# Tear MMP-9 Levels as a Marker of Ocular Surface Inflammation in Conjunctivochalasis

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**Purpose.** To evaluate the efficacy of surgical treatment for conjunctivochalasis by monitoring matrix metalloproteinase (MMP)-9 levels in the tears of patients with conjunctivochalasis before and after surgery and their correlation with clinical signs and symptoms.

**Methods.** Twelve eyes of patients with symptomatic conjunctivochalasis were included in this study as well as five eyes of healthy volunteers. Ocular surface inflammation was measured in terms of the concentration of pro–MMP-9 in tears, by enzyme-linked immunosorbent assay and zymography. Tear analysis was performed before and 1 month after surgery. The surgical technique consisted of the excision of redundant tissue and the use of organic glue for wound closure.

**Results.** The concentration of pro–MMP-9 was significantly higher in the conjunctivochalasis eyes than in the healthy controls (223.4 ± 74.53 ng/mL vs. 20.32 ± 5.21 ng/mL; $P < 0.001$). Tear pro–MMP-9 levels decreased significantly after conjunctival resection in patients with conjunctivochalasis without dry eye compared with patients with conjunctivochalasis and dry eye associated. Zymographic analysis indicated that MMP-9 is present in its active form only in conjunctivochalasis tears. After a follow-up of 4.9 ± 1.3 weeks, all operated eyes were found to have recovered a smooth and stable conjunctival surface, epithelial defects had improved, and epiphora had been resolved in 89% of cases.

**Conclusions.** Our results indicate that inflammation is likely to play a relevant role in the pathogenesis of conjunctivochalasis. Appropriate surgery decreases inflammatory activity, leading to symptom improvement, and tear analysis may facilitate the treatment of the ocular surface.

Keywords: inflammation, conjunctiva, MMP-9, tear, conjunctivochalasis

Conjunctivochalasis (CCh) is defined as a redundant, nonedematous conjunctiva, typically located below the bulbar conjunctiva interposed between the eyeball and the lower eyelid. Clinically, conjunctival folds, which generally affect elderly people, are a frequent cause of chronic ocular discomfort, such as irritation, epiphora, dryness, and blurred vision. Information regarding the pathogenesis of CCh is scanty and conflicting.[1]

Hughes[2] considers the formation of conjunctival folds to be a senile change. This involves the loss of subconjunctival connective tissue, leading to a loss of adherence of the bulbar conjunctiva to the sclera and a more superficial inferior fornix. Abnormalities in extracellular conjunctival components, such as degeneration of elastic fibers or increased collagenolytic activity, have also been reported. In contrast, Meller et al.[1,3] and Li et al.[4] have suggested that elastotic degeneration could be exacerbated by the presence of inflammation at the ocular surface. They report that inflammatory cytokines such as IL-1β and TNF-α in tear can in principle be derived from the cornea, and may be responsible for the increased levels of MMPs in cultured fibroblasts, derived from CCh patients. Tumor necrosis factor α and other interleukins, such as IL-1 and IL-6, are liberated immediately after an aggression to the eye and are considered to be "pro-inflammatory." Pflugfelder et al.[5] have reported an increase in the levels of IL-1α and IL-1β and coexpression with MMP-9 in the tears of patients with Sjögren's syndrome. These molecules are interrelated since IL-1β, a potent inductor of inflammation at the ocular surface, is activated extracellularly by proteases such as trypsin, plasmin, elastase, cathepsin-9, and MMP-9. Of all these proteins, MMP-9 is the enzyme that is the quickest and most effective in transforming IL-1β from its inactive to its active form. These findings suggest that both IL-1 and MMP-9 are implicated in inflammatory processes on the ocular surface, in such a way that a cytokine-mediated inflammation cycle is produced.[6] In an earlier study of our group, we have found that some proteins are overexpressed in tear from CCh patients compared with controls; these include MMP-9, IL-1β, and IL-6, which are all involved in inflammation, degradation, and remodeling processes.[7]

