BMJ Open Ophthalmology

Hypochlorous acid hygiene solution in patients affected by blepharitis: a prospective randomised study

Rita Mencucci ,[1] Alberto Morelli,[1] Eleonora Favuzza,[1] Angelo Galano,[2] Anna Maria Roszkowska,[3] Michela Cennamo[1]

To cite: Mencucci R, Morelli A, Favuzza E, *et al*. Hypochlorous acid hygiene solution in patients affected by blepharitis: a prospective randomised study. *BMJ Open Ophthalmology* 2023;**8**:e001209. doi:10.1136/bmjophth-2022-001209

Received 20 November 2022
Accepted 2 September 2023

[1]Department of Neurosciences, Psychology, Pharmacology and Child Health, University of Florence, Florence, Italy
[2]Clinical Microbiology and Virology Unit, Careggi Hospital, University of Florence, Florence, Italy
[3]Ophthalmology Clinic, Department of Biomedical Sciences, University Hospital of Messina, Messina, Italy

Correspondence to
Dr Rita Mencucci; rita.mencucci@unifi.it

ABSTRACT

Background/aims To investigate the clinical outcomes and antimicrobial activity of an hypochlorous acid hygiene solution compared with hyaluronic acid wipes for blepharitis treatment in patients with dry eye disease (DED).

Methods This study involved 48 eyes of 48 patients affected by blepharitis with mild to moderate DED. 24 patients were treated with a hypochlorous acid hygiene solution (HOCL group) and 24 patients were treated with hyaluronic acid wipes (HYAL group) for a period of 4 weeks. The following clinical outcomes were assessed before (V0) and after the treatment period (V1): non-invasive keratograph break up time (NIK-BUT), tear film BUT (TF-BUT) tear meniscus height (TMH), Keratograph meibography, Meibomian Gland Yield Secretion Score (MGYSS), Corneal Staining Score (CSS), Schirmer test I, Keratograph conjunctival redness score and Ocular Surface Disease Index (OSDI). Moreover, microbiological analysis of upper and lower eyelid margins was performed at V0 both before and 5 min after treatment.

Results After 1-month NIK-BUT and TF-BUT significantly increased in HOCL group, while they did not show a statistically significant difference in HYAL group compared with baseline. OSDI, TMH and MGYSS showed a significant difference in both groups, while Schirmer test, meibography, CSS and conjunctival redness score did not significantly change in both groups. Bacterial load showed a significant reduction in both groups, more pronounced in HOCL group compared with HYAL group.

Conclusions Hypochlorous acid hygiene solution can be securely employed in blepharitis treatment considering the satisfying clinical outcomes and antimicrobial activity compared with hyaluronic acid wipes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Blepharitis is a common chronic condition, which may be associated with dry eye disease and alteration of the resident microbiota. Hypochlorous acid may be considered a new therapeutic approach showing promising results in reducing local microbial load.

WHAT THIS STUDY ADDS

⇒ This study suggests that hypochlorous acid applied onto sterile wipes may represent a valid therapeutic option in blepharitis because of the clinical and antimicrobial efficacy compared with the standard hyaluronic acid wipes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Future larger multicentric study may investigate the efficacy of different antiseptic drugs exploring their use in the several fields of anterior segment conditions.

INTRODUCTION

Blepharitis is a common inflammatory condition of the eyelid margin, which may be associated with ocular surface disease and impairment of quality of life.[1]

The inflammatory process can involve both the anterior and posterior margins; anterior blepharitis affects the eyelid skin, base of the lashes and eyelash follicles, while in posterior blepharitis there is a dysfunction of the meibomian glands located on the posterior eyelid margin.[2] Meibomian gland dysfunction (MGD) is a cause of tear film (TF) instability and an important risk factor in dry eye disease (DED). The aetiology of blepharitis is not completely determined yet, it is multifactorial and may be infectious or non-infectious: primary blepharitis has been used to encompass rosacea, seborrhoea and hypersensitivity caused by staphylococcal toxins; secondary blepharitis refers to infectious processes, overcolonisation of eyelid bacteria or virus, or infestation by phthiriasis or Demodex.[1 3] The most frequent infectious causative agents isolated in blepharitis are represented by Gram-positive bacteria such as *Staphylococcus aureus*, coagulase-negative *Staphylococcus (CoNS)*, lipophilic *Corynebacterium* spp and *Propionibacterium acnes*. Conversely, anaerobes and Gram-negative bacteria were isolated in smaller quantities on the lids of patients with blepharitis as compared with the control patients and include *Pseudomonas*, *Proteus* and *Neisseria*.[2] Bacterial enzymes and toxin release (lipase, cholesterol esterase and different lipid acids) alter the secretion of

