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Confusing Factors in the Tear Break-Up Time Test: The Influence of Contact on the Ocular Surface: Cross-Sectional Study

Gözyaşı Kırılma Zamanı Testinde Yanıltıcı Faktörler: Oküler Yüzeye Temasın Etkisi: Kesitsel Çalışma

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ABSTRACT Objective: The purpose of the present paper was to examine the changes in the break-up time (BUT) value of the staining procedure in the fluorescein BUT test. **Material and Methods:** The natural BUT values of the participants were determined with a non-invasive BUT (NI-BUT) test. Then, the changes in the tears were re-evaluated with the topographic break-up test method after the staining procedure was simulated with strips that mimicked fluorescein staining but did not contain fluorescein. The BUT test performed after the staining simulation was named sham-hybrid BUT test (SH-BUT). Between the two tests, the first BUT value (BUT_{1st}), the second BUT value (BUT_{2nd}), the third BUT value (BUT_{3rd}), the fourth BUT value (BUT_{4th}), the fifth BUT value (BUT_{5th}), and the average value of the first 3 break-ups (BUT_{A3}) were compared. **Results:** Although the mean BUT_{1st} value in NI-BUT test was found to be 4.4±2.2 seconds, it was 9.4±5.1 seconds in the SH-BUT test (p=0.000). Mean values of BUT_{2nd}, BUT_{3rd}, BUT_{4th}, BUT_{5th} and BUT_{A3} parameters in NI-BUT and SH-BUT tests were found to be 5.2±2.3 and 10.6±4.9 (p=0.000); 5.9±3.1 and 11.9±4.7 (p=0.000); 6.4±3.1 and 12.5±4.4 (p=0.000); 6.8±1 and 12.9±4.3 (p=0.000) and 5.2±2.4 and 10.6±4.8 seconds (p=0.000) respectively. All parameters were found to be longer in the SH-BUT test at statistically significant levels. **Conclusion:** The staining procedure for the fluorescein BUT test causes a prolongation of the BUT test. It is important to consider the changes in BUT because of these effects when performing the fluorescein BUT test.

Keywords: Tear break-up time; fluorescein; dry eye; noninvasive break-up time test; tear film

ÖZET Amaç: Flöresein gözyaşı kırılma zamanı (F-GKZ) testindeki boyama prosedürü için oküler yüzeye temasın GKZ değerlerine etkisini araştırmaktır. **Gereç ve Yöntemler:** Katılımcıların doğal GKZ değerleri invaziv olmayan GKZ ile saptandı. Ardından flöresein boyamasını taklit edecek şekilde, ancak flöresein içermeyen striplerle, boyama prosedürü simüle edildikten sonra gözyaşında oluşan değişimleri topografik GKZ testi ile tekrar değerlendirdik. Boyama simülasyonu sonrası yapılan topografik GKZ testine sham-hybrid GKZ testi (SH-GKZ) dedik. İki test arasında ilk GKZ değeri (GKZ_{ilk}), ikinci GKZ değeri (GKZ_{ikinci}), üçüncü GKZ değeri ($GKZ_{üçüncü}$), dördüncü GKZ değeri ($GKZ_{dördüncü}$), beşinci GKZ değeri ($GKZ_{beşinci}$) ve ilk 3 GKZ'nin ortalama değeri ($GKZ_{3\ ortalama}$) karşılaştırıldı. **Bulgular:** İnvaziv olmayan GKZ testindeki ortalama GKZ_{ilk} değeri 4,4±2,2 sn iken, SH-GKZ testindeki bu değer 9,4±5,1 sn olarak bulundu (p=0,000). $GKZ_{(ikinci)}$ parametresinin invaziv olmayan GKZ testindeki ortalama değeri 5,2±2,3 sn iken, SH-GKZ testindeki bu değer 10,6±4,9 sn olarak saptandı (p=0,000). $GKZ_{üçüncü}$, $GKZ_{dördüncü}$, $GKZ_{beşinci}$ parametrelerinin invaziv olmayan GKZ testindeki ve SH-GKZ testindeki değerleri sırasıyla 5,9±3,1 ve 11,9±4,7sn (p=0,000); 6,4±3,1 ve 12,5±4,4 sn (p=0,000); 6,8±3,1 ve 12,9±4,3 sn (p=0,000) olarak saptandı. $GKZ_{3\ ortalama}$ parametresinin invaziv olmayan GKZ testindeki ortalama değeri 5,2±2,4 sn olarak saptandı buna karşın SH-GKZ testindeki bu parametrenin ortalama değeri 10,6±4,8 sn (p=0,000) olarak saptandı. Tüm parametreler SH-GKZ testinde istatistiksel olarak anlamlı bir şekilde daha uzundu. **Sonuç:** F-GKZ testindeki boyama prosedürü GKZ değerlerinde uzamaya neden olmaktadır. F-GKZ testi yapılırken GKZ değerlerindeki bu değişimler dikkate alınmalıdır.

