia marcescens*) cultured from the patient's corneal lesion.[13] Patients should therefore be advised that serum being a good culture medium, dispensed eyedrop bottles should be handled carefully to maintain hygiene and minimize the risk of microbial contamination. Patients should also be educated to report immediately to their treating doctor in case of symptoms such as increased pain, redness, or swelling in the eyes.

More recently, a randomized controlled trial conducted in Thailand found that all epitheliotrophic factors like EGF, fibronectin, platelet-derived growth factor-AB (PDGF-AB), and TGF-β1 were present in AS at baseline and did not decrease in concentrations in storage conditions (4°C for 1 week and at −20°C for 1 and 3 months). They also found significant improvement in symptoms and signs in patients with ADDE with the use of autologous serum eye drops.[16]

Experience-based Recommendations

Most experienced cornea and dry eye specialists like some of the authors on this review will agree that autologous serum eye drops are a critical part of the ocular surface disease management armamentarium. It can be considered as the fifth pillar of the therapeutic arsenal in addition to lubricants, topical and systemic immunosuppression, surgical techniques (like limbal transplantation, keratoprosthesis, and mucous membrane grafting), and scleral contact lenses.[31] In addition to its preservative-free lubricating and epitheliotropic properties, autologous serum eye drops are very effective in managing ocular discomfort and particularly neuropathic pain. An important component of serum eye drops is nerve growth

Table 3: Summary of studies on outcomes of autologous serum eye drops in aqueous deficiency dry eye disease (minimum sample size of 10)

First Author	Year	Design	Sample size	Avg duration (m)	Conc (%)	Outcome Assessment	Outcomes
Fox[4]	1984	Case series + Crossover	15	10.3	33	RB surface staining, subjective symptom score	Improvement noted in both surface staining and symptom score
Tsubota[5]	1999	Case series	12	0.5	20	RB & FS surface staining, subjective symptom score	Improvement noted in both surface staining and symptom score
Tananuvat[17]	2001	RCT	12	2	20	RB & FS surface staining, Schirmer's test, TBUT, CIC, subjective symptom score	No statistical difference between treatment and control groups
Ogawa[18]	2003	Case series	14	19.4	20	RB & FS surface staining, Schirmer's test, TBUT, CIC Corneal sensitivity score, subjective symptom score	Improvement in symptom score, surface staining scores & TBUT at 4 weeks[a]
Noble[19]	2004	Randomized controlled crossover trial	16	3	50	RB & FS surface staining, Schirmer's test, fluorescein clearance test, subjective symptom score, CIC	Improvement in symptom score and impression cytology grading favoring AS[b]
Kojima[20]	2005	RCT	23	0.5	20	RB & FS surface staining, Schirmer's test, TBUT, subjective symptom score	Improvement in surface staining, TBUT and subjective symptom score favoring AS[b]
Noda-Tsuruya[21]	2006	RCT	27	6	NA	RB & FS surface staining, Schirmer's test, TBUT & subjective symptom scores	Improvement in TBUT and RB staining favoring AS[b]
Yoon[22]	2007	Prospective case-control study	48	2	20	RB & FS surface staining, Schirmer's test, TBUT, CIC, corneal sensitivity score, tear clearance rate, subjective symptom score	Improvement in surface staining, TBUT, grade of conjunctival squamous metaplasia, goblet cell density, and subjective symptom score[b,c]
Lee[23]	2008	Case series	23	17.3	20	Surface staining, subjective symptoms	Improvement in surface staining and subjective symptoms[d]
Urzua[24]	2012	Double-blind RCT	12	0.5	20	Surface staining (OXFORD scale), TBUT, OSDI	Improvement favoring AS in OSDI score[b]
Lopez-Garcia[25]	2013	Double-blind RCT	26	2	20	RB & FS surface staining, Schirmer's test, TBUT, CIC, subjective symptom scores	Improvement in all parameters except Schirmer's test with treatment[e]
Celebi[26]	2014	Double-blind RCT	20	1	20	Surface staining (OXFORD scale), Schirmer's test, TBUT, OSDI	Improvement in symptom score (OSDI) & TBUT favoring AS[b]
Hussain[27]	2014	Case series	63	12	50	Surface staining (OXFORD scale), Schirmer's test, TBUT, OSDI	Improvement in surface staining, Schirmer's test, and symptom score[f]
Hwang[28]	2014	Comparative study	34	1	50	Surface staining (OXFORD scale), Schirmer's test, TBUT, subjective symptom score	Improvement in all parameters in the patients with primary Sjogren syndrome[g]
Liu[29]	2015	Case series	28	42.3	20	RB & FS surface staining, Schirmer's test, TBUT	Improvement in surface staining scores & TBUT[b,h]
Mahelkova[30]	2017	Case series	26	3	20	Surface staining (OXFORD scale), OSDI, IVCM	Improvement in surface staining, OSDI scores, & density of basal epithelial cells on IVCM after treatment

[a]Beyond 12 months of follow-up, 7 out of 14 patients maintained good response; [b]no differences in one or more of the other parameters studied; [c]greater improvement with umbilical cord serum compared to autologous serum in symptom score, surface staining and goblet cell density; [d]only descriptive statistics were used for analysis; [e]more improvement in group where sodium hyaluronate was used as diluent, compared to group where saline was used as diluent; [f]potential confounding factor: concomitant use of scleral contact lenses by 33% of patients; [g]no improvement in parameters in patients with secondary Sjogren's syndrome; [h]potential confounding factor: concomitant use of punctal plugs by 12 patients