the meibomian gland, disrupt TF homoeostasis through the degradation of lipid layer, favouring in turn bacterial growth and causing eyelid inflammation and keratitis.[4] If untreated, blepharitis and MGD can lead to chronic inflammation of the meibomian glands that can be resulted in complications such as marginal ulceration of cornea, conjunctival or corneal phlyctenulosis. Blepharitis and MGD are notoriously difficult to manage due to the lack of an evidence-based therapeutic approach that is simple and efficacious. Treatment goals in blepharitis are to decrease symptoms, the number of bacteria in eyelid margins and inflammation as well as to improve the function of meibomian glands. The therapeutic options range from eyelid hygiene with hyaluronic acid wipes or hyperthermic lid compress, lid margin massage with lash scrubs to the use of anti-inflammatory and antibacterial agents such as erythromycin, tobramycin, cyclosporine, local dexamethasone and oral doxycycline.[5] The use of antiseptics such as hypochlorous acid may be considered a new therapeutic approach for the treatment of blepharitis and this field of application has recently shown promising results in reducing local microbial load.[6 7]

This is the first study that aims to compare the efficacy of lid hygiene performed with eyelid wipes with hyaluronic acid (Lidcure, Medivis, Catania, Italy) and with 0.01% hypochlorous acid (Septavis, Medivis, Catania, Italy) in blepharitis patients by the clinical assessment of ocular surface characteristics, TF parameters, symptoms and by the quantification of ocular skin flora.

METHODS

This prospective randomised controlled study was conducted at Careggi Eye Hospital in Florence and included patients affected by blepharitis with mild to moderate DED. Patients were randomly enrolled in two groups depending on the treatment received: the first group (HOCL group) was treated with an hypochlorous acid hygiene solution applied onto sterile wipes and gently massaged onto the periocular skin of the closed superior and inferior eyelids for 1 min two times per day, while the second group (HYAL group) was treated with hyaluronic acid wipes used to gently clean away debris from the eyelid and eye lashes twice a day. The treatment period was 4 weeks for both groups.

Inclusion and exclusion criteria

Inclusion criteria comprised (1) age more than 18 years and (2) presence of clinical findings consistent with blepharitis or meibomian gland disfunction (MGD) on slit lamp examination (eg, eyelash crusting, eyelid margin/eyelash abnormalities or meibomian gland capping) and evidence of mild to moderate DED according to the following parameters: Ocular Surface Disease Index (OSDI) questionnaire score ≥13 and ≤32,[8] TF-Break up Time (TF-BUT) ≥5 and ≤10 s and Schirmer test I>5 mm and <15 mm.

Exclusion criteria comprised (1) documented history of major systemic and dermatological conditions (diabetes mellitus, rheumatism, immune diseases and other serious systemic diseases), (2) history of ocular surgery in the previous 3 months, (3) presence of allergies or hypersensitivity to topical medications, cleansing formulations, or shampoos, (4) history of use of isotretinoin within 1 year, cyclosporine-A 0.05% or lifitegrast 5%, (5) presence of active ocular inflammation or history of chronic, recurrent ocular inflammation within the prior 3 months (eg, retinitis, choroiditis, uveitis, iritis, scleritis, episcleritis, keratitis), (6) presence of eyelid abnormalities that affect lid function (entropion, ectropion, tumour oedema, blepharospasm, lagophthalmos, severe trichiasis, severe ptosis), (7) presence of ocular surface abnormalities that compromise corneal integrity (eg, previous chemical burn, recurrent corneal erosion, corneal epithelial defect, corneal dystrophy) and (8) pregnant or lactating patients.

Patients were not allowed to use topical or systemic antibiotics, topical ocular nonsteroidal anti-inflammatories and topical ocular glaucoma medications during the study.

