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A RETROSPECTIVE ANALYSIS OF CHARACTERISTIC FEATURES OF RESPONDER PATIENTS TO AUTOLOGOUS SERUM EYE DROPS IN ROUTINE CARE

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ABSTRACT

Purpose Autologous serum eye drops (ASEDs) are used worldwide to treat dry eye disease (DED). However, the biological composition of ASEDs has not been well investigated, and effectiveness predictive factors remain to be identified. The main objective of this study was to compare the response of patients treated with ASEDs biologically characterized and used for DED routine care.

Methods This retrospective observational study was conducted in a single university hospital, and included 50 patients (87 eyes) with DED refractory to conventional treatment and resulting from various etiologies with Ocular Surface Disease Index (OSDI) $\geq$ 20. Each patient used eight drops a day per treated eye with 20% diluted ASEDs. Undiluted serum extensive biological characterization were performed, and symptoms were recorded before the initiation of ASEDs and closer to the sixth month of treatment. Responders were defined as presenting an improvement from baseline $\geq$ 14 points in OSDI and/or $\geq$ 1 grade in corneal fluorescence staining for all eyes treated.

Results The OSDI and the Oxford scale were significantly reduced from 68.7 ± 23.2 to 54.8 ± 25.7 and 3.2 ± 1.5 to 2.1 ± 1.3 ($p \leq 0.0001$), respectively. A total of 68% of the patients were responders. Nonresponding patients had significantly higher epidermal growth factor concentrations in the serum compared to responders ($p = 0.017$).

Conclusions ASED administration resulted in significant clinical improvement in the management of DED. Biological differences observed between responders and nonresponders suggested that a better understanding of the biological activity of ASEDs is still required.

INTRODUCTION

The Tear Film and Ocular Surface Society Dry Eye WorkShop II (TFOS DEWS II) defines dry eye disease (DED) as: “a multifactorial disease of the ocular surface, characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles” (1). A classic scheme based on physiopathology distinguishes aqueous deficient and evaporative dry eyes, although a continuum between these two forms can exist. DED affects over 340 million people worldwide and has a high impact on a patient’s quality of life. The main symptoms include pain, burning sensations, eye fatigue, redness, blurred vision, discharge, contact lens intolerance, sensitivity to light, and a feeling of foreign bodies present in the ocular region. The treatment of DED includes various strategies such as unpreserved ocular lubricants, eyelid hygiene, and anti-inflammatory treatments. Autologous serum eye drops (ASEDs have been proposed since the 1970s to treat ocular surface disorders (2), and their use was first described in 1984 in patients with DED associated with keratoconjunctivitis sicca and Sjögren’s syndrome (SS) (3). ASEDs are typically used as a third-line option in the management of DED, whose etiologies are heterogeneous iatrogenicity, SS, neurotrophic keratopathy, graft-versus-host disease (GVHD), and ocular cicatricial pemphigoid. ASEDs are considered to be a safe and efficient way to treat DED because human serum is comparable to the pH and osmolarity of human tears (4), and potentially provides essential components to the ocular surface such as vitamin A, fibronectin, epidermal growth factor (EGF), and transforming growth factor (TGF)-β, that beneficially act on the proliferation, differentiation, and maturation of the ocular surface epithelium (5-8). ASEDs have also been shown to modulate stromal corneal wound healing by controlling matrix metalloproteinase activity (9). ASEDs contain interleukin-1 receptor antagonist (IL-1Ra), which has been shown to decrease signs of dry eyes in murine models (10,11). This anti-inflammatory potential of ASEDs may be of particular relevance because

the composition of tears from patients with DED involves high levels of inflammatory cytokines and chemokines (12-15). ASEDs are simply produced by centrifugation of non-anticoagulated peripheral blood, which can be followed by dilution in normal saline or artificial tears (5). However, ASED use remains controversial for two reasons: i) there does not exist any consensus regarding the manufacturing process, which could substantially differ with respect to the harvesting method (with or without anticoagulant), the dilution applied to the serum (from 20% to pure), the waiting time before centrifugation, or the centrifugation settings; and ii) the serum composition is highly variable from one DED patient to another, depending on the various etiologies and disease activities. Although many cytokines present in ASEDs have a beneficial role, an excess of TGF-β, interleukin (IL)-17, or interferon (IFN)-γ may lead to a decrease in epithelial growth and increased inflammation. For example, IFN-γ is known to promote squamous metaplasia and apoptosis, whereas IL-17 promotes epithelial barrier dysfunction (16). In the same manner, Hwang et al. (17) suggested that ASEDs might not be effective for the treatment of secondary SS, because these patients present elevated serum proinflammatory cytokine levels.