Conjunctivochalasis is characterized by a redundant bulbar conjunctiva and delayed tear clearance. This delayed clearance may be a consequence of a tamponade effect of the conjunctival folds on the lacrimal punctum, an obstruction at the level of the tear meniscus, a concomitant lid laxity, or a combination of all these factors.[8] Jordan and Pelletier,[9] using a

dye clearance test, have observed fluorescein retention in a patient with CCh, suggesting the presence of a functional blockade of tear clearance. We have studied the effect of removing the redundant conjunctiva and re-establishing tear clearance and its effects in the concentration of pro-inflammatory enzymes in tears. The accumulation of extracellular matrix–degrading enzymes in tears may contribute to the pathogenesis of this disease, since MMPs are a family of enzymes that participate in tissue remodeling[10–12] and, pathologically, they cause disruption or disintegration of extracellular matrices.[13–16] In the cornea, MMP-9 is the primary matrix-degrading enzyme produced by basal corneal epithelial cells[17] and neutrophils[18] and is known to degrade the major components of the epithelial basement membrane, such as collagen type IV[19] and VII,[20] and to impede re-epithelialization of the cornea.[21] This enzyme has been implicated in the early phase pathology of some diseases such as peripheral ulcerative keratitis,[22,23] corneal erosions,[24] rosacea,[25] pterygium,[26] and keratoconus.[27] Since tear film MMP content and clinical evidence of pathology progression have been reported to be correlated, it has been suggested that the zymographic visualization of these enzymes in tears could be reliably used to monitor disease activity.[28] Zymography is a frequently used technique, since most MMPs are secreted as inactive zymogens (pro-MMPs) that require extracellular matrix components for their activation.[29]

This study was designed to evaluate the efficacy of surgical treatment for this disease, by monitoring pro–MMP-9 levels in the tears as a marker related with inflammation and degradation of connective tissue before and after surgery and the correlation of these levels with clinical signs and symptoms.

## Methods

### Study Design

The present work was planned as two parallel studies. In the first study, we performed a longitudinal, prospective study to evaluate the response of the ocular surface to the surgical treatment for CCh, by monitoring pro–MMP-9 levels in the tears as a marker related with inflammation and degradation of connective tissue before and after surgery. The second was a case-control study to compare the levels of pro–MMP-9 in tears from healthy subjects with those from CCh patients before surgery in order to characterize the levels of pro–MMP-9 in normal tears.

### Patients

A total of 17 eyes from 17 patients were enrolled:12 patients (7 women and 5 men) with a mean age of 62.12 ± 14.08 years and diagnosis of CCh who were to undergo conjunctival resection surgery and 5 healthy volunteers (3 women and 2 men), with a mean age of 52.2 ± 24.1 years. This research was performed by medically qualified personnel after approval by the Instituto Clinico-Quirurgico de Oftalmologia Ethical Committee and in strict accordance with the tenets of the Declaration of Helsinki. Patients were recruited from the Cornea and Ocular Surface Unit of the Instituto Clinico-Quirurgico de Oftalmologia, Bilbao, Spain. Informed consent was obtained from all patients after the nature and possible consequences of the study had been explained. Tear samples were obtained from 1 eye from patients and volunteers. The patients included in this study were patients with symptomatic CCh who, upon not responding to pharmacologic treatment (topical steroids), were subjected to surgery (conjunctival exeresis).

The tear samples were taken before surgery and 1 month after surgery after at least 1 week without topical therapy. Tear sampling was carried out in the same manner pre and post surgery.

Clinical tests were not performed on the same day as tear collection, in order not to interfere with tear composition. Diagnosis was based on Lissamine green dye–assisted slit-lamp examination and the Yokoi classification was used to grade CCh severity.[30] The chalasia was higher than the height of the tear meniscus and apparent without forced blinking. The patients complained of eye symptoms such as foreign body sensation, epiphora, pain, or irritation.