Clinical parameters

Each patient underwent two visits: at baseline (V0) and after the 4-week treatment period (V1). During both visits the following clinical outcomes were assessed: OSDI questionnaire, TF-BUT, Corneal Staining Score (CSS) using the Oxford grading scale, Schirmer test performed without anaesthesia, best spectacle-corrected visual acuity (BSCVA) and intraocular pressure (IOP) measured by applanation tonometry (Goldmann applanation tonometer, Haag Streit, Bern, Switzerland). Meibomian Gland Yield Secretion Score (MGYSS) was assessed on the following: grade 0, clear; grade 1, cloudy expressed with gentle pressure; grade 2, cloudy meibum expressed with moderate pressure; and grade 3, dense meibum toothpaste-like. The upper and lower eyelids of each eye were scored separately with the total score ranging from 0 to 6. During the two visits, a study of the ocular surface was performed with Keratograph 5M (Oculus, Wetzlar, Germany) measuring the conjunctival redness score, the Tear Meniscus Height (TMH) and the Keratograph noninvasive tear BUT (NIKBUT), which is an objective non-contact measure of the time between a blink and the interruption of the rings reflected on the ocular surface. Finally, an infrared image of the upper and lower eyelid (meibography) was taken and graded according to the Keratograph grading system which takes into account the degree of the tortuosity and the extent of the atrophy of the meibomian glands.

Microbiological analysis

Moreover, to assess its antimicrobial activity of the product, at baseline visit 20 patients (the first 10 patients of HOCL group and the first 10 patients of HYAL group) underwent microbiological specimen collection of the upper and lower eyelids performed before and 5 min after the application of the product. Eyelid flocked swabs

(E-swabs, COPAN, Italy) were collected from each patient and afterwards sent in the provided liquid Amies solution to the laboratory of Microbiology Synlab (Florence, Italy). The identities and clinical details of the patients were masked to the microbiology personnel. Samples were cultured on appropriate agar-based media (chocolate, laked horse blood, chromogenic and Sabourad agar media). All plates were incubated at 37°C (ambient air) for 48 hours, except for chocolate agar medium that was incubated with a 5% CO2 supplement. In addition, three more cultures were carried on for each sample, using a 1:10, 1:100 and 1:1000 elution, respectively, in order to demonstrate the reproducibility of the test. The analyses performed after incubation were both qualitative (presumptive identification of different bacterial/fungal strains) and quantitative with colony-forming units (CFU)/mL for all strains. Each different type of colony was then identified using matrix-assisted laser desorption ionisation-time of flight (Bruker, USA).

Power calculation, statistics and study outcomes

A sample size of 48 patients has been estimated to provide 80% power to detect a significant difference between V0 and V1 treatment arms by using a two-sided t-test for paired data (type I error set to 0.05).

A 2 s change in NIK-BUT has been considered as clinically relevant and, based on previous studies,[9 10] the SD has been assumed 2.2 s. Planning to randomise a total of 48 patients (24 in each group) would allow for a 5% drop-out rate.

Statistical analysis was performed using SPSS software (V.28.0 for Windows; IBM SPSS). Normality of data distribution in both groups was assessed with Shapiro-Wilk test. Student's t-test was used for the interval scale parameters, while Fisher's exact test was used for categorical variables. The level of significance was characterised as p<0.05.

The primary objective of the study was to compare the difference in NIKBUT between V0 and V1 in the treatment groups. The secondary objectives were the changes in OSDI score, Schirmer I test, TF-BUT, TMH, CSS, ocular redness score, MGYSS and meibography measurements along with quantitative results of all colony types of bacteria.

RESULTS

A total of 48 participants (20 males and 28 females) with a median age of 68 years (range 46–79) were enrolled in this study during the period October 2021 to June 2022. No subject withdrew the study during the treatment period. The number of unused wipes was checked after the final visit in order to verify patients' compliance. No severe adverse events were found among participants and the products were well tolerated during the study period.

Clinical data

Demographic data of the two study groups are shown in table 1.

Table 1 Patient demographics

	HOCL group	HYAL group
Age (years)	67.1±6.9 (59–81)	±6.4 (60–85)
Male	10 (41%)	8 (33%)
Female	14 (59%)	16 (66%)
Right eye	11 (45%)	14 (58%)
Left eye	13 (55%)	10 (4%)

HOCL, hypochlorous acid hygiene solution; HYAL, hyaluronic acid wipes.

