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# Ocular Surface Temperature and Tear Film Matrix Metalloproteinase-9 Concentration in Sjögren Syndrome Patients

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## Abstract

**Purpose:** To evaluate the ocular surface temperature (OST) using an infrared thermography camera in Sjögren Syndrome (SS) patients compared with healthy subjects and correlate these results with the dry eye symptomatology, tear volume, stability and matrix metalloproteinase 9 (MMP-9) concentrations.

**Methods:** Twelve patients of primary SS (46.64 ± 13.34 years), and twenty volunteers (41.38 ± 9.67 years) without dry eye, participated in this study. OSDI questionnaire, Schirmer test, tear break up time (TFBUT), matrix metalloproteinase 9 (MMP-9) concentrations and OST were evaluated.

**Results:** Central cornea temperature was statistically higher in SS than in control group ($p = 0.014$), being 34.81 ± 0.37ºC and 34.25 ± 0.65ºC respectively. In the control group, the temperature increased in the periphery compared with central cornea, limbus and conjunctiva ($p<0.05$). However, no statistical differences were found in SS patients ($p>0.05$). The SS patients showed a significant lower Schirmer test and TFBUT compared with control group ($p<0.005$). Also, the OSDI score and MMP-9 concentration were statistically higher in SS patients compared with the control group ($p<0.05$). No correlation between central cornea temperature and TFBUT, OSDI and Schirmer test was found. However we found a strong positive correlation between central cornea temperature and MMP-9 concentration been 0.628 ($p=0.029$).

**Conclusion:** Central cornea temperature is higher in SS compared with healthy subjects. The strong positive correlation between MMP-9 concentration and central cornea temperature suggests that the high temperature in SS could be due to ocular surface inflammation.

**Keywords:** Corneal temperature; Sjögren syndrome; Dry eye symptomatology; Tear stability; Matrix metalloproteinase-9

## Introduction

Sjögren´s syndrome (SS) is a systemic autoimmune disease characterized by polyglandular tissue destruction mainly affecting the salivar and lacrimal glands [1]. This glandular inflammatory infiltrates produce an alteration in the secretory function and changes in tear composition incorporating proinflammatory markers as cytokines, chemokines and metalloproteinases [2]. Severe dry eye signs, including low tear volume, tear instability, inflammation of the ocular surface tissues and increased corneal staining are the main ocular manifestations of SS [3]. Primary Sjögren syndrome has a prevalence rate of 0.5% and occurs more frequently in women than in men with a ratio 9:1 [4]. It is diagnosed late, usually at 45 to 50 years of age, most often in an ophthalmologist's or optometrist's consulting room.

The objective ocular signs are mainly determined using the Schirmer test (without anaesthesia), tear film break-up time (TFBUT) and ocular surface staining with vital dyes. The most frequent symptoms in Sjögren patients are ocular discomfort, dryness and sensitivity to light [5]. In the early stages of the disease, when the typical signs and symptoms are often lacking or are not entirely expressed, diagnosis is complicated [6].

Moreover, ocular surface inflammation plays an important role in the pathogenesis of dry eye syndrome. This parameter has always been associated with a temperature increase in the affected tissue [7]. Some authors such as Chotikavanich and co-workers have associated dry eye with an over-expression and increase in MMP-9 corneal activity, proposing it as a biomarker for diagnosis, classification and management of this disease [8]. Previous research had already observed increased levels of MMP-9 in tear film of patients with several dry eye conditions including those diagnosed with Sjögren Syndrome [9]. Nowadays, it is proposed only as a biomarker for diagnosis of severe dry eye stages, since over-expression is rare in patients with moderate dry eye [10,11].

