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Autologous serum in treatment of dry eye disorder: An evaluation

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ABSTRACT

Dry eye syndrome is a common disorder of the tear film in which eye is unable to maintain a healthy layer of tears to coat it. Despite various conventional treatments such as ocular surface lubrication, artificial tears, protective glasses and punctal occlusion many patients continue to have signs and symptoms of dry eye. That's why autologous serum eye drops have been indicated for the treatment of dry eye. Therefore, to evaluate the effectiveness of the autologous serum eye drops in dry eye disorder, a total of 34 eyes of 20 patients were included in this study, who used autologous serum eye drops along with artificial tears. Out of 20 patients, 16 were males and 4 were females with M: F ratio of 4:1. The mean age of the patients was 51.95 years. Evaluations of patients were done before and after treatment in follow up, by determining the subjective and objective scores which showed that after follow up of 2 months all the subjective and objective scores were reduced in 28 eyes but 6 eyes did not show any improvement at follow up of 2 months as they stopped the drops after one month of use because of discomfort.

Keywords: Dry eye, autologous serum, subjective and objective scores.

INTRODUCTION

Normal sight depends on a moist ocular surface and this moisture is maintained by a complex interplay of various factors like sufficient quantity of tears, normal composition of tear film, normal lid closure and regular blinking of lids. Dry eye syndrome (DES) -- also known as dry eye or keratoconjunctivitis sicca (KCS) -- is a common disorder of the tear film that affects a significant percentage of the population, especially those older than 40 years of age. It is characterized by a disturbance of the tear film in which eye is unable to maintain a healthy layer of tears to coat it. This abnormality may result in disruption of the ocular surface and various changes like squamous metaplasia, epithelial defects and infections of corneal epithelium causing a variety of symptoms and signs which interfere with quality of life. So tear film is very important in the maintenance of an intact ocular surface and thus pathologies of the conjunctival and corneal epithelium are intimately related to tear film dysfunction (Mahajan, 2009).

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Three main layers make up this tear film. Innermost layer is mucin, middle is aqueous and the most superficial layer is of lipids. Defects of the aqueous layer are the most common cause of dry eye syndrome. Various conventional treatments such as ocular surface lubrication with frequent instillation of artificial tears, protective glasses, and punctal occlusion provide benefit to the patients (Young *et al*, 2004). However despite maximal therapy many patients continue to have signs and symptoms of dry eye. That's why treatment of ocular disorders with biological fluids has also been advocated (Rocha *et al*, 2000). More recently, autologous serum (AS) tears have been indicated for the treatment of dry eye. The rationale for this is based upon the fact that vitamins and growth factors present in tears, are also present in serum but lacking in artificial tears (Fox *et al*, 1984, Tsubota *et al*, 1999). Therefore the present study was designed to evaluate the effectiveness of the autologous serum eyedrops in relieving the symptoms and objective improvement in patients of dry eye.

MATERIALS & METHOD

The present prospective study was conducted in Department of Ophthalmology and Pharmacology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, from 16.07.11 to 16.03.12 (six months). The study included 34 eyes of 20 patients with dry eye disorder who used autologous serum eye drops along with artificial tears.

Criteria for patient selection

The study included the patients who satisfied the following criteria:-

- (1) Symptoms of dry eyes.
- (2) Break up time (BUT) <5 seconds & schirmer's test without anesthesia <5mm at 5 minutes.
- (3) Presence of Rose Bengal test and Fluorescein staining of ocular surface

Criteria for patient exclusion

Patients were not started on serum eye drops if patients have any of the following.

- (1) Patient with active microbial infection (acute herpes simplex/herpes zoster keratitis).
- (2) Vitamin A deficiency.
- (3) Progressive corneal melting due to any immunological process.
- (4) Recurrent corneal erosion syndrome.
- (5) Pregnant/ Lactating women.
- (6) Patient in whom venesection was not possible

Preparation of autologous serum

A total of 40 ml blood was taken by venepuncture from each patient under all aseptic precaution & collected in a sterile container. Blood was centrifuged at 4000rpm for 10 minutes. After separation of serum, it was packed into sterile dropper bottle preloaded with 3 ml unpreserved chloramphenicol (0.5% chloramphenicol with 1.5% boric acid, 0.3% borax and purified

water) or 3 ml saline. If 100% serum is desired, saline or chloramphenicol is not used. 6 ml of 100% serum or 6 ml of 50% serum eye drops were dispensed to the patients. Patients were asked to keep the bottle in dark to prevent the degradation of vitamin A and the bottle in use should be kept at home refrigerator at 5°C up to 1 month and -20°C up to 3 months. Each bottle was discontinued after 7 days of use (Fox *et al*, 1984, Tsubota *et al*, 1999).