Table 2 shows the clinical parameters assessed at V0 and V1 in HOCL and HYAL groups. At baseline, the groups were equally matched considering all the clinical parameters were similar between the two groups (all p>0.05).

NIKBUT average was 9.38 s±3.64 s at V0 and 12.12 s±4.21 s at V1 in HOCL group showing a statistically significant difference (p=0.02) while in HYAL group it was 8.50 s±3.39 s at V0 and 10.43 s±3.70 s at V1 without a statistically significant difference (p=0.06). The TF-BUT increased significantly in HOCL group from 5.70 s±1.30 s at V0 to 6.96 s±1.63 s at V1 (p<0.01) while TF-BUT in HYAL group increased not significantly from 5.75 s±1.18 s at V0 to 6.46 s±1.31 s at V1 (p=0.06).

NIKBUT and TF-BUT comparisons between HOCL and HYAL groups at V1 were the following: 12.12 s±4.21 vs 10.43 s ± 3.70 s, p=0.14 and 6.96 s±1.63 s vs 6.46 s±1.31 s, p=0.25, respectively.

TMH values increased significantly in both groups after the treatment period (0.23 mm±0.07 mm at V0 vs 0.31 mm±0.17 mm at V1 in HOCL group and 0.22 mm±0.09 mm at V0 vs 0.29 mm±0.11 mm at V1 in HYAL group, p=0.03 and p=0.04, respectively). OSDI score showed a statistically significant reduction in both groups after treatment period (HOCL group: 27.9±3.6 at V0 vs 23.2±4.1 at V1, p<0.01; HYAL group: 26.54±3.92 at V0 vs 22.29±5.19 at V1, p<0.01). Keratograph meibography did not show a significant decrease in both groups after treatment period for superior eyelid (1.5±0.5 at V0 vs 1.3±0.6 at V1, p=0.24 in HOCL group and 1.33±0.62 at V0 vs 1.17±0.55 at V1, p=0.33 in HYAL group) and inferior eyelid (1.6±0.7 at V0 vs 1.3±0.6 at V1, p=0.20 in HOCL group; 1.31±0.51 at V0 vs 1.25±0.49, p=0.67 in HYAL group). Schirmer test I in HOCL group increased from 8 mm±2 mm at V0 to 9 mm±2 mm at V1 (p=0.15) while in HYAL group it was 9 mm±3 mm at V0 and 10 mm±3 mm at V1 (p=0.18).

CSS did not show a significant reduction in both groups (HOCL group: 1.7±1.3 at V0 and 1.2±1.1 at V1, p=0.20; HYAL group: 1.2±0.9 at V0 and 1.1±1.0 at V1, p=0.78). Keratograph redness score in HOCL group was 2.0±0.6 at V0 vs 1.7±0.6 at V1, while in HYAL group it was 1.8±0.5 at V0 vs 1.7±0.5 at V1 (p=0.10 and p=0.40, respectively). MGYSS was reduced significantly in HOCL group (2.3±0.8 vs at V0 vs 1.5±0.7 at V1, p<0.01) and in HYAL group (2.2±0.5 at V0 vs 1.6±0.6 at V1, p<0.01).