Complementary biological analyses addressing the composition of ASEDs and the impact of the autologous patient's disease are therefore necessary. These analyses may reveal predictive factors to anticipate the response to treatment and optimize the use of ASEDs. The aim of this study was to analyze the response of patients presenting with DED who were treated with ASEDs in routine care in a university hospital, and to investigate the relationships between clinical results and the ASED composition by conducting a precise biological characterization of the administered ASEDs, including quantification of growth factors.

METHODS

Participant recruitment

This retrospective observational study was performed at a single university ophthalmology department (Timone Hospital, Marseille, France) between April 2014 and February 2018. Patients included males and females ≥ 18 years of age with various etiologies and presenting with severe DED with an Ocular Surface Disease Index (OSDI) ≥ 20 that were refractory to conventional treatment (artificial tears, eyelid treatment, or 0.05% cyclosporine ophthalmic emulsion ≥ 3 months). All patients included in the study had both OSDI and Oxford results available at initiation and the 6-month follow-up. Exclusion criteria were severe anemia, positive serology for HIV, hepatitis C virus, hepatitis B virus, syphilis, and active ocular infections. All patients provided informed consent and all procedures were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Efficacy assessments

The patient's subjective symptoms were assessed using the OSDI score, which is a reliable and valid test for quantifying the severity of dry eye symptoms. Objective symptoms were assessed using a combination of clinical examinations; corneal fluorescein staining (CFS) evaluates the severity of corneal epithelial punctuate erosion and estimates surface damage in dry eye patients. CFS was scored using the Oxford scale in which staining was represented by punctuate dots on a series of panels. Staining ranged as follows: i) from 0 (normal) to 5 (most severe); ii) in the presence or absence of blepharitis and the grade of conjunctival hyperemia (0, absence; 1, mild/moderate; 2, severe) was assessed using a slit lamp examination; iii) tear break-up time (tBUT) was measured using fluorescein that was instilled into the patient's tear film; the patient was asked not to blink while the tear film was observed under a broad beam of cobalt blue illumination; iv) the Schirmer's test determined aqueous tear production; and v)

the best-corrected visual acuity was scored using the logarithm of the minimum angle of resolution (LogMAR) chart to define the integrity of the visual system for each eye. The presence of blephatritis and conjunctival hyperemia, tBUT, Schirmer's test, and the LogMAR were analyzed when performed at initiation and at first follow-up.

These parameters were evaluated before the beginning of treatment and closer to the sixth month of treatment, over a maximum period of 13 months. A responder was defined as a patient displaying an improvement of ≥ 1 grade in CFS from baseline for all eyes treated and/or an improvement of ≥ 14 points in OSDI from baseline.

Eye drop serum preparation

To prepare the eye drops, 75 mL of peripheral blood was collected in non-anticoagulated tubes (Becton Dickinson, Sparks, MD, USA) from each subject's antecubital vein and allowed to clot for 1 h at room temperature. Following centrifugation at 3,000 × *g* for 10 min, 24 mL of serum was carefully isolated under sterile conditions in a laminar flow hood in the Cell Therapy Department of La Conception University Hospital. The serum was then diluted to 20% (v/v) concentration with a balanced saline solution to obtain a final volume of 120 mL of eye drop serum. The diluted serum was filtered through a 0.22 µm filter and divided in 24 5-mL vials, two of which were used for sterility testing. Aliquots of undiluted serum were used both for immediate measurements of biochemical parameters and frozen at -40°C for measurements of growth factors and cytokine levels.

Eye drop serum delivery

Following a 10 day period of quarantine at -40°C to validate sterility results, autologous serum eye drop vials were transferred to the pharmacy from La Conception University Hospital. Patients recovered four vials once a month to the pharmacy and used eight drops of autologous serum each day for one eye. Once thawed, the ASED vial was maintained at 4°C at the patient's home.

Biological parameter quantification

Eighteen pertinent biological parameters were measured from the autologous serum obtained from the first eye drop production of each patient. Cortisol was measured by an electrochemiluminescence technique corresponding to a Cobas e601 system (Roche Diagnostics, Rotkreuz, Switzerland). Albumin, IgA, and fibronectin were measured by an immunoturbidimetric technique using a Cobas 8000 system (Roche Diagnostics). Vitamin A and E were measured by high-performance liquid chromatography with a Shimadzu system (Shimadzu, Kyoto, Japan). A combination of 12 cytokines and growth factors [vascular endothelial growth factor (VEGF), EGF, IL-1Ra, fibroblast growth factor (FGF)-2, IFN-γ, IL-10, IL-1β, IL-6, tumor necrosis factor (TNF)-α, nerve growth factor (NGF), TGF-β1, and platelet-derived growth factor (PDGF) AA-BB or PDGF AB-BB] were measured using a Magpix instrument (Luminex xMAP Technology, Luminex, Austin, TX, USA) allowing simultaneous measurements of different analytes in small sample volumes.