Application of autologous serum

Patient was asked to apply autologous serum 8-10 times per day and examined weekly for two weeks and then two weeks interval for their subjective and objective scores which determine the efficacy of the treatment. Weaning of serum drops was done in patients who have beneficial effect and continued if there was any worsening of symptoms after withdrawal of serum drops.

Evaluation of patients

Evaluation of patients were done before as well as after initiation of treatment in follow up, by determining the subjective and objective scores.

Subjective evaluation was done by the following grading method:-0—no symptoms, 1- mild symptoms with no discomfort, 2- moderate symptoms with discomfort but no interference with daily activities, 3- severe symptoms with discomfort but no interference with daily activities, 4- very severe symptoms with discomfort and interference with daily activities.

Objective scores included ocular surface evaluation by double staining method according to the scoring system suggested by 'National Eye Institute Workshop On Dry Eye'. Rose Bengal staining (score 0—18) was used for bulbar conjunctival damage and fluorescein staining (score 0—15) for corneal damage. Two µl of preservative-free solution consisting of 1% rose bengal and 1% fluorescein dye were applied to the conjunctival sac (Toda *et al*, 1993).

The intensity of rose bengal staining was recorded in the temporal and nasal conjunctiva and the cornea, each graded on a scale of 0 to 3 points. Thus, the maximum score obtained from the staining of one eye is 9. Fluorescein staining was also rated from 0 to 9, but only in the cornea. Score of 3 or more for both the staining was the cut of value for diagnosis of dry eye disease.

OBSERVATIONS

The observations were based on the finding of dry eyes of same patients who used autologous serum along with artificial tears and were tabulated into 3 groups.

GROUP A- Observation before the use of autologous serum.

GROUP B- Observation after the use of autologous serum at 1 month.

GROUP C- Observation after the use of autologous serum at 2 months.

Patients were analyzed regarding: age, sex, subjective score and objective score (rose bengal & flourscein staining).

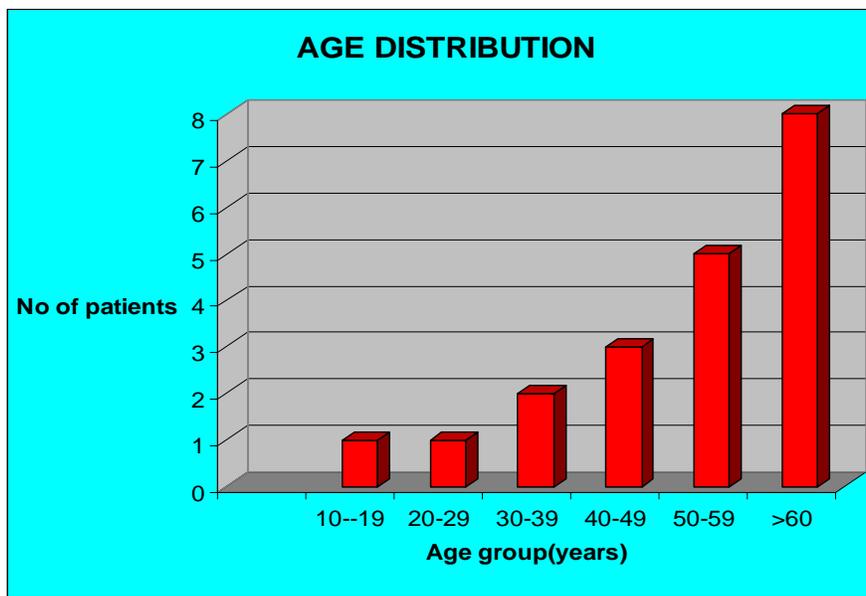


Fig. 1: Distribution of patients according to age.

Table. 1: Subjective Scores before and after use of autologous serum.