Table 2 Clinical assessments at V0 and V1 in HOCL and HYAL groups

	HOCL group			HYAL group		
	V0	V1	P value	V0	V1	P value
NIKBUT (s)	9.38±3.64 (2.68–16.65)	12.12±4.21 (3.63–19.56)	0.02*	8.50±3.39 (2.32–15.03)	10.43±3.70 (3.89–17.72)	0.06
TF-BUT (s)	5.70±1.30 (5–9)	6.96±1.63 (5–12)	<0.01*	5.75±1.18 (5–8)	6.46±1.31 (5–11)	0.06
TMH (mm)	0.23±0.07 (0.11–0.44)	0.31±0.17 (0.13–0.89)	0.03*	0.22±0.09 (0.12–0.47)	0.29±0.11 (0.15–0.54)	0.04*
MGYSS	2.3±0.8(1–5)	vs 1.5±0.7 (0–3)	<0.01*	2.2±0.5 (2-4)	1.6±0.6 (0–3)	<0.01*
Meibography sup	1.5±0.5 (0.5–2.5)	1.3±0.6 (0.5–2)	0.24	1.33±0.62 (0.0–2.5)	1.17±0.55 (0.5–2.0)	0.33
Meibography inf	1.6±0.7 (1.0–3.0)	1.3±0.6 (0.5–2.5)	0.20	1.31±0.51 (0.5–2.5)	1.25±0.49 (0.5–2.5)	0.67
Schirmer (mm)	8±2 (6-13)	9±2 (4-13)	0.15	9±3 (6-14)	10±3 (2-15)	0.18
CSS	1.7±1.3 (0–3)	1.2±1.1 (0–3)	0.20	1.2±0.9 (0–3)	1.1±1.0 (0–3)	0.78
OSDI	27.9±3.6 (20–32)	23.2±4.1 (15–28)	<0.01*	26.54±3.92 (18–32)	22.29±5.19 (14–31)	<0.01*
Redness	2.0±0.6 (1.0–3.1)	1.7±0.6 (0.9–3.2)	0.10	1.8±0.5 (0.8–2.6)	1.7±0.5 (0.6–2.5)	0.40

Data are shown in mean±SD (range).
*Statistically significant difference (p>0.05).
CSS, Corneal Staining Score; HOCL, hypochlorous acid hygiene solution; HYAL, hyaluronic acid wipes; MGYSS, Meibomian gland yielding secretion score; NIKBUT, non-invasive keratograph break up time; OSDI, Ocular Surface Disease Index; TF-BUT, Tear Film-BUT; TMH, tear meniscus height.

As shown in table 3, safety parameters such as IOP and BSCVA did not show significant differences in both groups after treatment period (all p>0.05).

Microbiological data

A total of 20 patients (10 patients of HOCL group and 10 of HYAL group) underwent microbiological analysis of the eyelid margins at baseline visit. Gram-positive bacteria were found to be the most representative species on the eyelid surface, while only two swabs led to the growth of a Gram-negative (*Klebsiella oxytoca* and *K. pneumoniae*), in addition to the Gram-positives. No fungi were found in all samples.

Among Gram-positive bacteria, CoNS were the most representative species (found in every sample), followed by *Staphylococcus aureus*, *Micrococcus luteus*, *Corynebacterium* spp (*C. accolens*, *C. tuberculostearicum*), *Streptococcus* spp (*S. canis* and *S. agalactiae*) and Bacillus cereus. Of all *CoNS*, *Staphylococcus epidermidis* was the most frequently isolated microrganism, followed by *Staphylococcus hominis, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus warneri, Staphylococcus haemolyticus, Staphylococcus pasteuri.* Table 4 shows the total bacterial species isolated from the eyelids at baseline.

Evaluation of total bacterial load at baseline, associated with all isolated strains, covered a range between 1×10^3 and 1×10^6 CFU/mL. Average pretreatment quantitative levels of total microbial load isolated from the upper and lower eyelids were $1.18\times10^6\pm2.4\times10^5$ CFU in HOCL group and $1.07\times10^6\pm1.95\times10^5$ CFU in HYAL group. Of note, the overall monomicrobism was observed in 35% of patients distributed between the two groups, while polymicrobism of two or three different bacterial species at baseline was observed in the 65% of patients.

Figure 1 shows the efficacy in terms of microbial reduction 5 min after the application of the products. In HYAL group the treatment provided a mild reduction (average 62.1%) of the total bacterial load with a mean post treatment value of $4.06\times10^5\pm7.40\times10^4$ CFU while in HOCL group the treatment provided a further severe lowering of the total microbial count (average 90.3%) with a mean post treatment value of $1.14\times10^5\pm2.31\times10^4$ CFU. In 3 out of 10 patients treated with hypochlorous acid hygiene wipes, a complete eradication (reduction of 100%) of isolated bacteria (*S. epidermidis, S. haemolyticus, S. hominis, B. cereus*) was registered.