Anahtar Kelimeler: Gözyaşı kırılma zamanı; flöresein; kuru göz; invaziv olmayan gözyaşı kırılma zamanı; gözyaşı filmi

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Dry eye disease (DED) is a multifactorial disease of tears and the ocular surface with tear film instability, ocular surface damage, inflammation and neurosensory abnormalities playing roles in its etiology. There are symptoms such as blurred vision and discomfort in DED, which is also characterized by findings such as tear film instability and ocular surface damage.[1] The diagnosis of DED is not as complicated as the definition of dry eye explained above. To put it simply, a questionnaire that identifies the patient's symptoms is performed along with the break-up time (BUT) test, which examines the tear film stability.[1,2] The combination of a score exceeding the normal threshold in the first item and a BUT value below the normal threshold in the second item is adequate for the diagnosis of DED.[2]

BUT test plays a central role in the diagnosis of DED.[2,3] BUT testing is performed in two ways. The first of these methods is the fluorescein BUT (F-BUT) test, which involves the observation of break-up in tears using fluorescein. The fluorescein in the strip is allowed to mix with the tear. The observation of the first black spot in the tear under cobalt blue light is noted with a time counter. If the detection time of the black spot is less than 10 seconds, it is considered an unstable tear film.[2-4] In the second method, which is called the non-invasive BUT (NI-BUT) test, which started to be used after the first test with its non-contact and non-invasive characteristic, the tears are examined with special modules or software added to specialized devices for tear film analysis or corneal topography devices and the resulting distortions are analyzed.[4] Although the BUT test is invaluable in DED, the changes caused by fluorescein in the tear film and its effects on BUT values raise questions.[4-7]

In our previous study, the tear film stained with fluorescein with the NI-BUT test was examined. We developed a new method called the hybrid BUT (H-BUT) test, which was created by combining the F-BUT test and the NI-BUT test. In the previous studies, we found that fluorescein caused a prolongation in BUT values in the H-BUT test.[6,7]

In the present study, the we observed the changes in the BUT test by simulating the staining procedure during the F-BUT test. In this way, we uncovered the situations confusing the F-BUT test. We simulated the operations performed in the F-BUT test as follows. During the staining of the tears with fluorescein in the F-BUT test, we examined the changes in the BUT value of the staining along with the changes in tears after the staining procedure simulated by using strips that mimicked fluorescein staining but did not contain fluorescein.

MATERIAL AND METHODS

Mardin Artuklu University Non-Invasive Clinical Research Ethics Committee (date: March 11, 2022; no: 2022/6) was received for the present study, which was conducted following the Declaration of Helsinki principles by obtaining consent forms from all volunteers. Our volunteer population consisted of individuals who applied to the eye clinic. Those who reported a previous cataract history, refractive or other ocular surgery in their CV were also excluded from the study. NI-BUT testing was performed before all the examinations to ensure that the tear film pattern was not altered by slit-lamp or other ocular examinations. We re-evaluated the changes in tears by using the topographic break-up test after simulating the staining procedure with strips that mimicked fluorescein staining without fluorescein (what the researchers mean by topographic method was the NI-BUT test). However, it was considered that the term "non-invasive" would be a "false name" because an invasive procedure such as staining was performed before the test. It was deemed appropriate to use the term "topographic break-up" instead. We called the procedure the sham-hybrid BUT (SH-BUT) test because it involved performing the changes in tears with a topographic break-up test after the staining was simulated with strips that did not contain fluorescein, imitating fluorescein staining.

Selection of the Participants: Only female participants who were aged between 18-30 were recruited in the study. The reason why we choose only the female gender and a narrow age range was that reflex tears because of contact with the ocular surface vary with age and gender.[8] The study procedure was performed as follows. Firstly, the natural state of tears was documented with the NI-BUT test. Then, the

changes that were caused by the staining simulation process in tear BUT values were examined with the SH-BUT test.

NI-BUT Test Procedure: The participants were explained in detail how the test would be performed and the situations they should pay attention to before the procedure, were asked to rest their chin and forehead on the apparatus and blink their eyes twice upon command. The device module automatically recorded and analyzed the distortions in the tears over time after the second blink. Those whose NI-BUT test duration was shorter than 10 seconds and without at least 3 break-ups were eliminated at this stage of the study. There was a 15-minute break after the NI-BUT test and the SH-BUT test was started.

