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Influence of Climate on Clinical Diagnostic Dry Eye Tests: Pilot Study

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ABSTRACT

Purpose. To analyze dry eye disease (DED) tests and their consistency in similar nonsymptomatic population samples living in two geographic locations with different climates (Continental vs. Atlantic).

Methods. This is a pilot study including 14 nonsymptomatic residents from Valladolid (Continental climate, Spain) and 14 sex-matched and similarly aged residents from Braga (Atlantic climate, Portugal); they were assessed during the same season (spring) of two consecutive years. Phenol red thread test, conjunctival hyperemia, fluorescein tear breakup time, corneal and conjunctival staining, and Schirmer test were evaluated on three different consecutive visits. Reliability was assessed using the intraclass correlation coefficient and weighted kappa (κ) coefficient for quantitative and ordinal variables, respectively.

Results. Fourteen subjects were recruited in each city with a mean (±SD) age of 63.0 (±1.7) and 59.1 (±0.9) years ($p = 0.08$) in Valladolid and Braga, respectively. Intraclass correlation coefficient and κ values of the tests performed were below 0.69 and 0.61, respectively, for both samples, thus showing moderate to poor reliability. Subsequently, comparisons were made between the results corresponding to the middle and higher outdoor relative humidity (RH) visit in each location as there were no differences in mean temperature ($p \geq 0.75$) despite RH values significantly differing ($p \leq 0.005$). Significant ($p \leq 0.05$) differences were observed between Valladolid and Braga samples on tear breakup time (middle RH visit, 2.76 ± 0.60 vs. 5.26 ± 0.64 seconds; higher RH visit, 2.61 ± 0.32 vs. 5.78 ± 0.88 seconds) and corneal (middle RH, 0.64 ± 0.17 vs. 0.14 ± 0.10; higher RH, 0.60 ± 0.22 vs. 0.0 ± 0.0) and conjunctival staining (middle RH, 0.61 ± 0.17 vs. 0.14 ± 0.08; higher RH, 0.57 ± 0.15 vs. 0.18 ± 0.09).

Conclusions. This pilot study provides initial evidence to support that DED test outcomes assessing the ocular surface integrity and tear stability are climate dependent. Future large-sample studies should support these outcomes also in DED patients. This knowledge is fundamental for multicenter clinical trials. Lack of consistency in diagnostic clinical tests for DED was also corroborated.

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Key Words: dry eye disease, diagnostic test, environmental conditions, reliability

Dry eye disease (DED) is a common disorder affecting between 5.5 and 33.7% of the general population, depending on the criteria used for its diagnosis.[1] It is characterized by ocular discomfort and pain, visual disturbance, tear film instability, increased tear osmolarity, and inflammation.[2] Clinical evidence indicates that the common diagnostic DED tests do not always correlate well with symptoms.[3,4] Besides, the ocular surface of any subject is always exposed to an environment that varies continuously during the day (i.e., outdoor vs. indoor) or among seasons. Thus, environment has been always considered as one of the possible factors implicated in the absence of relationship between DED signs and symptoms.[3,4] Additionally, previous authors have reported the lack of consistency in DED test outcomes across different days,[5,6] and this variability is not only a shortcoming for DED diagnosis but also a well-known challenge in the demonstration of therapeutic efficacy in clinical trials.[7]

Depending on the geographic area (i.e., desert vs. coast), at the same time point of the day, people will be exposed outdoors to diverse magnitudes of temperature, wind, draft, and relative humidity (RH), which could affect the ocular surface differently.

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Therefore, normal subjects who initially do not report DED symptoms and do not seek ophthalmic assessment could show different DED test scores depending on where they are living, because of the environment to which they are exposed. Consequently, the results of the common clinical DED test used worldwide performed in DED and non-DED subjects belonging to the same ethnicity could largely vary across the same country, resulting in a variation on the diagnostic criteria for each DED test and in a widening of the score range considered as within normal limits. Besides, normal subjects could be erroneously diagnosed of having DED if they are assessed after being exposed to adverse environments (i.e., air-conditioned waiting room, low RH, windy day, etc.), which can produce a transient worsening of the ocular surface.[8,9]

Therefore, the purpose of the present study was to analyze possible differences in DED test outcomes in similar normal population samples living in two geographic locations having different environmental climates as well as to assess the consistency of the DED test performed in both locations.