Subjective Score	Number of eyes(%)		
	Group --A Observation before use of autologous serum	Group --B Observation after use of autologous serum at 1 month	Group --C Observation after use of autologous serum at 2 months
4	20(58.82%)	6(17.64%)	-
3	11(32.35%)	8(23.52%)	02(7.14%)
2	03(8.82%)	20(58.82%)	24(85.71%)
1	-	-	02(7.14%)
0	-	-	-

Table. 2: Objective Scores (Fluorescin staining score) before and after use of autologous serum.

Fluorescin Staining Scores	Number of eyes(%)		
	Group--A Observation before use of autologous serum	Group --B Observation after use of autologous serum at 1 month	Group --C Observation after use of autologous serum at 2 months
0--3	-	-	01(3.6%)
4--6	01(2.94%)	13(38.83%)	27(96.4%)
7--9	18(52.9%)	16(47.05%)	-
10--12	15(44.1%)	5(14.70%)	-
13--15	-	-	-

TABLE. 3 :- Objective (rose bengal staining)Scores before and after use of autologous serum

Rose Bengal Staining Scores	Number of eyes(%)		
	Group--A Observation before use of autologous serum	Group --B Observation after use of autologous serum at 1 month	Group --C Observation after use of autologous serum at 2 months
3--6	01(2.95%)	6(17.64%)	13(46.42%)
7--10	10(29.54%)	17(50%)	15(53.50%)
11--14	23(67.6%)	11(32.35%)	-
15--18	-	-	-

RESULTS

The present study included 34 eyes of 20 patients having dry eye disorder. Out of these 20 patients, 16(80%) were males and 4 (20%) were females with a male female ratio of M : F - 4 :1. Distribution of patients according to age is shown in **Figure. 1** which shows that the maximum number of patients belongs to age group of >60 years and the mean age of the patient is 51.95 years. Regarding the subjective scores (**Table.1**), it was maximum in group A and least in group C. In group C, out of 34, 28 eyes (82.36%) show decrease in subjective score but 6 eyes

(17.64%) show no response to autologous serum at follow up of 2 months as they stopped the drops after one month of use because of discomfort. The results of objective scores were similar to subjective scores.

Fluorescein staining score (**Table.2**) and Rose Bengal staining score (**Table.3**) both were maximum in group A and lower in group C. Again a total of only 28 eyes (82.36%) show decrease in Fluorescein and Rose Bengal staining score and 6 eyes(17.64%) did not show any improvement at follow up of 2 months as they stopped the drops after one month of use because of discomfort.

DISCUSSION

Dry eye syndrome is a common clinical entity causing discomfort to many people (Pokupec *et al*, 2005). Standard substitution therapy with artificial tears like hydroxypropyl methyl cellulose and carboxymethylcellulose are effective in providing lubrication to the ocular surface, but are unable to substitute the nutrients and factors present in natural tears (Mahajan, 2009). This is why autologous serum come into focus, which contain various factors that are also present in natural tears. These factors include vitamin A, epidermal growth factor (EGF), transforming growth factor beta (TGF β), basic fibroblast growth factor, insulin like growth factor, substance P as well as proteins such as lactoferrin and lysozyme etc. (Rocha *et al*, 2000). Vitamin A plays a vital role in reducing the progress to squamous metaplasia in dry eye (Mahajan, 2009). EGF has been found to be helpful in healing of epithelial abrasions and it also facilitates epithelialisation because of its anti-apoptotic properties (Scardovi *et al*, 1993, Pastor and Calonge, 1992, Collins *et al*, 1994, Rodeck *et al*, 1997). Similarly transforming growth factor beta is also believed to control epithelial proliferation and to maintain cells in an undifferentiated state. Acidic and basic fibroblast growth factors (aFGF, bFGF) are also found to speed up healing of epithelial defects (Fredj-Reygrobellet *et al*, 1987).

Serum has also been shown to accelerate the migration of corneal epithelial cells *in vitro*; with upregulation of mucin expression and this may have beneficial effect in dry eye patient (Tsubota *et al*, 1999). Neural factors such as substance P are important for corneal epithelial migration. Serum also contains antiproteases such as beta-2 macroglobulins which has been thought to inhibit corneal collagenase and therefore, beneficial in conditions like alkali burns (Young *et al*, 2004). Proteins are also present in high concentration in serum, pre-albumin acts as a stabilizer for tear film and hence are important in the maintenance of its stability. Thus the rationale behind using autologous human serum in eye is that it contains the same growth factors that are present in tears. As a consequence it is now possible to create eye drops from the blood sample collected from the individual with dry eye. Furthermore serum also contains IgG, lysozyme and complement all of which may serve to additional anti-infective properties to a compromised surface. In this present study 34 eyes of 20 patients were examined for the evaluation of efficacy of autologous serum and patients were analyzed regarding age, sex, subjective score and objective score. The study showed that the maximum number of patients belonged to age group of >60 years and the mean age of the patient was 51.95 years. The results correspond to the study of Poon *et al*, 2001, who found that dry eye disease was frequent in individual above 50 years of age pointing towards aging and postmenopausal causes.

