Research Article

Autologous serum eye drops for ocular surface disorders- a clinical study

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Abstract

Introduction: Tears have antimicrobial, nourishing, mechanical and optical properties. Several tear factors that have been identified to be, of importance in the maintenance of normal corneal and conjunctiva epithelium. A lack of these factors results in severe ocular surface disorders. Autologous serum in the form of eye drops has been reported as an effective treatment for severe ocular surface disorders. The present study was designed to determine the efficacy and safety of autologous serum application in various ocular surface disorders. **Methods:** This is a prospective case analysis of 30 eyes of 18 patients between June 2009 & November 2010. The following tests were performed: Schirmer I, Basic secretion test, Tear film Breakup time, Fluorescein staining, Rose Bengal Staining. 20% concentration of Serum eye drop was used for 30 days and no other tear substitutes were prescribed to patients while during treatment. **Results:** After one month treatment marked improvement was observed - 19 eyes in Schirmer I, 18 eyes in Basic tear secretion, 23 eyes in tear film Break up time, 21 eyes in Fluorescein staining pattern and 29 eye in Rose Bengal staining pattern. **Conclusion:** It was observed that Hypovitaminosis A and Neurotrophic keratitis showed 100% improvement, kerato conjunctivitis sicca, sjogren's syndrome, persistent epithelial defect, Recurrent erosion syndrome, Marginal corneal ulcer also showed excellent results but Stevens - Johnson syndrome showed very poor response to treatment.

Keywords: serum, blood products, ocular surface disorders, dry eye.

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Introduction

Ocular surface disorders (OSD) has been defined as a group of disorders of diverse pathogenesis that results in decreased epitheliotrophic and antimicrobial properties of natural tears which ultimately leads to compromised wound healing process [1]. A pharmaceutical tear substitute produces very little effect on the ocular surface disorders. Eye drops prepared from autologous serum are a new treatment option for severe ocular surface disorders. Serum is fluid component of full blood that remains after clotting. It contains a large variety of growth factors, Vitamin, Immunoglobulin's in different concentration. TGF-B and vitamin – A, both are found in higher concentration in serum. In addition, serums also contains Ig-G, lysozyme, and complement which reduces the risk of contamination of sample and may reduce the risk of infection in an otherwise compromised ocular surface. These epitheliotrophic factors are thought to be responsible for the therapeutic effects of serum observed on ocular surface disorders [2].

In cell culture experiments, serum was found to be superior to preserved or unpreserved pharmaceutical products in the maintenance of human keratinocyte morphology and function. It was suggested that Retinoic acid in serum can upregulate (modify) some of the mucin on the ocular surface, particularly MUC1, MUC4 and MUC16 which are the membrane spanning mucin that allow the mucin core protein to remain associated with the epithelial cell. Vitamin A is found in tear fluid in much higher concentration in serum than in tear fluid and may decrease the overall ocular surface squamous metaplasia in severe dry eye diseases. It was also suggested that the epidermal growth factors and transforming growth factors in serum helps in healing of epithelial erosion and facilitate epithelization due to anti apoptotic properties [3].

There were many proteins such as albumin or globulin present in serum which can protect the degradation of important cytokines in serum even in frozen state.

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Therefore it was suggested in reference studies that serum eye drops may be safely frozen for up to three mouths [4]. The current study was conducted to review the local spectrum of indications and to examine the outcome of autologous serum eye drops usage

Material and Methods

The design of the study was a prospective study. We studied the use of Autologous serum as an eye drop in various ocular surface disorders in 30 eyes of 18 patients in total duration of one and half years. All apparently healthy patients attending ophthalmic OPD and indoor with severe dry eye symptoms and other ocular surface disorders were selected. Various ocular surface disorders which was included in this study were- Hypovitaminosis A (four patients), Neurotrophic keratitis(three patients), Keratoconjunctivitis Sicca i.e. Non Sjogren's Syndrome (two patients). Sjogren's Syndrome(two patients), Stevens-Johnson Syndrome(two patients), Persistent epithelial Defect(two patients), Recurrent erosion Syndrome(one patient) and Marginal corneal Ulcer(one patient).

The following tests were performed for clinical assessment of ocular surface disorders: Schirmer I Basic secretion test, Tear film Breakup time (TBUT), Fluorescein staining (Grading) and Rose Bengal Staining (Grading).