THE CONSTRUCTION AND PROCEDURE OF THE SH-BUT TEST

Clinical Background

Commercial standard fluorescein strips do not have a fluorescein-free form. The non-fluorescein part of the fluorescein strips, in other words, the part held by the physician, is very thick and harder than the fluorescein part and can also be uncomfortable for the patient. Contact of this part with the ocular surface may cause more reflex tear secretion. For these reasons, we needed to find a strip that had the closest characteristics and appearance to the fluorescein strips and was also sterile and non-toxic to the ocular surface. The commercial ready-made strips developed for the schirmer test (Schirmer Tear Test, Haag-Streit, UK) are suitable for use on the ocular surface. Also, these strips were used for the fluorescein staining procedure because they had similar characteristics to fluorescein strips and were sterile.

The SH-BUT test procedure was as follows. The schirmer tear test strips were gently applied to the inferotemporal-conjunctival areas twice when the patients were looking up. In this way, staining was simulated by applying strips to the conjunctival tissue without fluorescein. They were asked to blink their eyes twice with the command. The device module automatically records and analyzes distortions in tears over time after the second blink. The participants who kept their eyes open for less than 10 seconds in the SH-BUT test were excluded from the study. The BUT values of the participants whose tears did not break-up during the test were recorded as 17 seconds (for quantitative values).

The NI-BUT test and SH-BUT test were performed with the Sirius™ corneal topography device (Costruzione Strumenti Oftalmici, Srl, Italy). The device takes images from the ocular surface at 25 film frames per second with a videokeratoscope and analyzes the distortions in the tear film based on the video images with its special software, which provides numerical and locational data of the break-up formed in tears.[4,6,7,9]

THE PARAMETERS COMPARED IN THE NI-BUT TEST AND THE SH-BUT TEST (Figure 1A, Figure 1B)

1) First BUT value (BUT_{1st}): the value of the time when the first breakup occurs in seconds.

2) Second BUT value (BUT_{2nd}): the value of the time when the second breakup occurs in seconds.

3) Third BUT value (BUT_{3rd}): the value of the time when the third breakup occurs in seconds.

4) Fourth BUT value (BUT_{4th}): the value of the time when the fourth breakup occurs in seconds.

5) Fifth BUT value (BUT_{5th}): the value of the time when the fifth breakup occurs in seconds.

6) Average value of the first 3 break-ups (BUT_{A3}): average value of the first 3 break-ups in seconds.

7) Average value of the first 5 break-ups (BUT_{A5}): average value of the first 5 break-ups in seconds.

8) Average value of all break-ups (BUT_{avg}): average value of all break-ups during the measurement period for each participant in seconds.

9) The number of break-ups ≤5 seconds (NoB≤5sec): the number of break-ups less than or equal to 5 seconds in the tests for each participant.

10) The number of break-ups ≤7 seconds (NoB≤7sec): the number of break-ups less than or equal to 7 seconds in the tests for each participant.

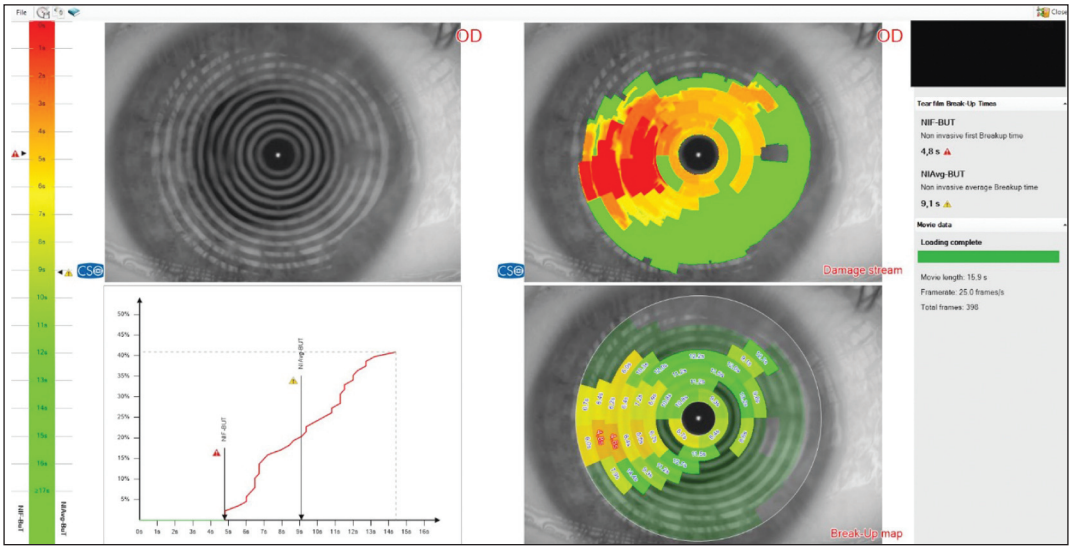

FIGURE 1A: The non-invasive break-up time test and display of parameters of one of the participants.