Statistical analysis

All data are presented as the mean ± standard deviation. Data were analyzed with GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Mean differences were compared using a nonparametric Mann–Whitney U test or one-way analysis of variance with Bonferroni post

hoc testing to make pairwise comparisons. The chi-square test was used to assess the response to treatments to patients involving co-medications or the etiology of DED. The coefficient of variation was obtained by division of the standard deviation by the mean.

RESULTS

Patient characteristics

Fifty patients (87 eyes) were included in the study; 32 were female (64%) and the mean age was 63 ± 16 years. DED was the consequence of various pathologies including GVHD (28%), neurotrophic keratitis (24%), SS (16%), ocular cicatricial pemphigoid (8%), and the remaining patients presented other isolated etiologies that were gathered under the term "others" (one with eye burn, one with CREST syndrome, and 10 with idiopathic ocular dryness). DED was characterized by an initial OSDI score of 68.7 ± 23.2, an Oxford scale of 3.2 ± 1.5, a tBUT of 4.1 ± 2.1 s, Schirmer's test of 5.6 ± 4.9 mm, and a LogMar of 0.5 ± 0.6. Finally, 92% of the patients were treated with ongoing hyaluronic acid-containing lubricant eye drops for DED. Local cyclosporine, corticoids, and scleral lenses were associated in 46%, 20%, and 16% of the patients, respectively. Eyelid treatments were systematically associated with patients presenting with blepharitis (62%, n = 31 patients). The demographics and clinical features of patients are listed in Table 1.

Biological characteristics of eye drop serum

The results of the extensive biological characterization performed on autologous serum of each patient before dilution are listed in Table 2. The eighteen relevant parameters were quantified and classified as a proinflammatory substance (TNF-α, IL-1β, and IL-6), anti-

inflammatory substance (cortisol, IL-10, IL-1Ra, and IFN-γ), with a positive impact on DED (IgA, vitamin A and E, fibronectin, and albumin), or a positive impact on cell proliferation (EGF, VEGF, PDGF AB-BB, NGF, FGF2, and TGF-β). High interindividual variations were observed in the levels of VEGF, IL-1Ra, FGF2, IFN-γ, IL-10, IL-1β, and IL-6, with a coefficient of variation > 200%. Conversely, only three parameters (albumin, vitamin A, and vitamin E) presented a coefficient of variation < 30%. However, analysis of variance and Bonferroni post hoc testing showed that the variations observed in the concentrations of the above parameters were not associated with any of the main eye dry etiologies (GVHD, neurotrophic keratitis, SS, and ocular cicatricial pemphigoid).

Efficacy of the eye drop serum

We investigated the efficacy of 20% autologous serum eye drop application with a mean follow-up of 6.5 ± 3.3 months corresponding to the time of the second evaluation performed by ophthalmologists. OSDI and the Oxford scale were significantly reduced from 68.7 ± 23.2 to 54.8 ± 25.7 ($p = 0.0001$) and 3.2 ± 1.5 to 2.1 ± 1.3 ($p < 0.0001$), respectively. The results of the Schirmer's test showed a trend toward improvement without reaching statistical significance ($p = 0.09$). However, tBUT ($p = 0.28$) and the LogMAR ($p = 0.23$) tests were not significantly modified following ASED application. Finally, statistical improvement ($p = 0.01$) was found in the conjunctival hyperemia grade, with 36/63 eyes improved, including 19 eyes with complete resorption of conjunctival hyperemia. No improvement was observed regarding blepharitis with only 4/66 eyes improved ($p = 0.11$). The efficacy results are detailed in Table 3 and Figure 1.