Different diagnostic techniques have been developed to evaluate and diagnose dry eye syndrome; however, many of these test are invasive. Some authors considered that the development of a more objective, reliable ocular surface test for evaluation of dry eye would aid in the diagnosis and monitoring of this disease. Previous studies demonstrated that measurement of corneal surface temperature by thermography application may reinforce the classical diagnostic techniques [12-14]. Infrared thermography is well-known for its ability to detect the pathological and physiological changes in the eye. The

ocular surface temperature can be used in the diagnosis of different ocular diseases [15]. However, to the best of our knowledge, no previous studies have reported correlation between ocular surface temperature and inflammation in Sjögren Syndrome patients.

Thus, the purpose of this study was to evaluate the ocular surface temperature using an infrared thermography camera, in eight different points, four being in the cornea (centre, nasal, temporal and inferior position), two in the limbus and another two in conjunctiva (nasal and temporal), in Sjögren Syndrome patients compared with healthy subjects and correlate these results with the dry eye symptomatology, tear volume, stability and MMP-9 concentration.

## Methods

### Subjects

A pilot, experimental, prospective study has been performed. Twelve patients of primary Sjögren Syndrome, who had received the diagnosis in the past five years, enrolled in the Spanish Association of Sjögren Syndrome, and participated voluntarily in the study. In all patients, the disease was diagnosed according to the American-European Consensus Group (AECG) on diagnostic criteria for Sjögren syndrome [3]. The ages of the participants ranged from 24 to 63 years old with an average age of 46.64 ± 13.34 years. A control group of 20 volunteers with neither Sjögren Syndrome, evidence of subjective symptoms or objective findings of dry eye, participated in the present study. The control group average age was of 41.38 ± 9.67 years ranging from 23 to 53 years old. All participants in the study were women. They were asked not to apply any artificial tear supplements at least two hours before experiments.

The study was conducted in compliance with good clinical practice guidelines, institutional review board regulations and the tenets of the Declaration of Helsinki [16]. The subjects signed an informed consent and were free to interrupt the session at any time. Moreover, the study was approved by the Ethics Committee (CEIC) of the Hospital Clínico San Carlos of Madrid prior to its commencement. Measurements were performed in one eye of each patient, selected randomly, during a unique visit. The order of measurement was OSDI, thermal analysis, Schirmer test, tear sample collection and TFBUT keeping 5 minutes between tests.

### Trials

In order to know the dry eye symptomatology, Ocular Surface Disease Index (OSDI) questionnaire was performed. The questionnaire, validated by different studies [17] is composed of 12 questions, each with five possible responses with a score between 0 and 4 (0=none of the time, 1=some of the time, 2= half of the time, 3=most of the time, 4=all of the time). This questionnaire was used to grade the degree of dry eye symptoms to aid in differentiating dry eye syndrome patients from healthy patients. The final score is between 0 and 100, where 100 corresponds the highest symptomatology of dry eye [17].

The tear collection was always performed after filling in the questionnaire. The volume of tears was collected using the Schirmer I test (Jones test). The Schirmer strip was placed on the temporal tarsal conjunctiva of the lower lid for 5 minutes with the eyes closed, and the volume of tears, as millimetres of moistened strip, was recorded [18]. For MMP-9 analysis with ELISA assay tears were collected gently from the conjunctival sac using a microcapillary tube (Brand® disposable Blaubrand, IntraMark; SIGMA-Aldrich), taking care not to produce reflex tearing. Prior to samples collection, 40 µl of non-preserved saline (sodium chloride 0.9%) was instilled into the inferior fornix to mix the tear fluid content, and instantaneously afterwards, tear was collected by capillary, following the procedure described by Markoulli et al. [19]. Following collection, samples were centrifuged at 10000 rpm for 5 minutes at 4ºC to remove cellular debris, and the supernatants were collected and stored at -80ºC until analysis.

After tear collection, fluorescein was applied to evaluate the break up time (TFBUT) which represents the time lapse from a blink to the first signs of tear film collapse. In order to warrant repeatability of the staining procedure, a solution was prepared using a 10% concentration of sodium fluorescein diluted in saline (NaCl 0.9 %). For each application, a micropipette with 5 µl of diluted fluorescein solution was applied to the inferior conjunctival sac and 20 seconds later was analyzed the average of three measurements was used. A chronograph was used to record the time to break the tear after the patient was asked to blink twice and maintain their eyes open.