METHODS

Participants

In this pilot study, Caucasian nonsymptomatic volunteers from the Iberian Peninsula living in Valladolid (interior city, Spain [Continental climate]) and Braga (coastal city, Portugal [Atlantic climate]) were included. They were recruited from university staff. The study protocols were approved by the University of Valladolid Ethics Committee. The study adhered to the tenets of the Declaration of Helsinki. All enrolled subjects were informed of the nature of the study and consent forms were signed. The same experienced examiner (MT) always performed the clinical evaluation in both cities and was always masked to the data previously obtained in each visit for each subject. Evaluations were performed on the same season (spring) of two consecutive years.

During a preliminary visit, volunteers were screened for inclusion criteria. The inclusion criteria were an Ocular Surface Disease Index score less than or equal to 12,[10] corneal fluorescein staining less than or equal to grade 1,[2] and Schirmer test without anesthesia greater than 5 mm in 5 minutes.[2] In addition, subjects had to be within normal limits in at least two out of the following three tests: fluorescein tear breakup time (T-BUT) greater than 7 seconds,[11] conjunctival lissamine green staining (Oxford scale) less than or equal to grade 1,[2,12] and phenol red thread test (PRTT) greater than 20 mm in 15 seconds.[13] Exclusion criteria for both populations were age younger than 40 years, contact lens wear, pregnancy or nursing, history of ocular surgery, and any acute or chronic ocular disease including patients with concomitant allergies (even if mild).

Only one eye of each subject was included and selected during the screening visit. The eye with the least corneal staining and symptomatic was selected.

Examination Procedure

Between 2 and 5 days after the screening visit, participants were evaluated on three different days; thus, we could assess the variability of the DED tests. Experimental sessions were separated by a minimum of 2 days and a maximum of 5 days.

The examinations were performed in the sequence outlined below in both populations:

PRTT: The test (Zone Quick Test; Menicon Company Ltd, Nagoya, Japan) was placed over the external canthus as recommended, and the length of wetting was read 15 seconds later.[13]

Conjunctival hyperemia: Bulbar hyperemia was scored based on the Efron scale.[14] Nasal and temporal areas were assessed independently; however, the final score was the average of both values.

Fluorescein T-BUT: Fluorescein T-BUT was measured after 5-μL instillation of 2% sodium fluorescein (Colircusí Fluoresceína 2%, Alcon Cusí, SA, Barcelona, Spain). The mean value of three consecutive measurements was recorded using a stopwatch.

Corneal fluorescein staining: Measurements were made 2 minutes after instillation of 5 μL of 2% sodium fluorescein (Colircusí Fluoresceína 2%). Corneal fluorescein staining was graded using the Oxford scheme that includes six severity grades (0 to V)[15] and the Baylor scheme.[16]

Conjunctival lissamine green staining: Lissamine green wetted strips (GreenGlo, HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA) were gently applied into the inferior fornix. Staining was evaluated 1 minute after the instillation following the Oxford scheme.[15]

Schirmer test without topical anesthesia: One Schirmer sterile strip (Tearflo, HUB Pharmaceuticals, LLC) was placed in the lateral canthus of the inferior lid margin. The wetted portion of the strip was measured after 5 minutes.[17]

Data Analysis

Data were expressed as the mean ± SEM. Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 19.0 for Windows; SPSS Inc, Chicago, IL) and R software by a licensed statistician. The reliability of diagnostics tests was assessed using the intraclass correlation coefficient (ICC)[18] for quantitative variables (PRTT, T-BUT, and Schirmer test) and κ coefficient[19] for ordinal-scale variables (corneal and conjunctival staining and conjunctival hyperemia). For life sciences, it has been commonly accepted that an ICC value greater than 0.90 represents excellent agreement, an ICC value between 0.89 and 0.75 indicates moderate agreement, and an ICC value less than 0.75 is not considered acceptable from a clinical standpoint. A κ coefficient value[19] between 1.0 and 0.81 is considered as excellent agreement, a value between 0.61 and 0.80 means substantial agreement, a value between 0.41 and 0.60 indicates moderate agreement, whereas a value between 0.21 and 0.40 shows only fair agreement. For comparisons between data obtained in both cities, the Mann-Whitney *U* test was used. p value less than or equal to 0.05 was considered significant for all statistical tests.