Relationship between patient and ASED baseline characteristics and response to treatment

Responders were defined as patients presenting an improvement from baseline ≥ 14 points in OSDI and/or ≥ 1 grade in CFS for all eyes treated. According to this definition, 34 patients (68%) were classified as responders. There was no age difference between the two groups (66.8 ± 14.7 years for responders vs. 59.1 ± 16.6 years for nonresponders; $p = 0.12$). Response was not associated with a dedicated etiology ($p = 0.33$) even though four patients presenting with ocular cicatricial pemphigoid and 10 out of the 12 patients with neurotrophic keratitis were responders. Regarding GVHD and SS, eight out of the 14 patients and five out of the eight patients were responders, respectively. Regarding the impact of co-medications, no statistical difference was observed ($p = 0.26$). Among the biological parameters studied, only EGF concentrations were significantly different between the two groups (Figure 2; $p = 0.017$), with higher concentrations in nonresponders (226.4 ± 112.0 pg/mL) compared to responders (153.0 ± 90.4 pg/mL). Five parameters (cortisol, VEGF, FGF2, IFN-γ, and IL-1β) also showed a trend toward increases in nonresponders without reaching statistical significance (Table 4; $p < 0.2$).

DISCUSSION

The pathogenesis of DED is not fully understood, but it is thought that inflammation plays a role in the development and persistence of DED symptoms (10). Conventional therapy includes artificial tears to provide additional lubrication. Although a large variety of artificial tears is available, none is considered the gold standard to substitute for tears, and one drawback is the possible presence of chemical preservatives. Because of the interesting

composition of anti-inflammatory molecules and growth factors, ASEDs are used worldwide to treat ocular surface disorders, and are well tolerated and free of any serious complications.

To the best of our knowledge, our study had the largest cohort of DED patients treated by ASEDs, together with an extensive biological characterization of the serum used for the production of eye drops. The results further supported the use of ASEDs in DED (18), because significant improvements in both the OSDI and the Oxford scale were observed. In previous studies, the biological composition of ASEDs was not well investigated. The main value of our study was therefore the opportunity to compare the use of ASEDs with clinical outcomes. We characterized 18 parameters for each ASED based on a literature review and their positive or negative impact on corneal healing (19-26). In the absence of consensus to define a responder, and based on the DED definition in TFOS DEWS II, the response was established as an improvement of ≥ 1 grade in CFS from baseline for all eyes treated and/or an improvement of ≥ 14 points in OSDI corresponding for this latter, to the minimal clinically important difference for severe DED (27). This applied to 68% of responders in our cohort. Our study revealed a significantly higher serum concentration of EGF in the nonresponder group. In our cohort, one nonresponder was characterized by extremely high concentrations of all cytokines and growth factors. However, after removing these values from this patient, the difference regarding EGF concentrations was maintained ($p = 0.04$). It is important to note that only 10 patients (20%) presented both improvement ≥ 14 points in OSDI and ≥ 1 grade in CFS for all eyes treated, suggesting that international consensus regarding the responder's definition for DED treatments is urgently needed. Despite this questionable issue regarding responder definition, our findings were confirmed by a statistical difference that was also observed between EGF concentrations of these 10 "very good" responders (146.0 ± 19.3) compared to nonresponders (226.4 ± 28.0; $p = 0.05$). These results are consistent with the study of Yan et al. (28), who assessed the optimal concentration of recombinant human EGF

on corneal epithelial wound healing. Proliferation of corneal epithelial cells *in vitro* was better with a concentration of 10 ng/mL than 20 ng/mL, suggesting that an excess of EGF reduces corneal epithelial wound healing. Another important finding of our study involved the concentrations of IL-1 and IFN-γ, which showed a trend toward higher levels in nonresponders. The proinflammatory cytokines IL-1 and IFN-γ have been reported to cause squamous metaplasia of epithelial cells and decrease goblet cell differentiation (29,30). Finally, the 20% dilution of serum we performed for the ASEDs was based on the results of Tsubota et al., who reported that the concentration of TGF-β in serum was five times higher than that in tears (31). A high concentration of TGF-β is expected to have anti-proliferative effects and might delay corneal epithelial wound healing. In our study, no link was found between TGF-β concentrations, which were similar in both groups, and their responses to treatments.

The impact of DED has already been investigated in previous studies, which reported that the composition of ASEDs from patients presenting with chronic renal failure was characterized by higher concentrations of epitheliotrophic factors (EGF, PDGF-AB, TGF-β1, and fibronectin) compared to healthy controls (32). Harloff et al. (33) showed that serum from healthy donors contained higher amounts of fibronectin and TGF-β compared to the serum of immunosuppressed patients with rheumatoid arthritis. However, in these two preclinical studies, changes in ASED composition did not significantly affect the stimulatory effects on proliferation, migration, and differentiation of human corneal epithelial cells. In clinical studies, higher expression levels of TGF-β1, IL-1β, IL-6, and TNF-α were found in ASEDs from patients presenting with active SS (based on a high erythrocyte sedimentation ratio and the presence of rheumatoid arthritis) compared with the inactive group. However, these differences were of limited therapeutic consequence because the OSDI and ocular surface staining were not different between the two groups (34). In our study, we found that the

majority of patients presenting with ocular cicatricial pemphigoid (100%) and neurotrophic keratitis (83.3%) were responders, whereas the response rate appeared lower in SS (62.5%) and GVHD (57.1%) patients. However, we failed to demonstrate DED etiology as a predictive factor of response using the chi-square test ($p = 0.33$).