$BUT_{1st(s)}$: The value of the time when the first breakup occurred in seconds=4.8 sec

$BUT_{2nd(s)}$: The value of the second breakup occurred in seconds=4.8 sec.

$BUT_{3rd(s)}$: The time when the third breakup occurred in seconds=5.5 sec.

$BUT_{4th(s)}$: The value when the fourth breakup occurred in seconds=6 sec.

$BUT_{5th(s)}$: The value of the time when the fifth breakup occurred in seconds=6 sec.

$BUT_{A3(s)}$: The average value of the first 3 break-ups in seconds (4.8+4.8+5.5)/3=5.03 sec.

$BUT_{A5(s)}$: The average value of the first 5 break-ups in seconds (4.8+4.8+5.5+6+6)/5=5.42 sec.

$BUT\leq_{A5(s)}$: The average value of all break-ups equal to or less than 5 seconds in seconds (4.8+4.8)/2=4.8 sec.

$BUT\leq_{A7(s)}$: The average value of all break-ups less than or equal to 7 seconds in seconds (4.8+4.8+5.5+6+6+6,2+6.4+6.4+6.4+6.7+6.7+6.7+6.9)/13=6.11 sec.

$BUT\leq_{A10(s)}$: The average value of all break-ups less than or equal to 10 seconds in seconds (4.8+4.8+5.5+6+6+6,2+6.4+6.4+6.4+6.7+6.7+ 6.7+6.9+7.2+7.9+8.4+8.6+ 9.1+9.3+9.3+9.8)/21=7.1 sec.

$BUT_{avg(s)}$: The average value of all break-ups during the measurement period for each participant in seconds=9.1 sec.

$Percent_{(\%)}$: It shows the percentage of the cornea in which break-up occurs during the measurement=41.

NoB-S-HMFLD (n): The number of break-ups in the superior hemi-field during the test=21.

NoB-I-HMFLD (n): The number of break-ups in the inferior hemi-field during the test=15.

NoB≤5s (n): The number of all break-ups less than or equal to 5 s during the test=2.

NoB≤7s (n): The number of all break-ups less than or equal to 7 s during the test=13.

NoB≤10s (n): The number of all break-ups less than or equal to 10 s during the test=21.

11) The number of break-ups ≤10 seconds (NoB≤10sec): the number of break-ups less than or equal to 10 seconds in the tests for each participant.

12) The number of break-ups occurring in the superior hemi-field (NoB-S-HMFLD): (The cornea was divided into upper and lower hemi-fields with an imaginary line that passed horizontally through the middle of the cornea). The NoB-S-HMFLD of the cornea during the test.

13) The number of break-ups occurring in the inferior hemifield (NoB-I-HMFLD): The NoB-I-HMFLD of the cornea during the test.

14) BUT $\leq_{A5\,(sec)}$: the average value of all break-ups in seconds equal to or less than 5 seconds.

15) BUT $\leq_{A7\,(sec)}$: the average value of all break-ups in seconds is equal to or less than 7 seconds

16) BUT $\leq_{A10\,(sec)}$: the average value of all break-ups in seconds less than or equal to 10 seconds

17) Percent (%): it shows the percentage of the cornea in which break-up occurs during the measurement.

18) The corneal surface for qualitative values was divided into 4 quadrants as 0-90 degrees; 90-180 degrees; 180-270 degrees, and 270-360 degrees. The