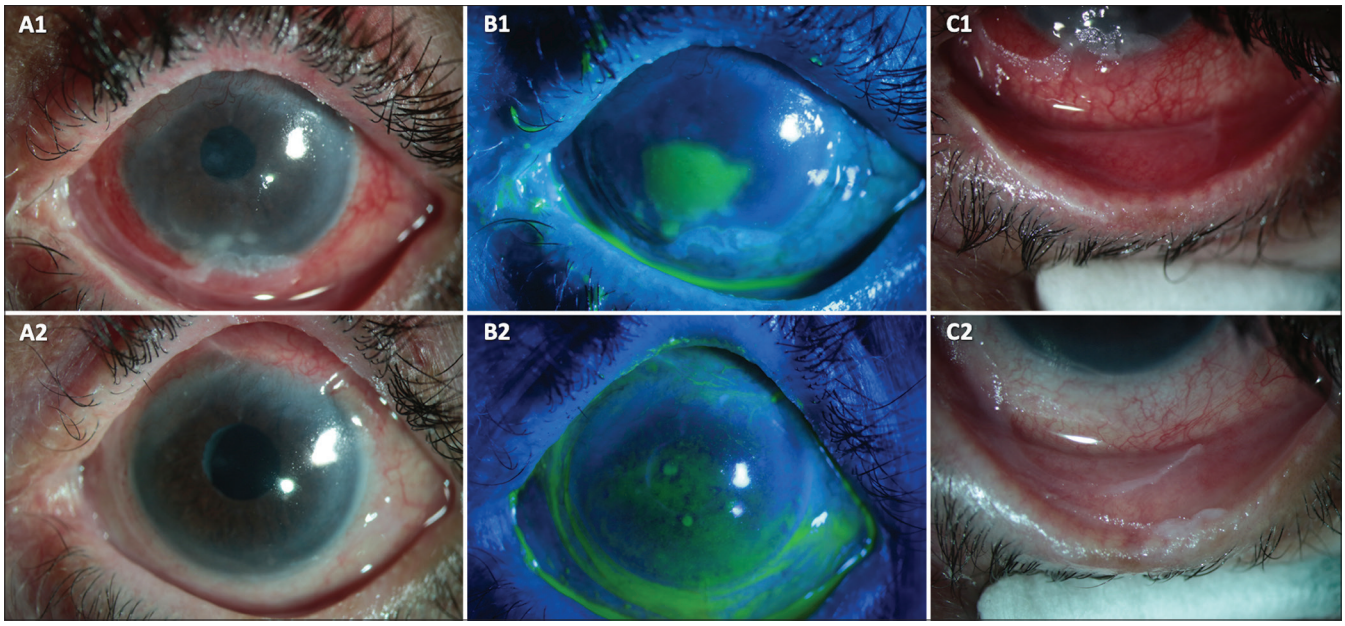

Figure 3: Before and after images of the left eye of a elderly gentleman with anti-glaucoma medication-induced cicatrizing conjunctivitis and aqueous deficiency dry eye disease. The patient was intolerant to any kind of topical medication, even preservative free lubricant eye drops. After 1 month of 20% autologous serum eye drops, the patient was symptomatically improved, with reduction in ocular surface keratinization (a2, c2) and corneal epithelial defect and ulceration (b2)

factor (NGF), which is present in much higher concentrations than natural tears. NGF plays a very important role in corneal nerve regeneration, through promotion of neuronal sprouting and rejuvenation of function of injured neurons.[32-34] Similarly, several studies have reported favorable outcomes of autologous serum therapy in neurotrophic keratitis.[12,21] A trial of autologous serum eye drops should be attempted in cases of dry eye disease with severe pain, with or without corneal epithelial defects. There are, however, important caveats to remember. The most important is proper patient selection. This is of specific relevance to developing countries like India, because not everyone has a refrigerator at home, or they may not be able to use or store the eye drops hygienically. This problem can be addressed by admitting the patient in the hospital for a week or ten days. It is also critical to understand that autologous serum eye drops are to be reserved for a crisis as it is not practically possible to maintain someone on this treatment long-term, because of the logistical hurdles. However, there are some sensitive patients who do not tolerate any topical medication, even unpreserved lubricating eye drops; and only respond to autologous serum therapy. This is particularly true for some patients with anti-glaucoma drug-induced ocular surface toxicity and GvHD patients [Fig. 3]. In such cases, they can be used in conjunction with scleral and soft bandage contact lenses.[35]

Conclusion

The idea of using autologous serum eye drops as a treatment for dry eye and ocular surface disorders is attractive because they contain growth factors that promote epithelial healing. A perusal of available literature leads us to conclude that there exists a reasonable amount of evidence to support the use of this therapeutic modality in ADDE. The duration for which autologous serum has been used in published studies is usually not more than a few weeks to months. This is probably due to logistical reasons and because conceptually it is a temporizing measure intended to tide over an acute ocular surface crisis. Preparation and dispensing of autologous serum eye drops require appropriate facilities to maintain sterility and specialized equipment and may be subject to rigorous stipulations depending on the local regulatory environment. Irrespective of applicable laws, we recommend that the highest standards of sterility and adherence to temperature requirements are maintained during preparation, dispensing, transport, storage, and usage of autologous serum eye drops to minimize chances of microbial contamination. From the patient's point of view, use of autologous serum eye drops usually involves multiple visits to the dispensing facility and may entail significant expense. Based on these practical realities, we prefer to use autologous serum eye drops for a short period of time in specific circumstances. In this context, autologous serum eye drops can be an extremely useful addition to the armamentarium of physicians dealing with severe dry eye disease and ocular surface disorders, helping them to improve patient comfort and achieve epithelial healing in these challenging cases.

Financial support and sponsorship

Hyderabad Eye Research Foundation (HERF), Hyderabad, Telangana, India.

Conflicts of interest

There are no conflicts of interest.

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