Exclusion criteria included the presence or history of any systemic or ocular disorder or condition (including ocular surgery, trauma, and disease) that could possibly interfere with the interpretation of the results. Current or recent use of topical ophthalmic or systemic medications that could affect the pathologic condition was also considered as an exclusion criterion. Similarly, subjects who wore contact lenses or who had lid congruity disorders, meibomian gland disease, blepharitis, a history of recent ocular surgery, systemic or topical drug use, or other systemic or ocular diseases with known association with ocular surface disease were also excluded from the study. However, patients diagnosed with aqueous-deficient dry eye were included in this study. They were analyzed in two different groups: patients with CCh and Schirmer test values $> 5$ mm and patients with CCh and Schirmer test values $< 5$ mm. The reason for not excluding this group was to understand the implication of dry eye in CCh, particularly when determining the most efficacious treatment for patients and to examine the involvement of the relaxation of the conjunctiva in the tear.

The healthy volunteers were subjected to an ocular surface examination to ensure that pathologies associated with the ocular surface were not present (absence of allergic or atopic history).

### Conjunctival Staining

One drop of 1% Lissamine green dye was instilled into the conjunctival sac. The patient was instructed to blink several times for a few seconds to ensure adequate mixing of the dye. Conjunctival folds were then emphasized, allowing the scoring of the CCh stage from 0 to 3 according to the Yokoi classification. Lissamine also stains the mucin-free epithelium but these observations were not used in this study.

### Schirmer Test

A standard Schirmer I test with topical anesthesia was performed. A sterilized strip of Schirmer-Plus Gecis (Neung sur Beuvron, France) was placed in the lateral canthus away from the cornea and left in place for 5 minutes. Readings were recorded in millimeters of wetting after 5 minutes.

### Tear Samples

For all experiments, tears were collected from the inferior lateral tear meniscus, minimizing irritation to the ocular surface or lid margin. Anesthetic drops were not instilled. We obtained tear samples (range, 2–5 μL) by using a microcapillary tube (intraMark; Blaubrand, Wertheim, Germany). After collection, the tear was introduced into a 0.5-mL tube (Eppendorf, Fremont, CA) and placed on ice. Samples were next centrifuged at 1145*g* for 20 minutes at 4°C to remove cellular debris. The supernatants were collected and stored in

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siliconized polypropylene microcentrifuge tubes (Eppendorf) at −80°C until analysis.

### Total Protein Content

Total protein concentration in each tear sample was estimated by means of the EZQ Protein Quantitation Kit (Molecular Probes, Carlsbad, CA) using bovine serum albumin as the standard. Briefly, 1 μL tear sample diluted in USD buffer (6 M urea, 10% sodium dodecyl sulfate [SDS], and 1 mM dithiothreitol) was applied to the assay paper. Signal intensity was recorded at 618 nm.

### Surgery

The present study was conducted with 12 CCh patients. Presurgical subjective symptoms included irritation, epiphora, and foreign body sensation. The inflammatory activity associated with the degrading of the extracellular matrix, which takes place in the bulbar conjunctiva, was evaluated by measuring the concentration of pro–MMP-9 in tear before and after surgery. Surgery involved the excision of the redundant conjunctiva by means of a modified version of the technique reported by Yokoi et al.[31]: the redundant conjunctiva was resected in the form of a half-moon, with two lateral discharge triangles in the nasal and the temporal sectors, in order to achieve a smoother conjunctival surface. For wound closure, we used an organic adhesive (Tisseel; Baxter, Deerfield, IL).

### MMP-9 Analysis

The concentration of total pro–MMP-9 (92 kDa) was determined by using a sandwich enzyme-linked immunosorbent assay (ELISA) with an MMP-9 ELISA development kit (Calbiochem, Darmstadt, Germany). This kit measures the concentration of enzyme in its inactive form (pro-enzyme), which is present in the tear. This inactive form is synthesized in the corneal epithelium and it is subsequently activated in tear, depending on the presence or absence of the corresponding activators or inhibitors (tissue inhibitors of metalloproteinases [TIMPs]). The assay was carried out in accordance with the instructions of the manufacturer.

### Zymography

This chromatographic technique is used for the detection of proteases. The protein that acts as a substrate of the proteases whose activity is to be measured is loaded into a polyacrylamide gel. Upon staining the gel with Coomassie Blue R-250 (Sigma-Aldrich, St. Louis, MO), unstained bands can be observed owing to the degradation of the corresponding proteins by the proteases. MMP-9 gelatinolytic activity was determined by using gelatin zymography.