Unfortunately, the abovementioned studies detailing the biologically active components of ASEDs remain an exception, and one of the main weaknesses is the complete absence of ASED characterization in the majority of studies on this topic (35-37). This is associated with a lack of standardization in the ASED preparation method (5), making it difficult to analyze the results of different studies. This is also consistent with the biological heterogeneity of ASEDs observed in our study, as evidenced by the high dispersion of the parameters assessed in the cohort. Taken together, these elements confirmed that ASEDs are a complex product with likely hundreds of active molecules, and that systematic characterization should be mandatory to identify positive and detrimental growth factors linked to the clinical response. Furthermore, new technologies are emerging such as quantitative suspension array technology, allowing simultaneous measurement of different cytokines and growth factors in small sample volumes (38).

Although our study had limitations, including the absence of a control group and a retrospective design associated with missing data for some scores, it provided further insights towards a better understanding of the biological activity of ASEDs in ocular surface disease.

In conclusion, our study showed that ASED administration provided a significant clinical improvement in the management of DED. Moreover, EGF levels in ASEDs were found to be higher in nonresponders, suggesting its potential value in the prediction of treatment outcomes. Because corneal epithelial wound healing is a complex process under the influence of various cytokines, growth factors, and interaction with the extracellular matrix, our results suggested the use of systematic biochemical quality control of ASEDs, as well as a record of

traceability data for patients in daily use. Further well-designed clinical trials, including comprehensive characterization of administered ASEDs, are needed to elucidate the mechanisms underlying its positive effect on DED, and to define optimized modalities for successful ASED therapy.

TABLES

Table 1. Baseline characteristics of patients treated with eye drop serum.

n = 50 patients, 87 eyes	
Sex (female/male)	32/18
Age (years, mean ± SEM)	63 ± 16
Disease characteristics (number of patients,%)	
Graft versus-host disease	14 (28%)
Neurotrophic keratitis	12 (24%)
Sjögren's syndrome	8 (16%)
Primary	5 (10%)
Secondary	3 (6%)
Ocular cicatricial pemphigoid	4 (8%)
Non-Sjögren-related dry eye disease	12 (24%)
Baseline score	
Ocular Surface Disease Index (n= 50 patients)	68.7 ± 23.2
Oxford score (n= 87 eyes)	3.2 ± 1.5
Tear break-up time (n = 82 eyes)	4.4 ± 2.5
Schirmer's test (n= 49 eyes)	7.5 ± 7.6
LogMAR (n = 79 eyes)	0.5 ± 0.6
Blepharitis (0, absence; 1, presence) (n = 82 eyes, % presence)	51, 62.2%
Conjunctival hyperemia (0, absence; 1, mild/moderate; 2, severe) (n = 85 eyes, % ≥ 1)	67, 78.8%
Ongoing local dry eye medications (number of patients,%)	
Cyclosporine (0.01%, 0.5%, or 2%)	23 (46%)
Corticoids	10 (20%)
Scleral lenses	8 (16%)
Eyelid treatments	31 (62%)
Hyaluronic acid-containing lubricant eye drops	46 (92%)

Table 2. Mean biological characteristics of patients serum used for eye drop preparations.