DISCUSSION

Blepharitis is a frequent inflammatory condition of the eyelid and represents one of the most important reasons for ophthalmological consult, affecting up to 47% of patients seen in clinical practice.[11 12] No standard of care has been approved for the treatment of blepharitis so far and further efforts are warranted for a better understanding of the etiopathogenetic mechanisms.[13 14]

Antiseptics are gaining interest in different fields in ophthalmology, from the prevention of postsurgical endophthalmitis to the treatment of anterior segment

Table 3 Safety parameters at V0 and V1 in HOCL and HYAL groups

HOCL group				HYAL group		
V0		V1	P value	V0	V1	P value
IOP (mm Hg)	12±2 (9–16)	13±2 (10–16)	0.69	13±2 (10–16)	13±1 (10–16)	0.78
BSCVA	0.03±0.06 (0–0.2)	0.03±0.05 (0–0.2)	1	0.04±0.07 (0–0.02)	0.04±0.08 (0–0.3)	1

Data are shown in mean±SD (range).
*Statistically significant difference (p<0.05).
BSCVA, best spectacle-corrected visual acuity; HOCL, hypochlorous acid hygiene solution; HYAL, hyaluronic acid wipes; IOP, intraocular pressure.

infections.[15] They provide a broad spectrum of efficacy thanks to the absence of induced microbial resistance which is in contrast to the growing concern about antibiotic resistance and the selection of multidrug resistant bacteria in ophthalmology.[16] Hypochlorous acid is an oxidising agent that shows its antimicrobial activity penetrating the cell wall and inhibiting DNA and protein synthesis by oxidation of thiol-containing proteins and enzymes.[17] Bitton *et al* recently performed an in vitro evaluation of 0.01% hypochlorous acid that showed to be effective in reducing *Staphylococcus* spp and *Pseudomonas aeruginosa* on bacterial isolates from patients with blepharitis and keratitis, maintaining low concentrations of *S. epidermidis* (part of the normal skin flora) with selective bactericidal activity.[7] A previous clinical study on the effect of hypochlorous acid through ultrasonic atomisation for Demodex blepharitis/MGD-DED demonstrated that pure hypochlorous acid can improve the eradication rate of the Demodex mite by shortening its average survival time.[18]

To the best of our knowledge, this is the first study to investigate the efficacy of two different products for the treatment of blepharitis combining the collection of clinical and antimicrobial parameters. We evaluated the efficacy in vivo of wipes containing 0.01% hypochlorous acid and wipes containing hyaluronic acid in improving clinical parameters and reducing the eyelid bacterial load in patients affected by blepharitis associated with mild to moderate DED.

Our clinical results showed in HOCL group a significant improvement in the following parameter: NIKBUT, BUT, TMH and OSDI score; instead, in HYAL group a significant improvement was observed only in TMH and OSDI score parameters. According to our results, we postulate that the improvement in TF stability seen in HOCL group could be related to the reduction of bacterial load and the subsequent lipolytic exoenzyme activity with the improvement of meibomian secretion which may justify a better symptomatic response.

Microbiological analysis supported our clinical findings, showing a significant microbial reduction in both groups, more pronounced in HOCL group with a mean reduction of 90% compared with the 62% of HYAL group. Both products showed an influence, with no difference among different bacterial strains, suggesting a wide spectrum activity towards inhabitants of the eyelid microbiota. The most representative species were found among Gram+, in particular CoNS and *S. aureus*. The most common Gram bacteria were klebsiella spp and proteus mirabilis. Other studies investigated the bacterial composition of donor conjunctiva in corneal transplantation, showing similar results compared with our baseline microbial assessments since the most common bacteria isolates were CNS and *S. aureus*.[19 20] The antimicrobial activity seen in HYAL group may be due to the mechanical removal of residual debris and the subsequent action in reducing the resident microbial colonies. In HOCL group, the combination of the mechanical activity of the sterile wipe contained in the product and the chemical activity of hypochlorous acid was responsible for the higher rates of bacterial eradication.