### Ocular surface thermal analysis

A thermal digital camera (FLIR A325; FLIR Systems Inc., MA, USA), with an accuracy of ± 2%, temperature ranging from −20°C to 120°C, thermal sensitivity of 50 mK, resolution of 320 × 240 pixels and frame rate of 9 Hz were used for evaluating the ocular surface temperature. For ocular surface temperature measurements sensitivity of 0.98 was assumed [20]. The thermal camera was placed in a slit lamp support and the patient was accommodated in a chin rest to take three thermal images of the eye previously selected in scotopic conditions and always at the same distance of the detector. The room temperature was specifically set and controlled at all times at 21°C and humidity remained at 27.0%. Patients were adapted to room conditions for at least 20-25 minutes before ocular surface temperature measurements [21].

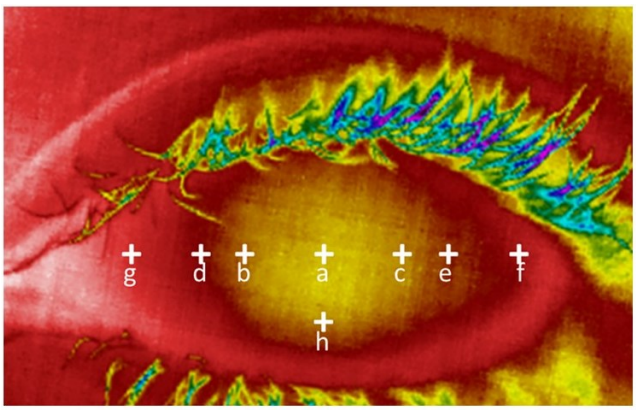


**Figure 1:** Scheme of ocular surface points where temperature was measured. Eight points were evaluated, **a:** central cornea; **b:** nasal cornea; **c:** temporal cornea; **h:** inferior cornea; **d:** nasal limbus; **e:** temporal limbus; **f:** temporal conjunctiva; **g:** nasal conjunctiva. b, c and h points were placed at 5 mm of a point. d and e point were placed at 6.2 mm of a point. And finally, f and g were placed at 9 mm of a point.

Subjects were asked to blink normally for a few seconds and then to open their eyes as wide as possible as the scan was obtained. Eight

different points were measured: Central, Nasal, Temporal and Inferior cornea; nasal and temporal Limbus; and nasal and temporal conjunctiva (Figure 1). All images were analysed with the FLIR IR monitor software provided by the thermal camera manufacturer. Temperatures below 32.1ºC and above 37.2ºC were excluded in any point measured [22]. In corneas out of 12 mm to 12.8 mm of diameter, the limbus points were also excluded.

### Statistical analysis

The presented data were analysed using the statistical software SPSS 22.0 (SPSS, Inc., Chicago, IL). The values presented are the means ± SD of the experiments performed. Normal distribution of variables was assessed by the Shapiro-Wilk normality test. Sample size calculations were performed with statistical software (Granmo 6.0; Institut Municipal d'Investigacion Medica, Barcelona, Spain). With an accepted two-sided statistical significant threshold of 0.05 and a risk of 0.20, for a standard deviation 0.6 units to the mean and in order to detect a difference of 0.7 units or more and taking into account a 2:1 group ratio (control group to Sjögren Syndrome group), at least 18 subjects were needed in the first group and 9 in the second, to find statistically significant differences.

Differences between the Sjögren Syndrome group and control group were estimated by the Student's t-test for independent samples. The temperature variation from the corneal centre to conjunctiva was analysed by related-samples two-way ANOVA analysis of variance, with Bonferroni's adjustment in order to avoid the experimental error rate. To correlate the Schirmer test, TFBUT, OSDI, MMP-9 concentration with ocular surface temperature, Pearson bivariate regression was used. P<0.05 was considered statistically significant.