	Mean ± SD	Min-Max	Coefficient of variation (%)
TNF-α (pg/mL)	10.11 ± 8.68	0–39.26	85.87
IL-1β (pg/mL)	2.49 ± 8.21	0–62.06	330.30
IL-6 (pg/mL)	16.19 ± 45.35	0–281.44	280.11
Cortisol (nmol/L)	298.15 ± 113.27	4.9–559.2	37.99
IL-10 (pg/mL)	2.28 ± 5.81	0–22.98	255.21
IL-1Ra (pg/mL)	128.53 ± 408.05	0–2524.32	317.47
IFN-γ (pg/mL)	34.66 ± 113.78	0–877.48	328.28
IgA (g/L)	2.16 ± 1.40	0.16–6.02	65.01
Vitamin A (mg/L)	0.55 ± 0.16	0.24–0.96	29.33
Vitamin E (mg/L)	14.23 ± 3.57	7.2–29.2	25.07
Fibronectin (g/L)	0.34 ± 0.11	0.15–0.75	31.50
Albumin (g/L)	44.01 ± 2.88	37.6–49.2	6.55
EGF (pg/mL)	189.67 ± 101.18	42.53–482.25	53.35
VEGF (pg/mL)	316.60 ± 846.82	0–6453.87	267.47
PDGF AB-BB (ng/ml)	65.53 ± 28.35	16.67–152.85	43.27
NGF (pg/mL)	2.18 ± 2.79	0.06–11.96	128.53
FGF2 (pg/mL)	127.93 ± 320.56	0–2272.96	250.59
TGF-β1 (ng/mL)	74.74 ± 26.17	39.76–152.87	35.00

TNF-α, tumor necrosis factor-α; IL, interleukin; IGA, immunoglobulin A; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; FGF2, fibroblast growth factor 2; TGF-β1, transforming growth factor-β1; IFN-γ, interferon-γ; IL-1Ra, interleukin-1 receptor antagonist.

Table 3. Key efficacy variable changes before and after eye drop treatment.

	Before treatment	After treatment	p
OSDI, n = 50 patients	68.7 ± 23.2	54.8 ± 25.7	**0.0001**
Oxford, n = 87 eyes	3.2 ± 1.5	2.1 ± 1.3	**< 0.0001**
tBUT (s), n = 67 eyes	4.1 ± 2.1	4.4 ± 1.8	0.28
Schirmer's test (mm), n = 20 eyes	5.7 ± 4.9	7.8 ± 4.6	*0.09*
LogMAR, n = 72 eyes	0.5 ± 0.6	0.4 ± 0.6	0.23

OSDI, ocular surface disease index; tBUT, tear break-up time; LogMAR, logarithm of the minimum angle of resolution.

Table 4. Comparison of mean biological characteristics of serum from responder and non-responder patients.

	Responders	Non responders	p
TNF-α (pg/mL)	10.0 ± 7.6	10.2 ± 9.7	0.95
IL-1β (pg/mL)	0.2± 0.8	4.73 ± 15.6	0.10
IL-6 (pg/mL)	12.8 ± 49.5	19.6± 41.1	0.64
Cortisol (nmol/L)	264.6± 125.1	331.7 ± 101.5	0.10
IL-10 (pg/mL)	1.6 ± 4.9	2.94 ± 6.73	0.43
IL-1Ra (pg/mL)	103.3 ± 444.6	153.8 ± 371.5	0.70
IFN-γ (pg/mL)	4.0 ± 8.9	65.4 ± 218.6	0.10
IgA (g/L)	2.2 ± 0.1	2.1 ± 1.5	0.65
Vitamin A (mg/L)	0.6 ± 0.2	0.5± 0.1	0.65
Vitamin E (mg/L)	14.3± 4.3	14.1 ± 2.8	0.93
Fibronectin (g/L)	0.3 ± 0.1	0.3 ± 0.1	0.51
Albumin (g/L)	43.9± 3.2	44.1 ± 2.6	0.89
EGF (pg/mL)	153.0 ± 90.37	226.4 ± 112.0	0.017
VEGF (pg/mL)	132.3± 104.4	500.9 ± 1589.2	0.18
PDGF AB BB (ng/mL)	72.37 ± 28.55	58.69 ± 28.15	0.12
NGF (pg/mL)	2.2± 3.0	2.2± 2.5	0.96
FGF2 (pg/mL)	51.4± 78.2	204.4 ± 563.0	0.12
TGF-β1 (ng/mL)	74.7± 26.3	74.8 ± 26.0	0.99

TNF-α, tumor necrosis factor-α; IL, interleukin; IGA, immunoglobulin A; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; FGF2, fibroblast growth factor 2; TGF-β1, transforming growth factor β1; IL1Ra, interleukin-1 receptor antagonist.

FIGURES

Figure 1. Ocular Surface Disease Index (OSDI), ocular surface staining grades, tear film break-up time (tBUT), Schirmer's test, and LogMAR before and after 6.5 ± 3.3 months of autologous serum eye drop applications. Significant improvement was observed in OSDI and Oxford scores.

Figure 2. Comparison of serum epithelial growth factor levels between responders and nonresponders. The mean concentration of epidermal growth factor in nonresponders was significantly higher compared to responders.