RESULTS

Screening Visit

Fourteen subjects (7 men and 7 women; mean [±SD] age, 63.07 [±1.76] years; age range, 50 to 74 years) were evaluated in

TABLE 1.
Reliability of DED tests (ordinal variables) in Valladolid (Continental climate) and Braga (Atlantic climate)

Ordinal-scale variables	Valladolid κ coefficient (95% CI)	Braga κ coefficient (95% CI)
Conjunctival hyperemia		
Nasal	0.49 (0.16–0.79)	0.43 (0.26–0.64)
Temporal	0.21 (0.0–0.35)	0.61 (0.23–0.90)
Corneal staining (Oxford scale)	0.41 (0.06–0.67)	0.08 (0.0–0.28)
Corneal staining (Baylor scale)		
Central*	0.33 (0.23–0.43)	—
Nasal	0.49 (0.05–0.91)	0.00 (0.0–0.0)
Temporal	0.30 (0.0–0.58)	0.00 (0.0–0.0)
Superior*	—	—
Inferior	0.33 (0.0–0.70)	0.00 (0.0–0.18)
Conjunctival staining (Oxford scale)		
Nasal	0.58 (0.31–0.77)	0.36 (0.27–0.39)
Temporal	0.59 (0.16–0.82)	0.48 (0.0–0.87)

*All subjects showed grade 0; thus, reliability analysis could not be carried out.

Valladolid (Spain) between April and June 2011. During the same months in 2012, 14 sex-matched and similarly aged subjects (7 men and 7 women; mean [±SD] age, 59.07 [±0.87] years; age range, 54 to 66 years) were evaluated in Braga (Portugal) during the same months of 2012. The age of the two groups was not significantly different ($p = 0.10$).

Reliability of DED Tests

The reliability of the Schirmer test, PRTT, and fluorescein T-BUT for Valladolid subjects was 0.61 (95% confidence interval [CI], 0.31 to 0.83), 0.40 (95% CI, 0.07 to 0.71), and 0.11 (95% CI, 0.0 to 0.49), respectively, whereas ICCs for Braga subjects were 0.69 (95% CI, 0.42 to 0.87), 0.55 (95% CI, 0.24 to 0.80), and 0.14 (95% CI, 0.0 to 0.51), respectively. The reliability of DED tests using ordinal-scale variables is detailed in Table 1. All DED tests performed showed either poor or moderate intervisit agreement and were thus not reliable. There were no significant differences ($p \geq 0.05$) between the variability observed for all DED tests performed in both cities in terms of ICC and κ coefficients, except for temporal conjunctival hyperemia and nasal corneal staining.

DED Test Outcomes in Both Cities

Because of the lack of reproducibility of the DED tests performed in each city, it is not recommended to compute average data obtained during the three visits to compare DED test scores between groups, from a statistical viewpoint. Thus, we ranked each of the three visits depending on the outdoor RH value as recorded from each National Meteorological Office (Spain and Portugal) (Table 2). Then, we compared DED test outcomes obtained for the middle and higher RH visits as there were no significant ($p \geq 0.75$) differences in temperature between both cities in contrast to the lower RH visit (Table 2). There were significant ($p \leq 0.005$) differences in mean RH between cities during the middle and higher RH visits (Table 2), as expected based on each local climate. For the middle RH visit, we found significant ($p \leq 0.05$) higher values for corneal fluorescein and lissamine green conjunctival staining in Valladolid subjects compared with those of Braga ones, as well as significant lower T-BUT scores (Table 3). For the higher RH visit, we also found significant ($p \leq 0.05$) higher values for corneal fluorescein and lissamine green conjunctival staining as well as conjunctival hyperemia in Valladolid subjects and marked lower T-BUT scores (Table 4).