This may be because of aqueous layer abnormality. Regarding the sex distribution, in our study M: F ratio was 4:1 but we didn't find any study, showing any sex preponderance. Subjective score was based on the grading of symptoms as mentioned above. The score was least in group C in which 28 eyes (82.36%) showed decrease in subjective score but 6 eyes (17.64%)

did not show response to autologous serum at follow up of 2 months as they stopped the drops after one month of use because of discomfort. Our findings were consistent with the study of fox *et al*, 1984 and Tsubota *et al*, 1999, who found decrease in subjective as well as objective scores by the use of autologous serum diluted to 30% and 20% in saline respectively.

The objective scores were measured by Rose Bengal staining and fluorescein staining. Normally rose Bengal is a water soluble dye that stains degenerated and dead cells as well as mucous fibrils. The stain produce a punctuate coloration along the lacrimal revus. Current understanding for Rose Bengal staining of ocular surface is due to loss of normal mucin layer in dry eye disease, allowing the dye to stain live epithelial cells that would normally be protected by mucin in healthy eyes. Recent researches have suggested that staining in dry eye is associated with a altered glycosylation of the surface mucin of apical conjunctival cells. Thus although stained cells are not necessarily degenerated, they may suffer from impaired expression of membrane mucin. Impression cytology studies also suggest that autologous serum leads to upregulation of goblet and mucin expression in dose dependent manner. Fluorescein is a water soluble dye that can be excited by illumination with near ultraviolet light such as that produced by cobalt blue filters. In dry eye patients, fluorescein accumulates in the intracellular spaces resulting from discontinuity in the corneal epithelium. The thickness in the tear film and its integrity can be assessed by the intensity of the coloration over the ocular surface. Patients with reduced tear volume or tear film thickness usually exhibit a less intense fluorescence despite good illumination. In this study both rose bengal staining as well as fluorescein staining were least in group C and maximum in group A after 2 months of treatment. Thus the objective scores were improved by the use of autologous serum. Our findings were consistent with the study of fox *et al*, 1984 and Tsubota *et al*, 1999, as mentioned above.

The results were similar to the studies of Kojima *et al*, 2005, who compared the artificial tears and autologous serum in dry eye patients and found that there was significant improvement in tear film break up time, subjective score, fluorescein and rose bengal staining in patients assigned to autologous serum. The results also correspond with the study of Creuzol *et al*, 2004, who applied the 20% autologous serum in eyes of sjogren's syndrome and found to be very effective in improving fluorescein staining as well as subjective symptoms of burning, foreign body sensation and dryness. Poon *et al*, 2001, recently also reported their experiences using 20% autologous serum drops to treat 11 dry eyes of 9 patients with KCS, and noted 60% improvement based on fluorescein, rose bengal staining and subjective symptoms score.

In contrast, study done by Goto *et al*, 2001, recommended that autologous serum should only be considered as a second choice only if punctal occlusion is not effective. They treated 11 patients with superior limbic keratoconjunctivitis with 20% autologous serum drops for 4 weeks, and noted partial efficacy in 82% of them using the fluorescein and rose bengal staining as well as subjective symptom score. They also pointed out in the study that autologous serum drops might also provide lipids, a very

important component of the tear, but did not perform any measure of lipid components. Besides this in our study 6 eyes showed no response to autologous serum at follow up of 2 months who stopped the drops after one month of use because of discomfort on instillation of autologous serum eye drops.

CONCLUSION

Autologous serum drops were found to be effective in improvement of symptoms and objective signs in severe dry eye disorder. The drawbacks of this treatment is to obtain blood from patients. Thus, the development of an ideal artificial tear substitute containing these essential components would be ideal. But until such medication is available, the use of autologous serum may be of great benefit for the dry eye patients.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest to disclose

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