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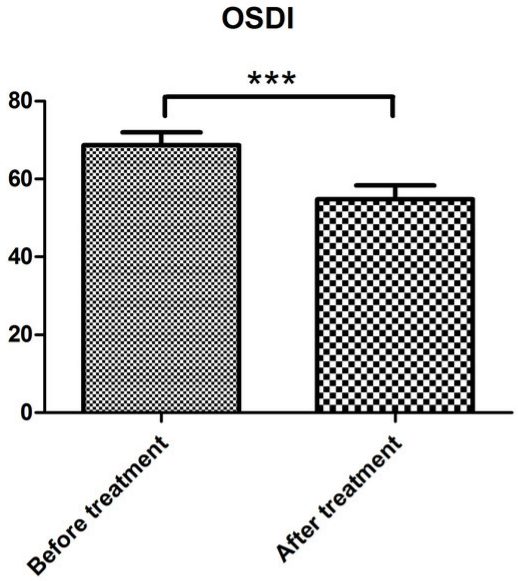
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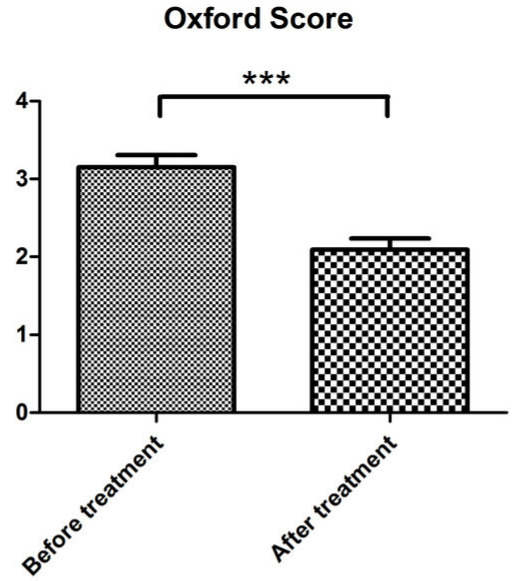
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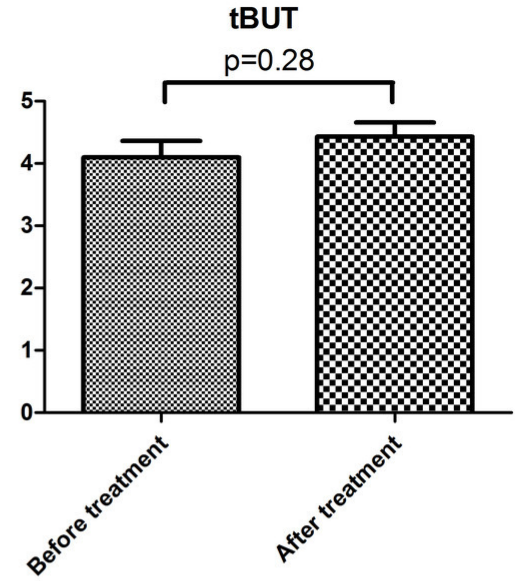