DISCUSSION

This pilot study compared DED test data obtained from two normal adult samples from two different geographical locations but similar latitudes (Valladolid, 41.65 degrees; Braga, 41.54 degrees), with the aim of evaluating the possible differences associated to climate factors (Continental vs. Atlantic),[20,21] which are not

TABLE 2.
Mean outdoor RH and temperature recorded in Valladolid (Continental climate) and Braga (Atlantic climate) during the three visits

Visit ranking	City	RH, mean ± SEM, %	p*	Temperature, mean ± SEM, °C	p*
Lower RH	Valladolid	36.8 ± 1.9		21.6 ± 1.5	
	Braga	75.8 ± 1.6	0.001	16.6 ± 0.7	0.008
Middle RH	Valladolid	52.6 ± 3.7		16.0 ± 0.9	
	Braga	81.2 ± 1.2	0.003	16.5 ± 0.7	0.76
Higher RH	Valladolid	62.4 ± 5.4		17.4 ± 1.0	
	Braga	85.2 ± 2.0	0.005	17.2 ± 0.3	0.75

Data were obtained from the National Meteorological Office of each country.
*Comparison by Mann-Whitney *U* test between both cities.

TABLE 3.

Comparison of DED test outcomes between Valladolid (Continental climate) and Braga (Atlantic climate) obtained during the middle RH visit

Variable	City	Mean ± SEM	p*
PRTT	Valladolid	19.21 ± 1.83	0.37
	Braga	21.43 ± 1.69	
Conjunctival hyperemia (mean)	Valladolid	0.89 ± 0.09	0.06
	Braga	0.61 ± 0.09	
Fluorescein T-BUT	Valladolid	2.76 ± 0.60	0.0006
	Braga	5.26 ± 0.64	
Corneal staining (Oxford scale)	Valladolid	0.64 ± 0.17	0.01
	Braga	0.14 ± 0.10	
Corneal staining (Baylor scale—total score)	Valladolid	2.14 ± 0.83	0.02
	Braga	0.43 ± 0.17	
Conjunctival staining (mean)	Valladolid	0.61 ± 0.17	0.03
	Braga	0.14 ± 0.08	
Schirmer test (no anesthesia)	Valladolid	11.93 ± 2.20	0.73
	Braga	14.29 ± 2.78	

*Comparison by Mann-Whitney *U* test between both cities.

under human control. For the inclusion criteria, we recruited only adult volunteers because we wanted to obtain data from nonsymptomatic subjects having an age range similar to the one commonly found in DED patients.[22] Besides, we allowed participants to show up to a grade 1 (Oxford scheme) corneal staining because Dundas et al.[23] have already reported that up to 79% of healthy young subjects could show some degree of corneal staining. In case of fluorescein T-BUT, we selected a previously proposed[11] cutoff value of 7 seconds because fluorescein disturbs tear film and real noninvasive T-BUT values are 4.0 seconds longer on average.[24]

In our study, we assessed the reliability of commonly used DED tests in the clinical setting. Their low reproducibility from a clinical standpoint has been reported previously[5,6] and it is a well-recognized problem when assessing DED therapeutic efficacy in clinical trials.[25] In our study, the DED test showing the best reproducibility was the Schirmer one, being slightly higher in the Atlantic climate city sample (0.69 vs. 0.61). Nonetheless, even the Atlantic climate city ICC value was not close to the one commonly recognized as clinically acceptable (0.75).[18] In our study, we obtained slightly higher ICC values for the Schirmer test than those reported by Nichols et al.[6] who found a value of 0.48. When comparing our outcomes and those reported by Nichols et al.,[6] it must be taken into account that they have assessed DED patients during two visits and we evaluated nonsymptomatic volunteers during three visits. Regarding the DED tests that use ordinal variables to grade patients (Table 1), we obtained κ coefficient values less than 0.61, which means that the agreement among days was only moderate or poor. We did not find differences in day-to-day variability between both cities for the vast majority of the DED test; however, it must be taken into account that our sample

TABLE 4.

Comparison of DED test outcomes between Valladolid (Continental climate) and Braga (Atlantic climate) obtained during the higher RH visit

Variable	City	Mean ± SEM	p*
PRTT	Valladolid	18.07 ± 1.96	0.60
	Braga	19.21 ± 2.09	
Conjunctival hyperemia (mean)	Valladolid	1.10 ± 0.12	0.009
	Braga	0.57 ± 0.11	
Fluorescein T-BUT	Valladolid	2.61 ± 0.32	<0.0001
	Braga	5.78 ± 0.88	
Corneal staining (Oxford scale)	Valladolid	0.60 ± 0.22	0.05
	Braga	0.0 ± 0.0	
Corneal staining (Baylor scale—total score)	Valladolid	2.57 ± 0.92	0.06
	Braga	0.28 ± 0.46	
Conjunctival staining (mean)	Valladolid	0.57 ± 0.15	0.05
	Braga	0.18 ± 0.09	
Schirmer test (no anesthesia)	Valladolid	12.50 ± 2.26	0.66
	Braga	13.80 ± 2.28	