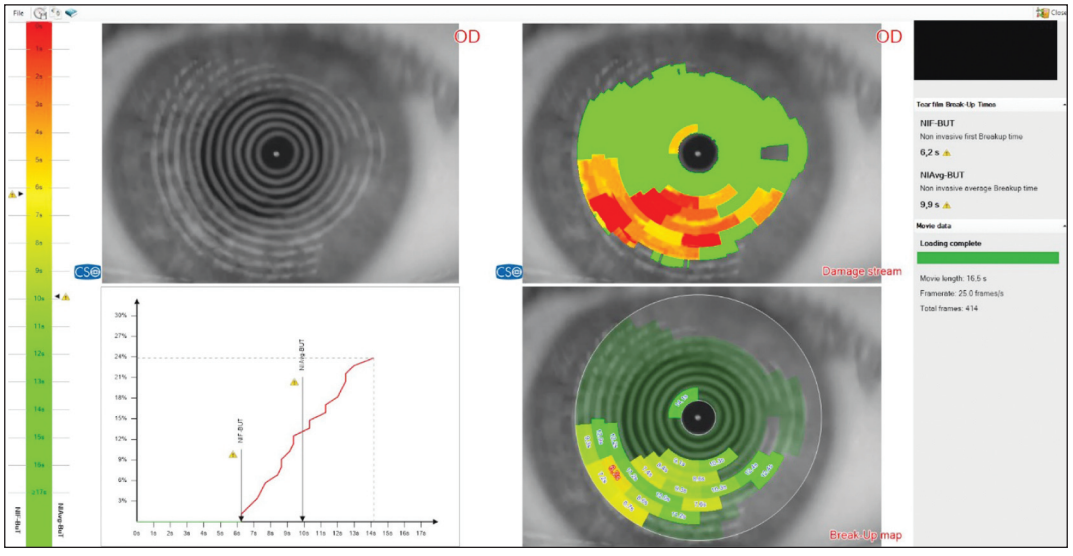

FIGURE 1B: The sham-hybrid break-up time test results of the same participant given in 1A.
$BUT_{1st(s)}$: The value of the time when the first breakup occurred in seconds=6.2 sec.
$BUT_{2nd(s)}$: The value of the time when the second breakup occurred in seconds=6.7sec.
$BUT_{3rd(s)}$: The value of the time when the third breakup occurred in seconds=7.2 sec.
$BUT_{4th(s)}$: The value of the time when the fourth breakup occurred in seconds=7.4 sec.
$BUT_{5th(s)}$: The value of the time when the fifth breakup occurred in seconds=7.6 sec.
$BUT_{A3(s)}$: The average value of the first 3 break-ups in seconds (6.2+6.7+7.2)/3=6.7 sec.
$BUT_{A5(s)}$: The average value of the first 5 break-ups in seconds (6.2+6.7+7.2+7.4+7.6)/5=7.02 sec.
$BUT\leq_{A5(s)}$: The average value of all break-ups is less than or equal to 5 seconds in seconds (N/A).
$BUT\leq_{A7(s)}$: The average value of all break-ups is less than or equal to 7 seconds in seconds (6.2+6.7)/2=6.45 sec.
$BUT\leq_{A10(s)}$: The average value of all break-ups less than or equal to 10 seconds in seconds (6.2+6.7+7.2+7.4+7.6+8.4+8.6+8.6+9.1+9.3+9.3)/11=8.03sec.
$BUT_{avg(s)}$: The average value of all break-ups during the measurement period for each participant in seconds=9.9 sec.
Percent $_{(\%)}$: It shows the percentage of the cornea in which break-up occurs during the measurement=24.
NoB-S-HMFLD (n): The number of break-ups in the superior hemi-field during the test=1.
NoB-I-HMFLD (n): The number of break-ups in the inferior hemi-field during the test=20.
NoB≤5s (n): The number of all break-ups less than or equal to 5 s during the test=N/A.
NoB≤7s (n): The number of all break-ups less than or equal to 7 s during the test=2.
NoB≤10s (n): The number of all break-ups less than or equal to 10 s during the test=11.

quadrants in which the first 3 break-ups occurred were compared between the two tests.

18a) QUAD $_{(1st\ breakup)}$: the quadrant where the first break-up occurred.

18b) QUAD $_{(2nd\ breakup)}$: the quadrant where the second break-up occurred.

18c) QUAD $_{(3rd\ breakup)}$: the quadrant where the third break-up occurred.

19) The cornea surface was divided into upper and lower hemi-fields by a fairly horizontal line that passed through the middle, and a comparison was made between the two tests in terms of the hemi-fields where the first break-up occurred.

Hemi-field $_{(1^{st}breakup)}$: It shows in which hemi-field the first break-up occurs (superior/inferior hemi-field).

STATISTICAL METHOD

The mean, standard deviation, median, lowest, highest, frequency, and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured with the Kolmogorov-Smirnov test. The Wilcoxon test was used to analyze the dependent quantitative data and the McNemar test was used to analyze the qualitative dependent data. The "Statistical Package for Social Sciences (SPSS) for Windows, version 28.0. (IBM Corp. Armonk, NY, U.S., 2021)" was used in the analyses.

RESULTS

The present study was conducted with 44 female participants the ages of whom were between 18-30. The first 5 BUT values were statistically and significantly longer in the SH-BUT test than in the NI-BUT test ($p<0.05$) (Table 1). No significant differences were detected in the localization comparisons between the two tests ($p>0.05$) (Table 2).