## Results

No statistically significant age differences were detected between groups (p=0.205; Student's t-test for independent samples). The Horizontal Visible Iris Diameter (HVID) for all Sjögren Syndrome patients and control group was between 12.0 mm to 12.8 mm and therefore, it was not necessary to avoid any temperature measurement in the limbus zone.

| Ocular Surface Temperature (°C) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Central Cornea | Nasal Conjunctiva | Nasal Limbus | Nasal Cornea | Temporal Limbus | Temporal Cornea | Temporal Conjunctiva | Inferior Cornea |
| Control | Mean (SD) | 34.15 ± 0.53 | 35.78 ± 0.48 | 35.05 ± 0.61 | 34.77 ± 0.61 | 34.69 ± 0.71 | 35.01 ± 0.52 | 35.73 ± 0.49 | 35.03 ± 0.63 |
| | P value | -- | <0.001* | <0.001* | <0.011* | <0.001* | <0.001* | <0.001* | <0.001* |
| Sjogren Syndrome | Mean (SD) | 34.80 ± 0.41 | 35.51 ± 1.14 | 35.10 ± 0.53 | 34.94 ± 0.38 | 34.77 ± 1.01 | 34.93 ± 1.01 | 35.43 ± 1.07 | 35.44 ±1.38 |
| | P value | -- | 0.041* | 0.176 | 0.872 | 0.93 | 0.137 | 0.004* | 0.09 |

**Table 1:** Ocular surface temperature in different points of the ocular surface. *p value<0.05. Studnets t-test for related samples; central cornea vs. other ocular surface points.

| Test | Control | Sjogren Syndrome | P value |
|---|---|---|---|
| Schirmer (mm) mean (SD) | 20.22 ± 12.09 | 7.64 ± 8.78 | 0.025* |
| TBUT (s.) mean (SD) | 6.03 ± 2.86 | 3.91 ± 1.95 | 0.030* |
| OSDI (0-100) mean (SD) | 15.07 ± 13.18 | 59.56 ± 33.90 | >0.001 |
| MMP-9 (ng/ml) mean (SD) | 2.82 ± 3.01 | 6.51 ± 5.50 | 0.02* |

**Table 2:** Summary of paramenters evaluated for the different groups. p value <0.05 (Students t-test for independent samples; Sjogren Syndrome vs. Control group).

Central cornea temperature in Sjögren Syndrome group was statistically higher than control group (p=0.014), being 34.81 ± 0.37ºC and 34.25 ± 0.65ºC respectively. No statistical differences in the rest of cornea, limbus or conjunctiva temperature points evaluated were found (p>0.05) (Figure 2). In the control group, the temperature is increased in the periphery of the cornea compared with central cornea (p<0.05). Also, the limbus temperature and conjunctiva temperature were statistically higher than the corneal temperature (p<0.05). However, no statistical differences between corneal periphery, limbus temperature and central corneal temperature in Sjögren Syndrome patients were found (p>0.05). Only significant differences between conjunctival temperature and central corneal temperature were found (p<0.05). All means, standard deviation and p values are showed in Table 1.

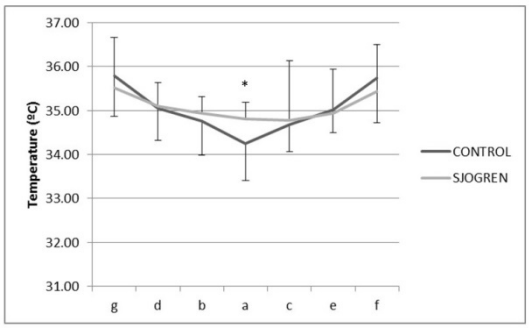


**Figure 2:** Ocular surface temperature variation in the horizontal line. Central cornea temperature in Sjögren Syndrome group was statistically higher than in the control group. The temperature was increased in the periphery compared with central cornea in both groups, being significant for all points in control group and only for conjunctiva points in Sjögren Syndrome group. *p<0.05 control group vs. Sjögren Syndrome group; Student's t-test for independent samples.