Ten micrograms of a pool of patients tears was added to a 5 μL SDS sample buffer (2X Novex Tris-glycine; Invitrogen, Carlsbad, CA) and the volume was adjusted to 12 μL with ultrapure laboratory-grade water (MilliQ; Millipore R&D Systems, Billerica, MA). An MMP-9 standard (50 ng/mL) (Millipore R&D Systems) was activated by incubating with 1 mM 4-aminophenyl-mercuric acetate (Sigma-Aldrich) at 37°C for 1 hour. A 1 μL sample of this was then added to 2X sample buffer (5 μL) and topped up to a volume of 12 μL with ultrapure laboratory-grade water (MilliQ). This was incubated for 30 minutes at room temperature. Each sample was resolved by using 10% zymography gelatin gel (Novex) under denaturing, but nonreducing conditions. Gels were subjected to electrophoresis at a constant voltage (120 V) for 3.5 hours in running buffer (glycine, Tris base, and 1% SDS) at 4°C. The gels were washed with 2.5% (vol/vol) Triton X-100 (Astral Scientific, Amresco, Solon, OH) for 1 hour, since proteolytic activity can be reversibly inhibited by SDS during electrophoresis and recovered by incubating the gel in aqueous Triton X-100. This was then decanted and the gels were equilibrated with developing buffer (Novex Zymogram Developing Buffer) for 30 minutes. The gels were then transferred to fresh developing buffer and incubated overnight at 37°C for 16 to 20 hours. Proteins were detected by staining with Coomassie Blue R-250 (manufacturer name, city, state or country) for a minimum of 2 hours and destaining in 30% (vol/vol) ethanol, 10% (vol/vol) acetic acid. Disodium ethylenediaminetetraacetic acid, which is known to inhibit MMPs, was added to a separate renaturing and developing buffer. We used a pool of tears from healthy volunteers to ascertain whether MMP-9 (active form) was also present in healthy tears.

### Statistical Analysis

Statistical analyses were performed with SPSS 19.0 (SPSS Sciences, Chicago, IL) commercial software. Data were expressed as mean ± SD. The normality of data was verified with the Kolmogorov-Smirnov test. Statistical comparisons of tear pro–MMP-9 levels between CCh subjects and normal control subjects were performed by using the Mann-Whitney *U* test. The Wilcoxon test was used to compare presurgical and postsurgical levels of pro–MMP-9 in tears from CCh patients ($P < 0.05$ was considered to be statistically significant). Correlations between tear pro–MMP-9 levels and conjunctival folding scores were determined by Spearman correlation ($P < 0.01$).

## Results

After a follow-up of 4.9 ± 1.3 weeks, 12 eyes were found to have an improvement, characterized by a smooth and stable conjunctival surface and the absence of corneal epithelial damage. Epiphora was resolved in 89% of eyes that previously presented with this feature. In all patients significant symptom relief was obtained for heaviness (100%) and pain (100%), blurred or reduced vision in 10 of the 12 patients (83.33%), redness in 9 patients (75%), burning in 8 (66.6%) patients, and foreign body sensation in 7 patients (58.33%).

The mean conjunctival folding scores were 2.67 and 0.49 in the CCh patients (before surgery, Fig. 1A) and control subjects, respectively. Statistically significant differences were found in the staining of the inferior conjunctival bulb of CCh patients before surgery versus healthy controls ($P = 0.002$). After the surgical removal of the redundant conjunctival folds, staining values were similar for both groups (0.25 ± 0.45) (Fig. 1B).

The mean Schirmer values were 10.75 ± 1.03 and 11.4 ± 2.6 mm in the presurgical CCh patients and control volunteers, respectively. Patients who underwent surgery did not show significantly different Schirmer values 1 month after surgery. Four patients presented Schirmer values ≤ 5 mm, which is the criterion to diagnose a patient with aqueous-deficient dry eye. Thus, these patients had both CCh and aqueous-deficient dry eye (DE) diseases. Since it is important to understand what is happening at the ocular surface and in the tear in particular in this type of patient (these two pathologies often present together), we decided not to eliminate these patients from the study and separate them into two different groups (CCh and CCh+DE). The values of Lissamine green staining and Schirmer test of these groups and control group are shown in Table 1.