Schirmer – I: This test measures both basic and reflex secretion. Schirmer strips were used. It is a 5 X 35 mm strip of Whitman filter paper- 41 and it was bent at the notch which was situated 5 mm away from tip. The smaller end of filter paper strip was inserted gently in lower cul- de-sac at lateral one third between lid and bulbar conjunctiva of unanaesthetized eye. After 5 minute the wet area from notch to distal end of wet area was measured. Less than 10 mm of wetting after 5 minute is diagnostic of aqueous tear deficiency [5].

Basic Secretion Test: It was performed after the instillation of tropical anesthetic agent followed by lightly blotting residual fluid out of the inferior fornix although normal measurements are quite variable, repeated measurement of less than 5 mm of wetting with anesthesia can be highly suggestive of aqueous tear deficiency and 5-10 mm is equivocal [5] .

Schirmer - II:-This measures the reflex secretion. After instillation of topical anesthetic agent, paper strip is inserted into the inferior fornices and cotton - tipped applicator is used to irritate the nasal mucous. Wetting of less than 15 mm after 5 minutes is consistent with a defect in reflex secretion [5].

Tear Film Break up Time:-Defined as the interval following a blink to first occurrence of dry spots on cornea. Individual was asked to look up and a drop of 2 % Fluorescein dye (autoclaved) was instilled in lower cul-de- sac excess of dye was whipped off. Dry spots appearing in less than 10 seconds are considered abnormal [6,7].

Fluorescein Staining (Grading) [8]: After 2 minutes of instillation of 2% dye, eye is thoroughly washed with saline or water. The eye is then viewed through blue filter of slit lamp and score is calculated. Score is given according to protocol suggested at the national eye institute workshop on dry eye. Cornea is divided into 3 zones - upper, middle and lower. Staining score of each zone 0 to 3 resulting in cumulative score 0 to 9. Grading of Fluorescein staining on the cornea is done as follows –

0	-	Negative (No Staining)
1	-	Scattered minute (Mild Staining)
2	-	Moderate Spotty (Moderate Staining)
3	-	Diffuse Blotchy (Severe Staining)

A Score above 1 point is considered abnormal.

Rose Bengal Staining [9]:- Instillation is best preceded by topical anesthesia to limit stinging sensation and care should be taken to remove the excess dye from the lid margins to avoid unsightly staining of the lid skin.. After this eye is viewed with slit lamp and staining score calculated according to **"Van Bjisterveld Score".** According to this visible ocular surface is divided into three zones- corneal, temporal bulbar and nasal bulbar conjunctiva. Staining score of each zone is 0 to 3, resulting in cumulative score 0 to 9. The grading of staining on cornea is done as follows-

0	-	Negative (No Staining)
1	-	Scattered minute (Mild Staining)
2	-	Moderate Spotty (Moderate Staining)
3	-	Diffuse Blotchy (Severe Staining)

A van Bjisterveld score above 1 point is considered abnormal.

Method of blood collection and serum preparation: 30 ml of blood was collected from anterior cubital vein. It was allowed to stand for 2 hours to allow the blood to clot and retract fully. Serum was then separated from the clot of blood by centrifugation at rate of 3000 rpm for 15 minute. Serum was separated in sterile manners under laminar flow and collected in sterile glass bottles, which was sterilized with UV rays for at least 15 minutes. Serum was diluted to 20 % concentration with saline (0.9% Nacl)

Microbiological examination of serum was performed before application. Drops were stored in bottles at -4^{o} C in freezer in hospital refrigerator. The vial to be used for the day was kept to +4 ^{o} C in refrigerator and instilled 1 hourly / day [8]. Last specimen was again sent for culture and sensitivity.

Treatment Schedule

After preparation of serum eye drops, treatment was started to the patient for duration of 30 days. No other tear substitutes were given. Ciprofloxain (0.3%) eye drop was used for antibiotic coverage in all patients during treatment. Follow up was done after 3 month.

Results

We studied the use of autologous serum as an eye drop in various ocular surface disorders in 30 eyes of 18 patients. In present study, age of patients ranged from 16 years to 70 years. Mean age was 43 years. Maximum numbers of patients were found in 51-75 year age group – i.e. (7 patients). Male: female ratio was 7:2. Most of ocular surface disorders were Bilateral(12). Maximum numbers of patient were observed of Hypovitaminosis A. In present study 21 eyes had Schirmer-I value less than 10 mm before treatment but after treatment Schirmer- I improved markedly and most of eyes (19) eyes achieved Schirmer- I value >10 mm and only two eyes had the Schirmer- I value less than 10 mm after treatment and they were cases of Stevens-Johnson syndrome [Table 1].