Regarding tear volume, the Sjögren Syndrome patients showed a significant lower Schirmer test value compared with control group (p=0.025). The tear stability was significantly lower in the Sjögren Syndrome group compared with control group (p=0.030). Also, The OSDI score and MMP-9 concentration were statistically higher in Sjögren Syndrome patients compared with healthy subjects being (p<0.001) and (p<0.05) respectively, shown in Table 2.

No correlation between central cornea temperature and any other parameter evaluated was found. The Pearson's correlation coefficient was 0.171 (p=0.595), -0.67 (p=0.835) and 0.008 (p=0.981) for central cornea temperature and TBUT, OSDI and Schirmer test respectively. However we found a strong positive correlation between central cornea temperature and MMP-9 concentration been 0.628 (p=0.029) in Sjögren Syndrome patients (Figure 3).

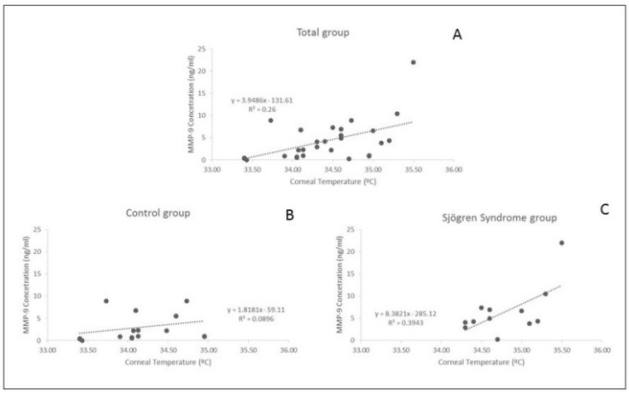


**Figure 3:** Relationship between central cornea temperature and MMP-9 concentration in total group and segmented in control group and Sjögren syndrome group. Pearson correlation was statistically significant r=0.628 (p=0.029) in Sjögren Syndrome patients.

## Discussion

Infrared thermal images have been used in the last decades for measuring the ocular surface temperature, since its main advantage is being a non-invasive test [23,24]. Infrared thermography is based on considering the ocular surface as an efficient radiator of the infrared region of the electromagnetic spectrum [12]. Further studies have suggested that the important role of tear film in ocular surface temperature is its efficiency as an absorber and radiator of infrared radiation [25,26]. Therefore, It is considered that OST would correspond to tear film temperature and this technique could be used to increase the understanding of ocular physiology [25,27]. Our results show that central cornea temperature is higher in Sjögren Syndrome compared with healthy patients. Purslow et al. and Morgan et al. described in two different studies that the mean ocular surface temperature across the anterior eye appears higher in patients with tear film disorders which matches with our results. The first group evaluated the OST for 8 s while Morgan and co-workers analysed this parameter 4 and 5 s after blinking. Furthermore they found that this parameter was also higher in dry eye patients who displayed either a fast tear break-up time or poor Schirmer´s test results [15,28]. However, Kamao et al. did not find differences in ocular surface temperature between groups [29]. The lack of consensus between studies could probably be due to some factors like different devices used for measuring ocular surface temperature, different room conditions, or different dry eye severity criteria recruitment. In the case of the Kamao study, the room temperature and humidity was higher than the one in our study, allowing a better stability of tear film in dry eye patients.

Dry eye patients usually relate an increase in blinking frequency due to discomfort [30]. On the other hand, ocular temperature has been shown to increase when the eye is closed and also blinking spreads the tear over the ocular surface warming immediately after each blinking [26,31]. These facts could explain the higher central cornea temperature in Sjögren Syndrome compared to healthy subjects.