Pro–MMP-9 levels were analyzed in the tears of CCh patients with Schirmer values > 5 mm (CCh), in CCh patients with Schirmer values ≤ 5 mm (CCh+DE) and compared with

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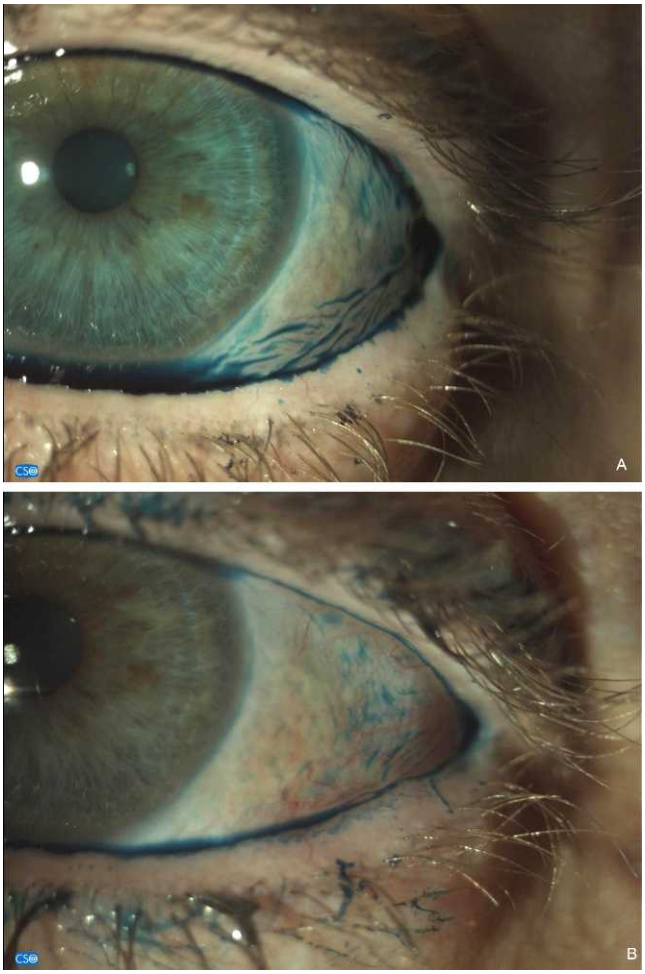

FIGURE 1. Conjunctivochalasis. (**A**) Before surgery, abundant folds were highlighted by the Lissamine green dye. The tear meniscus is significantly disrupted. (**B**) After surgery, both conjunctival smoothness and the tear meniscus have been restored.

healthy subjects (Ct) before surgery. Pro–MMP-9 levels were significantly higher (11-fold) in the eyes with CCh (CCh + CCh+DE) than in the normal eyes (Ct) (223.4 ± 74.53 ng/mL vs. 20.32 ± 5.21 ng/mL; $P < 0.001$).

Tear samples were taken from all patients before and 1 month after the surgery and levels of pro–MMP-9 were quantified. Pro–MMP-9 levels were much higher in CCh patients before surgery than afterwards (Table 2). In the CCh group the levels dropped 5.31 times after surgery (207.74 ± 74.89 ng/mL vs. 39.1 ± 20.6 ng/mL; $P = 0.012$) (Fig. 2), while in CCh+DE group the difference was less (2.33-fold) in the levels of pro–MMP-9 after surgery (254.55 ± 73.7 vs. 109.05 ± 5.27 ng/mL, $P = 0.068$). In this group no statistically significant difference was observed before and after surgery, suggesting an inflammation related to the dry eye. The height of the folds and