Table - 1:-Comparison of Schirmer I in various ocular surface disorders.

S.N.	Diagnosis	Before Treatment		After Treatment	
		<10mm (No. of eyes)	≥10mm. (No. of eyes)	<10mm (No. of eyes)	≥10mm. (No. of eyes)
1.	Hypovitaminosis – A	4	4	0	8
2.	Neurotrophic Keratitis	1	2	0	3
3.	Kerato conjunctivitis Sicca (Non Sjogren's Syndrome)	5	1	0	6
4.	Sjogren's Syndrome	4	0	0	4
5.	Stevens - Johnson Syndrome	4	0	2	2
6.	Persistent epithelial defect	2	1	0	3
7.	Recurrent erosion Syndrome	0	1	0	1
8.	Marginal corneal Ulcer.	1	0	0	1
	Total	**21**	**9**	**2**	**28**

In present study eye 27 eyes had basic tear secretion value <10mm, only 3 eyes has Basic tear secretion value was more than 10 mm before treatment. After 1 month of treatment 18 eyes improved Basic tear secretion to > 10mm. All patient with Hypovitaminosis A, Neurotrophic keratitis achieved Basic tear secretion value >10mm.[Table 2].

Table - 2:-Comparison of Basic Tear Secretion in various ocular surface disorders.

S.N.	Diagnosis	Before Treatment		After Treatment	
		<10mm (No. of eyes	≥10mm. (No. of eyes	<10mm (No. of eyes	≥10mm. (No. of eyes
1.	Hypovitaminosis – A	8	0	0	8
2.	Neurotrophic Keratitis	2	1	0	3
3.	Kerato conjunctivitis Sicca (Non Sjogren's Syndrome)	6	0	2	4
4.	Sjogren's Syndrome	4	0	1	3
5.	Stevens - Johnson Syndrome	4	0	4	0
6.	Persistent epithelial defect	2	1	2	1
7.	Recurrent erosion Syndrome	1	0	0	1
8.	Marginal corneal Ulcer.	0	1	0	1
	Total	**27**	**3**	**9**	**21**

Table - 3:-Comparison of Tear film Breakup Time in various ocular surface disorders

S.N.	Diagnosis	Before Treatment		After Treatment	
		<10sec (no. of eyes)	≥10sec (no of eyes)	<10sec (no. of eyes)	≥10sec (no of eyes)
1.	Hypovitaminosis – A	8	0	0	8
2.	Neurotrophic Keratitis	3	0	0	3
3.	Kerato conjunctivitis Sicca (Non Sjogren's Syndrome)	6	0	1	5
4.	Sjogren's Syndrome	4	0	4	0
5.	Stevens - Johnson Syndrome	4	0	0	4
6.	Persistent epithelial defect	3	0	1	2
7.	Recurrent erosion Syndrome	0	1	0	1
8.	Marginal corneal Ulcer.	1	0	0	1
	Total	**29**	**1**	**6**	**24**

After treatment there was marked improvement in TBUT was observed in 23 eyes [Table 3]. In the present study 15 eyes had grade-2, 10 eyes had grade-3 staining pattern. After treatment most of eyes showed good epithelization i.e. - 21 eyes and become grade-0, most of those patients had Hypovitaminosis A and Neurotrophic keratitis, but satisfactory epithelization was not achieved in eyes with Stevens Johnson syndrome [Table 4].

Table 4:- Comparison of fluorescein staining (Grading) in various ocular surface disorders.

S.N.	Diagnosis	Before Treatment (Grade)				After Treatment (Grade)			
		O	1	2	3	O	1	2	3
1.	**Hypovitaminosis – A**	0	0	6	2	8	0	0	0
2.	Neurotrophic Keratitis	0	1	1	1	3	0	0	0
3.	Kerato conjunctivitis Sicca (Non Sjogren's Syndrome)	0	1	3	2	5	1	0	0
4.	Sjogren's Syndrome	0	2	2	0	2	2	0	0
5.	Stevens - Johnson Syndrome	0	0	1	3	0	4	0	0
6.	Persistent epithelial defect	0	0	2	1	2	1	0	0
7.	Recurrent erosion Syndrome	0	1	0	0	1	0	0	0
8.	Marginal corneal Ulcer.	0	0	0	1	0	1	0	0
	Total	**0**	**5**	**15**	**10**	**21**	**9**	**0**	**0**

In present study 29 eyes showed grade-1 in Rose Bengal staining pattern at the start of study, out of which 25 eyes become grade-0 after treatment. 100% improvement was observed in eyes with Hypovitaminosis A, Neurotrophic keratitis kerato conjunctivitis sicca, Sjogren's syndrome, recurrent erosion syndrome, Marginal corneal ulcer. However eyes with Stevens - Johnson syndrome showed no significant improvement in rose Bengal staining pattern after treatment [Table 5].