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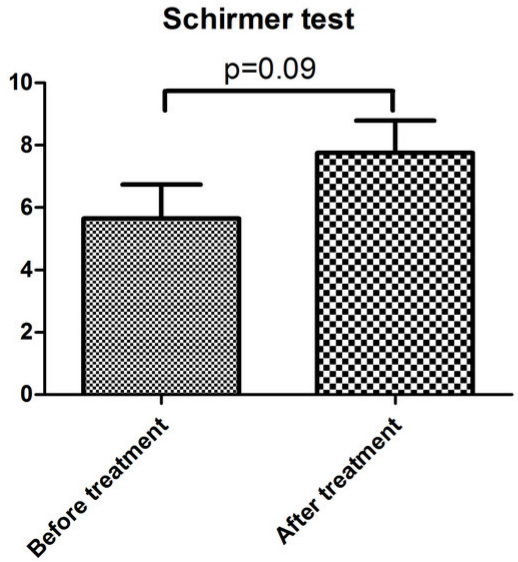
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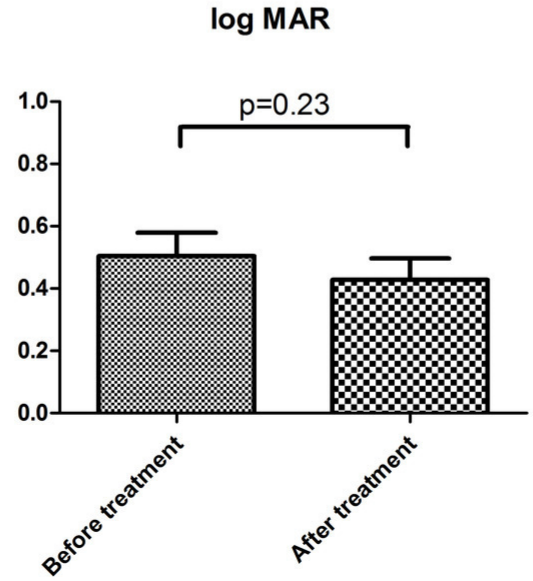
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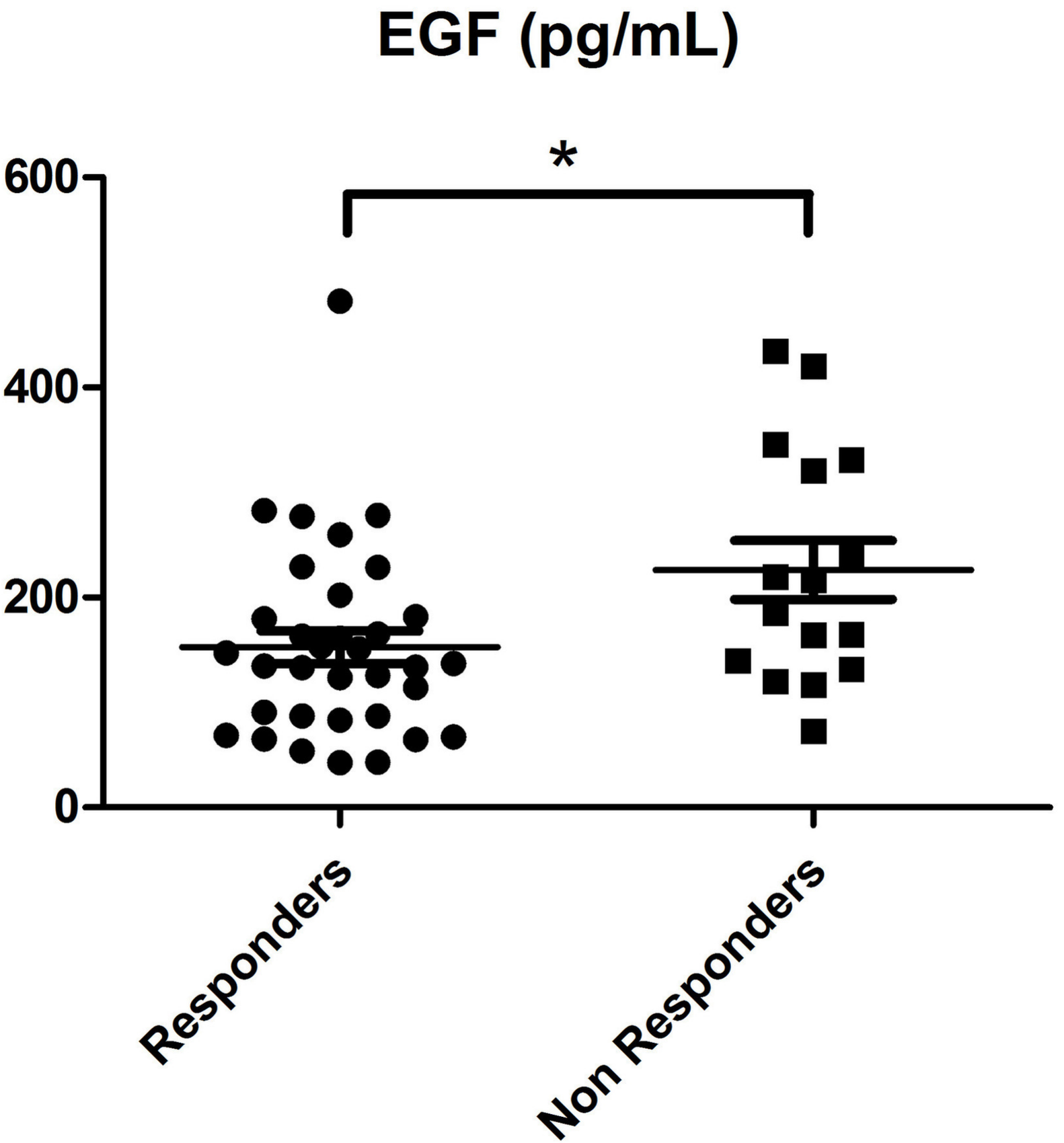
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