TABLE 1. Tear Volume and Ocular Surface Findings in Patients With CCh and Healthy Control Subjects

| Group | Subjects, *n* | Conjunctival Staining | Schirmer I Test, mm |
|---|---|---|---|
| CCh | 8 | 2.75 ± 0.46 | 10.75 ± 1.03 |
| CCh+DE | 4 | 2.7 ± 0.5 | 3.25 ± 0.95 |
| Ct | 5 | 0.4 ± 0.54 | 11.4 ± 2.6 |

TABLE 2. Levels of Pro–MMP-9 (ng/mL) in Tears From CCh Patients Before and After Surgery and From Healthy Subjects (Controls)

| Group | Pro–MMP-9 Presurgery, ng/mL | Pro-MMP-9 Postsurgery, ng/mL |
|---|---|---|
| CCh | 207.74 ± 74.89 | 39.1 ± 20.6 |
| CCh+DE | 254.55 ± 73.7 | 109.05 ± 5.27 |
| Ct | 20.32 ± 5.21 | N/A |

N/A, not applicable.

the concentration of protein present in tears were found to be positively correlated ($P = 0.002$).

To determine whether activated MMP-9 was present in the tears of patients with CCh, a selection of tear samples was assayed for gelatinolytic activity. Tear samples from healthy subjects were assayed as control. Of the tear samples assayed, only those that exhibited the highest overall protease and zymographic MMP activities contained activated enzyme and were from patients with CCh. Figure 3 shows a Coomassie Blue–stained gel of tear samples from control subjects and CCh patients. The clear bands against the stained gelatin background are areas of gelatinolytic activity. These bands are consistent with those previously identified and correspond to active forms of MMP-9 and MMP-2. Although we did not quantify MMP-2 levels in tear, zymography showed that this enzyme was present and active in the tears of CCh patients. On the basis of the size of the band, we can infer that its activity is lower, probably because it is present in a lower concentration. Thus, the zymography technique also verified that the MMP-9 enzyme in the tears of CCh patients is indeed present in its active form.

## DISCUSSION

This preliminary study on tears of patients with CCh showed the presence of accumulated molecules in the tears of these cases that did not respond to pharmacologic therapy and underwent surgical treatment consisting of conjunctival resection.

An improvement of symptoms and clinical signs was seen in these patients after the restoration of the tear meniscus as can be observed in Figure 1, evaluated with the use of the Lissamine green dye test. One of the difficulties in ocular surface disease is the lack of clinical evidence of inflammation in situations in which active inflammation is known to be present. Conjunctival resection was indicated in those patients who presented a severe degree of CCh and symptoms that did not respond to pharmacologic therapy. For an objective quantification we decided to measure a marker (MMP-9) that is present in tears and which, on the basis of earlier studies, is known to be altered in this pathology.[7]

Ward et al.[32] have also found increased levels of TNF-α, IL-1β, IL-6, IL-8, and IL-12 in tears of CCh patients. They reported that silent inflammation originating from the vascular endothelium, due to build up of reactive oxygen species and increased oxidative stress status, may initiate NF-κB pathway–mediated inflammation, with elevation of tissue cytokines. Such elevated levels of inflammatory markers measured in tears in this disease condition may result from similar events or may be partly due to mechanical rubbing of the chalatic conjunctiva during blinking and ocular movements, resulting in the release of cytokines by the conjunctival epithelium or by endothelia of the conjunctival vessels into the tear film.

The stimulated production of MMP-9, as well as of cytokines that stimulate MMP-9 production (IL-1, IL-6, TNF-α, and TGF-β1) by the ocular surface epithelium has been confirmed at the