Table - 5:-Comparison of Rose Bengal staining (Grading) in various ocular surface disorders.

S.N.	Diagnosis	Before Treatment (Grade)				After Treatment (Grade)			
		O	1	2	3	O	1	2	3
1.	Hypovitaminosis – A	0	6	2	0	8	0	0	0
2.	Neurotrophic Keratitis	0	3	0	0	3	0	0	0
3.	Kerato conjunctivitis Sicca (Non Sjogren's Syndrome)	0	5	1	0	6	0	0	0
4.	Sjogren's Syndrome	0	3	1	0	4	0	0	0
5.	Stevens - Johnson Syndrome	0	1	3	0	0	4	0	0
6.	Persistent epithelial defect	0	3	0	0	3	0	0	0
7.	Recurrent erosion Syndrome	1	0	0	0	1	0	0	0
8.	Marginal corneal Ulcer.	0	0	1	0	1	0	0	0
	Total	**1**	**21**	**8**	**0**	**26**	**4**	**0**	**0**

Discussion

Autologous serum contains various factors like growth factors, vitamins, fibronectin and other components so serum can better support proliferation and migration of ocular surface epithelial cells. Autologous serum eye drops have been reported to be effective for the treatment of severe dry eye-related ocular surface disorders. Present study includes some ocular surface disorders like- Hypovitaminosis A, Sjogren's syndrome, Non Sjogren's syndrome, Stevens-Johnson syndrome, recurrent or persistent corneal erosions, persistent epithelial defect, Neurotrophic keratopathy, Mooren's ulcer [10].

The preparation of serum eye drops in our study was similar to the Standard Operating Procedure (SOP) at university of Luback, Germany [2]. The eye drop frequency of 1 hourly for 30 days was similar to that used by Rocha et al (2000)[11]and Tsubota et al (1996)[12] and De Souza et al (2001)[13]. In the present study 20% concentration of serum eye drops were used and this concentration was similar to that used by Tsubota et al (1999)[5], Tananuvat et al (2001)[14], Takamura et al (2001)[15], Ogawa et al (2003)[16] Del Castillo et al (2003)[17], Goto et al (2002)[18].

There was improvement in schirmer test in present study. Other studies which showed some important in Schirmer test were Tsubota et al (1999)[5], Noble et al (2004)[19]. We also found marked improvement in TBUT. The studies published in literature have not evaluated change in tear breakup time after treatment with autologous serum eye drops.

In this study, after treatment most of eyes showed good epithelization i.e. - 21 eyes (70%). Tsubota et al (1999)[5] and Poon et al (2001)[20] showed 55% success, Tananuvat et al (2001)[14] showed 39% improvement while Ogawa et al (2003)[16] showed 61% improvement in Fluorescein staining pattern.

Improvement in Rose Bengal staining pattern found in the present study was higher than that of other studies. Fox et al [4]showed 41% improvement, Tsubota et al [5] showed 68% improvement, Tananuvat et al [14] reported while - 33% and Ogawa et al [16] showed 44% improvement.

No patient had any adverse effects such as superficial punctate keratitis sloughing, delayed epithelial healing, stromal melting. 36 samples were cultured and all samples were sterile after 48 hours of incubation. Lagnadol et al [21] showed growth of streptococcus epidermis and streptococci viridens. No other study has reported contamination of serum eye drop during treatment.

It was observed that Hypovitaminosis A and Neurotrophic keratitis showed 100% improvement in all parameter after 1 month treatment. Keratoconjunctivitis sicca, sjogren's syndrome, persistent epithelial defect, Recurrent erosion syndrome, Marginal corneal ulcer also showed excellent results but eyes with Stevens - Johnson syndrome showed very poor response to treatment.

So treatment with autologous serum is an efficient method to provide a number of growth factors that have been reduced in ocular surface disorders.

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