The limitations of our study were the involvement of a relatively small number of patients as well as the limited time of follow-up during the treatment period. Other

Table 4 Total bacterial species of the skin below the upper and lower eyelids

Gram positives	Coagulase-negative Staphylococcus (CONS)	Gram negatives
CONS	Epidermidis	*Klebsiella oxytoca*
Staphylococcus aureus	*Staphylococcus hominis*	*Klebsiella pneumoniae*
Micrococcus luteus	Haemolyticus	*Proteus mirabilis*
Corynebacterium Tubercolostearicum	Capitis	
Corynebacterium accolens	Warneri	
Streptococcus canis	Pasteuri	
Streptococcus agalactiae	Simulans	

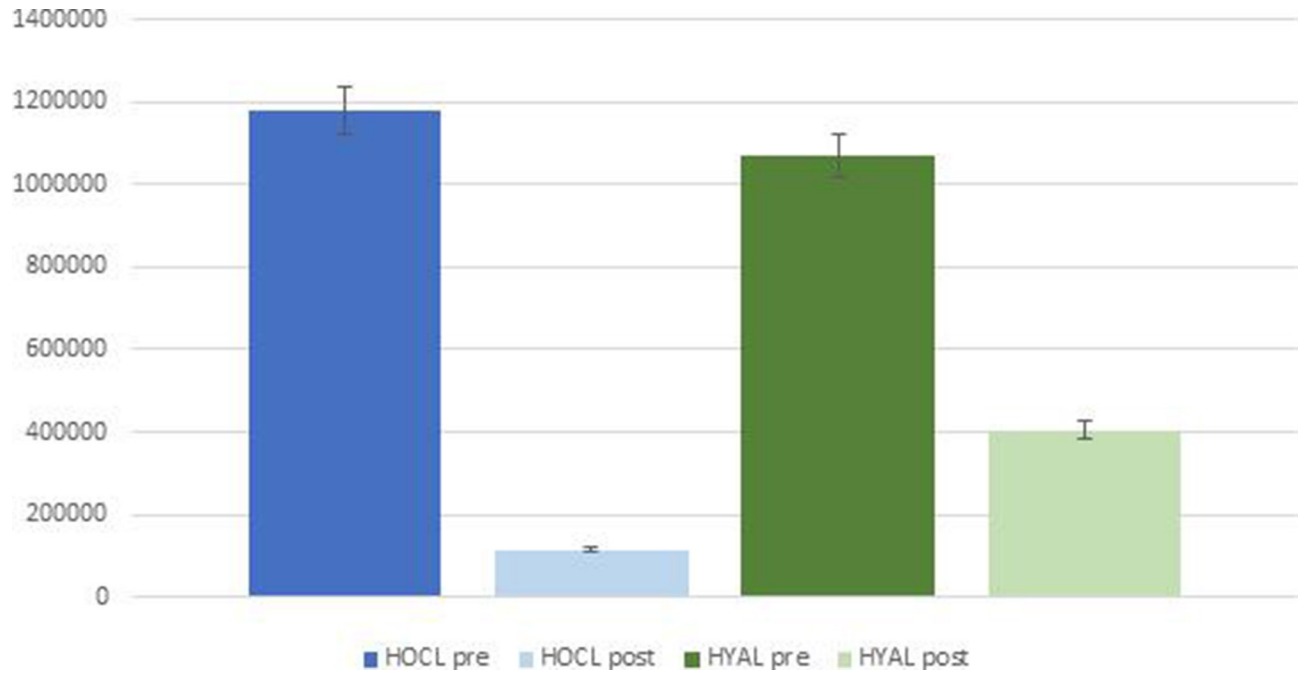

Figure 1 Average total CFU on the eyelid surface in HOCL group at baseline (HOCL pre), 5 min after the application of hypochlorous acid solution (HOCL post), in HYAL group at baseline (HYAL pre) and 5 min after the application of hyaluronic acid wipes (HYAL post). CFU, colony-forming unit.

limitations of our study were the lack of the follow-up after the discontinuation of the treatment to verify the long-term efficacy of hypochlorous acid in chronic blepharitis, and the absence of analysis of tear inflammatory molecules levels that might be correlated with blepharitis symptoms. Further larger multicentric studies are warranted to assess the best treatment options for blepharitis.

According to our findings, wipes containing hypochlorous acid can be safely used in blepharitis considering the satisfying clinical and microbiological results along with the absence of adverse effects seen in our study. Wipes containing only hyaluronic acid seem to be less effective despite a similar safety profile.

In conclusion, products containing antiseptic agents, such as hypochlorous acid, can be considered a valid option in the treatment of blepharitis associated with DED. Furthermore, the antimicrobial activity showed by hypochlorous acid may extend its employment in the field of prophylaxis of several ocular procedures.