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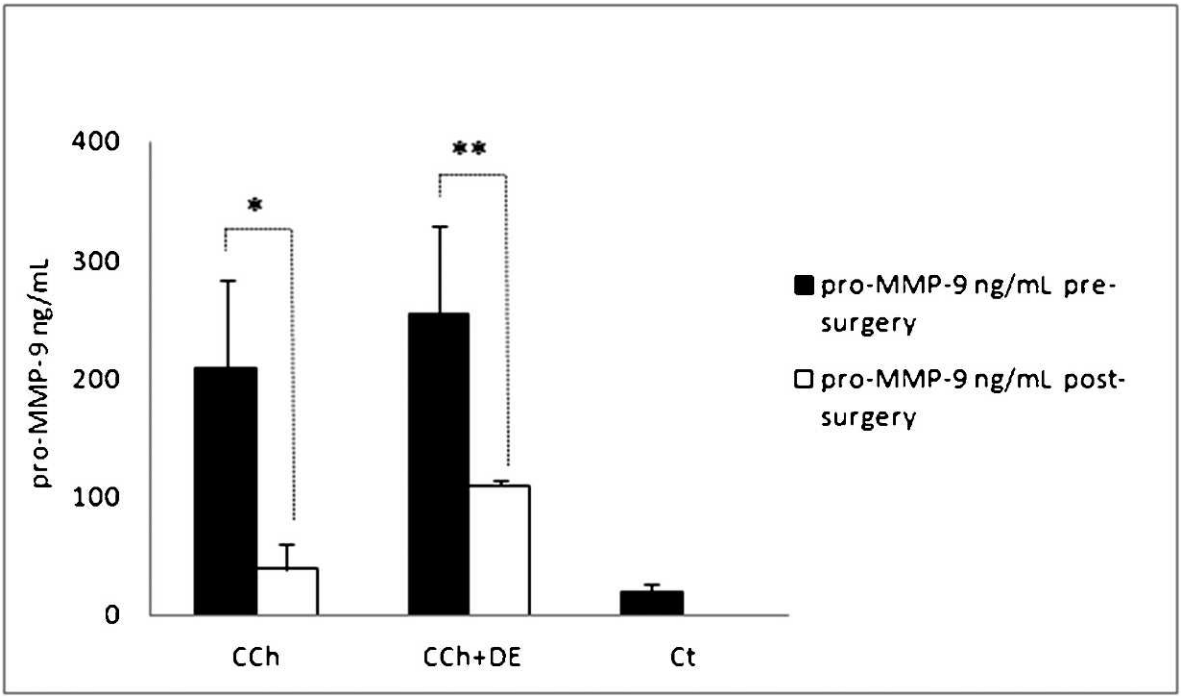


**Figure 2.** The concentration of pro–MMP-9 (ng/mL) in tear samples from conjunctivochalasis (CCh and CCh with dry eye) and healthy control subjects (Ct). *Pro–MMP-9 (ng/mL) levels in the tears of patients with CCh before and 1 month after surgery; $P < 0.012$. **Pro–MMP-9 (ng/mL) levels in the tears of patients with CCh + DE before and 1 month after surgery; $P < 0.068$. In this group no statistically significant difference was observed ($P < 0.05$).

transcriptional level by semiquantitative real-time PCR.[33] Among several proteases, MMP-9 is the most efficient activator of the IL-1β precursor.[34] Therefore, increased MMP-9 activity at the ocular surface could in principle exacerbate inflammation in CCh. Indeed, MMP-9 has been demonstrated to accelerate epithelial regeneration in the cornea by modulating the inflammatory response in the healing cornea.[35] These findings indicate that CCh is capable of initiating an escalating cycle of cytokine and proteinase activity which, if unchecked, can have deleterious consequences for the ocular surface.

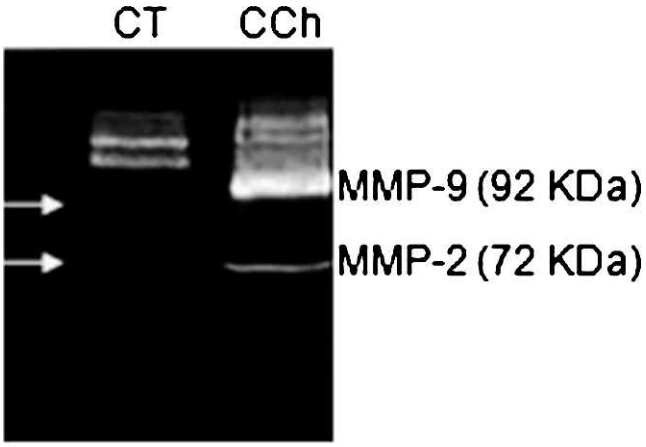


**Figure 3.** A Coomassie Blue–stained gel of tear samples from control subjects (Ct) and CCh patients. The clear bands against the stained gelatin background are areas of gelatinolytic activity. The 92-kDa and 72-kDa bands, indicated with *arrows*, correspond to the active forms of MMP-9 and MMP-2, respectively.