Another factor, which could influence the ocular surface temperature, is inflammation. It plays an important role in dry eye disease; this is why it is included into the dry eye definition proposed by Dry Eye Workshop report in 2007 10. It has been considered that inflammation is a concomitant process related to ocular dryness; therefore an increase in one of the inflammation markers in patients sounds reasonable [32]. In this sense, increases in ocular surface temperature have been observed when blood flow increases during inflammation of the anterior eye, finding a correlation between ocular redness and ocular surface temperature [7,33]. Also, some authors suggested that posterior segment inflammation can also cause an increase in the ocular surface temperature [20,34]. A possible limitation of this study could be the fact to only measure MMP-9 as an inflammatory marker. There are another cytokines and interleukins involved in the dry eye pathophysiology being IL-, IL-6, IL-8 and TNF-α most studied 10, but MMP-9 has been proposed the main inflammatory biomarker in dry eye, being the only proinflammatory molecule measurable in our clinical practice for dry eye diagnosis. To corroborate this relationship between ocular surface temperature and inflammation would be interested to measure another inflammatory molecules and ocular surface temperature in the same visit.

No differences were found in peripheral cornea, limbus and conjunctiva when comparing Sjögren Syndrome and control group. As in our results, Morgan et al. described that the ocular surface temperature differences between dry eye patients and healthy patients

in the limbal and peripheral cornea areas were lower than the central cornea but they did not show any statistical analysis [28]. Regarding conjunctiva temperature, Kamao et al., showed similar results to those of our study, but without significant differences between groups [29].

It has been described that ocular surface temperature in the healthy eye is warmer in the limbus and conjunctiva by 0.45ºC to 1.5ºC than the central cornea. Our findings correlate well with the previous literature since we found statistical differences of 0.75ºC and 1.5ºC for limbus and conjunctiva compared with central cornea, respectively [26,33]. For Sjögren Syndrome, the temperature was higher in the limbus and conjunctiva than the central cornea but less significant than the one obtained in healthy subjects. This temperature distribution in both groups could be explained by the blood flow from the warmest areas of the ocular surface, as a conjunctiva and limbus, and avascular feature of the cornea. It is probably that blood flow could have more influence in the ocular surface temperature in the periphery than surface inflammation, and therefore only in the avascular cornea is possible to see the temperature differences between groups.

Dry eye symptomatology was assessed with OSDI questionnaire, showing a very high score in Sjögren Syndrome patients. Moreover, the Schirmer test and TFBUT were lower in Sjögren Syndrome compared with the control group. Our results match with other previous studies [35]. Those studies have found a correlation between ocular surface temperature and tear film stability and with an increase in the rate of evaporation [25].

The dry eye patients participating in our study did not present this correlation. The reason for that might be the time of measurement. Purslow et al. described the variation of temperature after blinking. They related that immediately after blinking, the ocular surface temperature rose to decrease quickly during the time without blinking [15]. The correlation was found to compare the ocular surface temperature at 5 to 10 seconds of blinking with tear film stability and our study measured the temperature just after blinking.

Although no significant effect of gender on the ocular surface temperature has been observed in previous studies [33,36], our study was performed only in women patients with the aim to avoid any bias due to gender, because it has been described that the female/male ratio in prevalence data is close to 9:1 [4].

A limitation of our study could be the small sample size. Some difficulties exist to find larger sample sizes due to low prevalence of Sjögren Syndrome [1]. However it is enough to find significant correlations between the parameters evaluated. Another limitation of our study could be that we only analyzed the ocular surface temperature just after blinking. Further studies could be interesting to evaluate the relationship between the dynamics of tear film temperature and inflammation. Moreover, it could be interesting to evaluate the effect of some anti-inflammatory drugs used in these dry eye patients, such as cyclosporine, on ocular surface temperature.

In conclusion, the central corneal temperature in Sjögren Syndrome patients was higher than in healthy patients. Furthermore, there is a strong positive correlation between the central corneal temperature and the matrix metalloproteinase 9 concentration indicating that the higher OST in Sjögren patients could be due to inflammation. This parameter could probably be used for the diagnosis together with other classical diagnostic tools and to target the therapeutic management of this disease in patients treated with anti-inflammatory drugs.

## Conflict of Interest Statement

All authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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