*Comparison by Mann-Whitney *U* test between both cities.

size was low and this fact might tend to produce unavoidably wider 95% CI ranges, which can make more difficult to show differences between groups assessed from a statistical viewpoint. Besides, knowing the expected variability of DED test for a certain location can be worthwhile for selecting sites and timing for DED studies and especially for DED clinical trials. However, the best approach for this purpose might be the use of controlled environmental exposures (i.e., goggles, environmental chambers),[8,25] so that DED patients can be always evaluated under the same environmental conditions regardless of the season or time of day.

One of the main causes of the DED test variability has been commonly assigned to the environment.[2,7,25] Consequently, we decided to perform the DED tests in two similar populations living in Valladolid (Continental climate city, Spain) and Braga (Atlantic climate city, Portugal), cities located in the same peninsula (Iberian Peninsula, Europe) with a 400-km distance between them. Once we confirmed the low reproducibility obtained in the DED tests performed in both cities, we selected the DED test outcomes corresponding to the visits having the middle and higher RH value for each subject included in the study, because these visits showed only a marked difference in average RH values (Table 2). It has been previously demonstrated that there is an inverse relationship between RH and evaporation rate of the tear film in normal and DED patients.[26] Consequently, we observed that fluorescein T-BUT scores corresponding to the Continental climate city were reduced compared with those to the Atlantic climate city (Tables 3 and 4), which might have also affected the integrity of the cornea and the conjunctiva (higher staining scores).

Differences between groups (Tables 3 and 4) in clinical DED tests might not be clinically relevant when assessing moderate to severe DED patients; however, our outcomes clearly show that the ocular surface is unavoidably affected by the surrounding environment, despite not being a high desiccating one (i.e., desert). When we selected the data corresponding to the middle and higher RH visits, we observed significant differences in DED tests between both samples and that T-BUT values for the Continental climate city group were similar to those expected in mild-moderate DED patients (2.7 ± 0.6 and 2.6 ± 0.3 seconds).

The main limitation of the present study is that the sample size might not be very large; however, this pilot study provides evidence showing that the ocular surface of nonsymptomatic adults is differently affected in subjects having the same ethnicity but living in different geographical locations under dissimilar climates. A second limitation is that we did not include DED patients; thus, we cannot completely assure, based on our study, that their ocular surface is also affected in exactly the same way. However, previous authors[26] have already showed under controlled conditions that the tear evaporation rate is inversely proportional to the RH value in DED patients in a similar magnitude to healthy subjects; consequently, ocular surface in DED patients should be at least similarly affected by the environment. Another limitation was that, to compare DED test outcomes between visits, we grouped results obtained for each subject depending on the outdoor RH value observed for each visit, despite it being obvious that the population wastes time not only under outdoor conditions but also under indoor ones. However, it must be also taken into account that the expected variation (decrease) in indoor RH from the outdoor one should be similar in both cities because the recommended indoor temperature is between 21 and 24°C and the average outdoor temperature was around 17°C in both cities when tests were performed. Finally, bias could have been introduced unconsciously by the examiner when carrying out subjective DED tests (i.e., corneal staining) because of hypothetical expected outcomes from different RH values. Nonetheless, data were not statistically analyzed until all participants finished the study, so that the examiner was masked to previous DED test scores.

In conclusion, we showed that the integrity of the ocular surface of nonsymptomatic adults having the same ethnicity can vary depending on the surrounding environmental conditions. Subjects exposed to higher RH might show healthier ocular surface in terms of corneal integrity and tear film stability. This pilot study was performed in south Europe (Iberian Peninsula); however, these findings can be applied worldwide. Nonetheless, future studies with a larger sample are needed to support our findings, especially in DED patients. Besides, our outcomes also showed that the consistency of the common DED tests performed in any outpatient clinic might be too low to differentiate real DED patients from borderline ones owing to DED test variability associated to climate conditions. These findings stress the importance of incorporating to the design of DED clinical trials the exposition of participants to controlled environmental conditions; thus, at least on one occasion, all of them are evaluated under the same environment to overcome the shortcomings related to the different climates observed in multicenter clinical trials.[7]

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