DISCUSSION

We found in the present study that the fluorescein staining procedure caused a prolongation in quantitative BUT values. We detected significantly longer BUT_{1st}, BUT_{2nd}, BUT_{3rd}, BUT_{4th}, and BUT_{5th} parameters in the SH-BUT test. To eliminate the misleading effect of the testing time, we determined that the average value of the first 3 break-ups (BUT_{A3}) and the average value of the first 5 break-ups (BUT_{A5}) were longer in the SH-BUT test in the present study. We also found that the value in the BUT_{avg} parameter, which is the average value of all break-ups in the test for each participant and varied depending on the test duration, which is automatically appointed by the device, was longer in the SH-BUT test (Table 1). However, we did not detect significant differences between the two tests in terms of qualitative values such as the quadrant where the first 3 break-ups occurred and the hemi-field where the first break-up occurred (Table 2).

The tears is a secretion produced by the lacrimal and accessory glands, Meibomian glands, and goblet cells of the conjunctiva. Tear secretion occurs in four ways. 1- secretion in the closed eye is the tear se-

TABLE 1: Comparison of the parameters in the NI-BUT and SH-BUT tests.

	NI-BUT		SH-BUT		
	X̄±SD	Median	X̄±SD	Median	p[w]
Age (years)	23.4±4.0	23.0			
$BUT_{1st(s)}$	4.4±2.2	4.0	9.4±5.1	7.5	0.000
$BUT_{2nd(s)}$	5.2±2.3	4.8	10.6±4.9	9.1	0.000
$BUT_{3rd(s)}$	5.9±3.1	5.4	11.9±4.7	12.0	0.000
$BUT_{4th(s)}$	6.4±3.1	5.7	12.5±4.4	13.6	0.000
$BUT_{5th(s)}$	6.8±3.1	6.0	12.9±4.3	14.0	0.000
$BUT_{A3(s)}$	5.2±2.4	4.8	10.6±4.8	9.1	0.000
$BUT\leq_{A5(s)}$	3.8±0.8	4.0	3.9±0.8	4.2	0.260
$BUT\leq_{A7(s)}$	5.1±1.2	5.2	5.5±1.2	5.7	0.173
$BUT\leq_{A10(s)}$	6.4±1.9	6.7	7.5±1.2	7.9	0.044
$BUT_{avg(s)}$	7.4±3.1	7.9	12.2±3.8	11.9	0.000
NoB-S-HMFLD (n)	6.1±5.5	5.0	1.5±3.4	0.0	0.000
NoB-I-HMFLD (n)	11.1±8.6	10.0	5.6±6.8	3.0	0.001
NoB≤5s (n)	4.0±5.9	2.0	0.5±1.3	0.0	0.000
NoB≤7s (n)	7.9±8.7	5.5	1.2±2.3	0.0	0.000
NoB≤10s (n)	12.9±11.0	9.5	4.1±6.8	2.0	0.000
$BUT_{A5(s)}$	5.7±2.6	5.3	11.3±4.5	10.2	0.000
Percent (%)	20.0±13.5	16.0	8.2±10.1	5.0	0.000

[w]Wilcoxon test; NI-BUT: Non-invasive break-up time test; SH-BUT: Sham-hybrid break-up time test; SD: Standard deviation; BUT_{1st}: The time when the first breakup occurs in seconds; BUT_{2nd}: The time when the second breakup occurs in seconds; BUT_{3rd}: The time when the third breakup occurs in seconds; BUT_{4th}: The time in seconds at which the fourth breakup occurs; BUT_{5th}: The time when the fifth breakup occurs in seconds; BUT_{A3}: The average value of the first 3 break-ups in seconds; $BUT\leq_{A5}$: The average value of all break-ups in seconds equal to or less than 5 seconds; $BUT\leq_{A7}$: The average value of all break-ups in seconds is equal to or less than 7 seconds; $BUT\leq_{A10}$: The average value of all break-ups in seconds less than or equal to 10 seconds; $BUT_{avg(s)}$: The average value of all break-ups in seconds during the measurement period for each participant; NoB-S-HMFLD: The number of break-ups in the superior hemi-field during the test; NoB-I-HMFLD: The number of break-ups in the inferior hemi-field during the test; NoB ≤5s: The number of all break-ups less than or equal to 5 s during the test period; NoB ≤7s: The number of all break-ups less than or equal to 7 s during the test period; NoB ≤10s: The number of all break-ups less than or equal to 10 s during the test period; $BUT_{A5(s)}$: The average value of the first 5 break-ups in seconds; Percent (%): It shows the percentage of the cornea in which break-up occurs during the measurement.

TABLE 2: Distribution of breakup locations between the tests.