Although tear clearance was not measured in this cohort of patients, delayed tear clearance is a well-known feature of CCh patients, which may be related to interference with tear meniscus circulation and to mechanical obstruction of the puncta by the folds. In any event, tear retention may increase the concentration of pro-inflammatory proteins already present in tears. Indeed, delayed tear clearance has been shown to be associated with increased tear film and ocular surface inflammation.[36] It is still uncertain how increased tear film and ocular surface inflammation may be related to collagenolytic activity, which has been proposed by Meller and Tseng[1] to be one of the central events in the pathogenesis of conjunctival laxity.

In the present study, we found overexpression of MMP-9 (active and inactive forms), which could be involved in the degeneration of gelatin, type I and IV collagen of the basal membrane, and of elastin. Levels of the MMP-9 enzyme were measured in the tears of CCh patients both before and 1 month after surgery (more than a week without topical treatment), and a reduction in the concentration of the enzyme was found once the redundant folds of the bulbar conjunctiva had been removed by surgery and tear clearance normalized. The conjunctival epithelium was evaluated by means of a slit lamp, verifying the absence of the conjunctival folds and the re-establishment of the smoothness of the conjunctival epithelium. These data correlated with the disappearance of symptoms and the reduction in the levels of MMP-9 in tears.

We have included in our study patients with symptomatic CCh who did not respond to pharmacologic treatment (topical steroids). After surgery, the patients were subsequently treated for a few weeks with lubricants and topical steroids, and a washout period of 1 week was allowed before the tear samples were obtained. We consider that it is unlikely that these treatments could influence the tear composition when

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compared with presurgical values, since this therapy was used in a similar manner.

In this study, we included patients with CCh and patients with CCh and hyposecretive dry eye and we decided to separate them into two groups. Although we are aware that the number of patients in this study was low, we considered it as a pilot study to verify the effectiveness of the chosen protein as suitable marker for use in clinical assays to evaluate the efficacy of different kind of therapies. Moreover, despite the fact that our study involved a small number of patients, the results were statistically very significant.

We included the group of patients with dry eye and CCh because in clinical practice both conditions are frequently associated and probably the therapeutic approach has to be specific. It has been suggested by Liu[37] that the loose conjunctiva frequently found in patients with an unstable tear film can be exacerbated by interrupting the formation of the inferior tear meniscus. It is important to distinguish between an unstable tear film from dry eye and another that develops as a result of CCh. Moreover, the instability of the tear film may not be due to an aqueous deficiency in the tear, but rather to a lipid deficiency, the latter idea being supported by the pattern of staining of the nonexposed zone presented by patients. Our studies revealed that following removal of the folds, the tear level of MMP-9 in patients with CCh and dry eye did not recover as well as in patients with a low Schirmer test value.

We found that there is an inflammatory component present in CCh tears, which is responsible for the inflammation of the lower bulbar conjunctiva, leading to discomfort in these patients. The determination and monitoring of MMP-9 levels in tears may be a very useful tool for the objective evaluation of responses to pharmacologic and/or surgical treatments. In this study, we found a good response to surgery in terms of the disappearance of symptoms and reduced MMP-9 levels in tears.

The main objective of this study was not to find a causal relationship between MMP-9 and CCh, but rather to find a marker that is known to be altered in this pathology and analyze whether its level is modified after a given treatment.

To conclude, to the best of our knowledge, this is the first study to objectively demonstrate the efficacy of surgical treatment for CCh, in terms of reduced levels of MMP-9 in tears in response to treatment. With this quantitative assay, new doors are opened in this area of research and the present findings indicate that MMP-9 quantification is a good candidate for inclusion in future clinical trials of treatment for several ocular surface disorders.

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