Contributors RM, AM, MC, AG, AMR and EF equally contributed to design, acquisition of data, analysis and interpretation of data and manuscript preparation. RM wasresponsible for the overall content as guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by Local Ethics Committee (University of Florence, Area Vasta Centro Ethics Committee, protocol number: 19220_spe). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iD

Rita Mencucci http://orcid.org/0000-0003-1856-6363

REFERENCES

1 Gostimir M, Allen LH. Is there enough evidence for the routine recommendation of eyelid Wipes? a systematic review of the role of eyelid Wipes in the management of Blepharitis. *Can J Ophthalmol* 2020;55:424–36.
2 Stroman DW, Mintun K, Epstein AB, *et al*. Reduction in bacterial load using Hypochlorous acid hygiene solution on ocular skin. *Clin Ophthalmol* 2017;11:707–14.
3 Putnam CM. Diagnosis and management of Blepharitis: an Optometrist's perspective. *Clin Optom* 2016;8:71–8.
4 Sung J, Wang MTM, Lee SH, *et al*. Randomized double-masked trial of eyelid cleansing treatments for Blepharitis. *The Ocular Surface* 2018;16:77–83.
5 Sabeti S, Kheirkhah A, Yin J, *et al*. Management of Meibomian gland dysfunction: a review. *Survey of Ophthalmology* 2020;65:205–17.
6 Romanowski EG, Stella NA, Yates KA, *et al*. In vitro evaluation of a hypochlorous acid hygiene solution on established Biofilms. *Eye Contact Lens* 2018;44:S187–91.
7 Bitton E, Ngo W, Dupont P. Eyelid hygiene products: A Scoping review. *Contact Lens and Anterior Eye* 2019;42:591–7.
8 Schiffman RM, Christianson MD, Jacobsen G, *et al*. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol* 2000;118:615–21.
9 Fogagnolo P, Favuzza E, Marchina D, *et al*. New therapeutic strategy and innovative lubricating ophthalmic solution in minimizing dry eye disease associated with cataract surgery: a randomized, prospective study. *Adv Ther* 2020;37:1664–74.
10 Simmons PA, Carlisle-Wilcox C, Vehige JG. Comparison of novel lipid-based eye drops with aqueous eye drops for dry

eye: a multicenter, randomized controlled trial. *Clin Ophthalmol* 2015;9:657–64.
11 Amescua G, Akpek EK, Farid M, *et al*. Blepharitis preferred practice pattern®. *Ophthalmology* 2019;126:56–93.
12 Nelson JD, Shimazaki J, Benitez-del-Castillo JM, *et al*. The International workshop on Meibomian gland dysfunction: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci* 2011;52:1930–7.
13 Lindsley K, Matsumura S, Hatef E, *et al*. Interventions for chronic Blepharitis. *Cochrane Database Syst Rev* 2012;2012:CD005556.
14 Duncan K, Jeng BH. Medical management of Blepharitis. *Curr Opin Ophthalmol* 2015;26:289–94.
15 Kanclerz P, Myers WG. Potential substitutes for Povidone-iodine in ocular surgery. *Eye* 2021;35:2657–9.
16 Thomas RK, Melton R, Vollmer PM, *et al*. In vitro antibiotic resistance among bacteria from the Cornea in the antibiotic resistance monitoring in ocular microorganisms surveillance study. *Optom Vis Sci* 2021;98:1113–21.
17 Kiamco MM, Zmuda HM, Mohamed A, *et al*. Hypochlorous-acid-generating electrochemical scaffold for treatment of wound Biofilms. *Sci Rep* 2019;9:2683.
18 Li Z, Wang H, Liang M, *et al*. Hypochlorous acid can be the novel option for the meibomian gland dysfunction dry eye through ultrasonic atomization. *Dis Markers* 2022:8631038.
19 Okumura Y, Inomata T, Miyamoto S, *et al*. Donor characteristics and risk factors for Methicillin-resistant *Staphylococcus aureus* contamination in storage medium for corneal transplantation: a 10-year retrospective study. *Transpl Infect Dis* 2019;21:e13123.
20 Inomata T, Ono K, Matsuba T, *et al*. Pre-banking microbial contamination of donor conjunctiva and storage medium for penetrating Keratoplasty. *Jpn J Ophthalmol* 2017;61:369–77.