		NI-BUT		SH-BUT		
		n	%	n	%	p^N
$QUAD_{(1st\ breakup)}$:	0-90	5	15.2%	2	6.1%	0.221
	90-180	6	18.2%	3	9.1%	
	180-270	18	54.5%	18	54.5%	
	270-360	4	12.1%	10	30.3%	
$QUAD_{(2nd\ breakup)}$:	0-90	1	3.2%	2	6.5%	0.598
	90-180	7	22.6%	3	9.7%	
	180-270	19	61.3%	20	64.5%	
	270-360	4	12.9%	6	19.4%	
$QUAD_{(3rd\ breakup)}$:	0-90	3	11.1%	1	3.7%	0.215
	90-180	2	7.4%	8	29.6%	
	180-270	19	70.4%	13	48.1%	
	270-360	3	6.8%	5	18.5%	
$Hemifield_{(First\ breakup)}$:	**Superior**	15	34.1%	5	15.2%	0.146
	Inferior	29	65.9%	28	84.8%	

^{N}McNemar test; NI-BUT: Non-invasive break-up time test; SH-BUT: Sham-hybrid break-up time test; The corneal surface for qualitative values was divided into 4 quadrants as 0-90 degrees; 90-180 degrees; 180-270 degrees, and 270-360 degrees. The quadrants in which the first 3 break-ups occurred were compared between the two tests;

$QUAD_{(1st\ breakup)}$: The quadrant where the first break-up occurred.

$QUAD_{(2nd\ breakup)}$: The quadrant where the second break-up occurred.

$QUAD_{(3rd\ breakup)}$: The quadrant where the third break-up occurred.

The cornea surface was divided into upper and lower hemi-fields by a fairly horizontal line that passed through the middle, and a comparison was made between the two tests in terms of the hemi-fields where the first break-up occurred. $Hemi\text{-}field_{(1st\ breakup)}$: It shows in which hemi-field the first break-up occurs (Superior/Inferior Hemi-field).

creted during sleep. 2- basal tear secretion is the secretion occurring when the eye is open. 3- emotional tear secretion is the secretion occurring with sadness and emotional stimulation. 4- reflex tear secretion is the secretion of the ocular surface resulting from chemical or mechanical stimulation.[1,11,12] Any contact with the ocular surface epithelium, cornea, and conjunctiva leads to the formation of secretions from the lacrimal gland, goblet cells in the conjunctiva and meibomian glands.[1,10-13]

In their study conducted with gas estesiometer, Acosta et al. reported that the sensation thresholds for mechanical, chemical, and thermal stimulation of the cornea and conjunctiva were similar, although less in the conjunctiva.[14] To the best of our knowledge, there is no other study investigating how contact with the ocular surface causes changes in the BUT values for the fluorescence staining. In the present study, we found that reflex tear secretion, which resulted from the staining procedure, caused a prolongation in BUT values. The results of the present study show that the mechanical effect (staining-touching) is effective in reflex tear secretion and therefore in BUT values in addition to the chemical factor (fluorescein). In their study, Sheppard et al. reported that nasal stimulus caused a prolongation of the Schirmer test in the early and late periods.[15] In their study, Friedman et al. reported that nasal stimulation caused decreased conjunctival staining and improved dry eye symptoms. On the other hand, they also reported that nasal stimulus did not cause any changes in BUT values.[16] In their study, Cohn et al. reported that nasal stimulus did not cause any changes in the corneal staining scores or BUT values, but caused an increase in the Schirmer scores.[17] Many studies are reporting that intranasal tear neurostimulators, which activate reflex tear secretion, cause an increased tear secretion. However, no significant changes were detected in the BUT values with nasal stimulus.[15-17] This may cause us to ask the following question; “does contact with the ocular surface and contact with the nasal area cause different tear film structures?” the answer to this question can be found with a detailed examination of the layers that make up the tear film (lipid, aqueous, and mucus layers) and other components that make up the tear. However, it was determined

that the nasolacrimal reflex that is activated by nasal stimulation provides an increase in the aqueous component of the tear by 1/3.[18] In their study, Gumus et al. reported that nasal stimulation also increased secretion in goblet cells.[19] In both studies, the changes in tears caused by the nasolacrimal reflex activated by nasal stimulation were examined.[18,19] In the present study, the tear film stability was examined by directly contacting the ocular surface - by activating the oculolacrimal reflex arc. Although the nasolacrimal reflex arc and the oculolacrimal reflex arc end up in the same efferent ends, it is considered that their complex interactions may cause different results.[20] This information may explain the different results in the BUT values between previous studies and ours.[15-18] However, further studies to be conducted to investigate tear content are needed to determine this. In their study, Lam et al. reported that staining tears with fluorescein strips caused an approximately 0.03 mm increase (12%) in tear meniscus height.[21] In their study, Gumus et al. reported a 28% increase in tear meniscus height because of nasal stimulus in dry eye patients. In addition to the differences in the methods, the differences in the location of the stimulation may also have played roles in the differences in the changes in tear meniscus height between the two studies.[19,21] In their study, Jordan and Baum reported that the tear turnover rate increased at a rate of 300% when the lower eyelid margin was stimulated with a mild stimulus, even in an eye whose reflex tear secretion was reduced by an anesthetic agent. In the same study, the physiological flow value of tears was determined to be approximately 1 µL/min, but the flow value was approximately 2.7-4.4 µL/min with stimulation of the lid margin.[22] In the study that was conducted by Sørensen and Jensen it was reported that the tear flow rate in the fellow eye of the eye stimulated with a strip was 4.4 µL/min (approximately 4-fold the normal).[23]

In the study that was conducted by Johnson and Murphy it was found that fluorescein strips caused a change in BUT value equivalent to 1 µL fluorescein solution and the BUT value was statistically and significantly longer with 2.7 µL fluorescein solution than the values with lower doses. In the same study, when the amount of fluorescein solution was increased from 2.7 µL to 7.4 µL, they did not detect any significant increases in the BUT value and argued that a 2.7 µL fluorescein solution caused thickening of the tears and therefore a longer BUT.[24] In the present study, we detected increased BUT values by simply touching the conjunctival tissue, without adding any substance to the tear. Our results cannot be explained by the assumption that the amount of fluorescein solution is associated with a longer BUT value as a result of the thickening of the tear. Our results show that other factors may also affect the BUT value as well as the externally administered fluorescein solution. Before explaining other factors, the researchers think it would be useful to briefly explain how tear film stability was disrupted and how a break-up occurred in tears.

Break-ups or dry spots in the tear occur as a result of diffusion of the superficial lipid layer to the mucus-aqueous interface. The speed at which the surface lipid layer reaches the deeper layers of the tear depends on the thickness of the aqueous layer. According to this assumption, the thickness of the aqueous layer and tear film stability are directly proportional.[25] In the study conducted by Sharma and Ruckenstein the importance of the mucus layer in tear film stability was emphasized. The interaction of the mucus layer, which has a thickness of approximately 0.03 µm, with the lipid and aqueous layers, which are the other layers of the tear, provides that the tear remains stable for a certain period, in other words, remains without any break-up. Previous studies argued that break-up in the tear occurred at a much later period because of evaporation and lipid deficiency.[26] As a side note, the only source of the mucus layer is the surface epithelium of the cornea and conjunctiva and the goblet cells in the conjunctiva.[26-28] According to previous studies, either a thin aqueous layer or a dysfunction in the mucus layer plays roles in the deterioration of tear film stability, in other words, in the formation of a break-up in the tear in a short period.[25,26]

What did we do to the eye when testing the SH-BUT? We touched the conjunctiva twice with the strip while performing the fluorescein staining procedure and activated the reflex tear arc. We created a

dominant and high-volume tear secretion of the aqueous component with the active reflex tear arc, in which the lacrimal gland secretion was at the forefront. We also ensured the formation of mucus-based secretion from goblet cells and asked the participants to blink twice for the SH-BUT test. We ensured that this reflex tear was released by blinking mixed with the lipid and mucus layer.[13-19] In this way, according to the model suggested by Holly et al. that thicker tear film had a longer BUT value, we found that it was more stable and therefore had a longer BUT value.[26] According to the break-up model of Holly, the results of the present study seem to be consistent with the increase in reflex tear secretion.[25]

We did not perform a volumetric evaluation of the reflex tear secretion resulting from touching the ocular surface. i) we did not perform the Schirmer test because it would cause reflex tear secretion. ii) the tear meniscus height was not evaluated because the researchers did not have a non-contact and non-invasive device to detect the tear meniscus height. We considered the fact that how much reflex tear secretion increased in volume was not investigated as the limiting factor of the study.

CONCLUSION

We examined the changes in the BUT test by simulating the fluorescein BUT test, it was concluded that touching the ocular surface causes reflex tear secretion, which fulfills its responsibility and makes tears more stable. Although this is essential for the continuity of the ocular surface and tear film, it causes a prolongation of BUT values. The changes in the BUT value occurring because of the effects of reflex arcs must not be ignored while performing the BUT test.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Yakup Acet;* ***Design:*** *Yakup Acet;* ***Control/Supervision:*** *Yakup Acet;* ***Data Collection and/or Processing:*** *Yakup Acet, Yaşar Dağ;* ***Analysis and/or Interpretation:*** *Yakup Acet;* ***Literature Review:*** *Yakup Acet;* ***Writing the Article:*** *Yakup Acet, Yaşar Dağ;* ***Critical Review:*** *Yakup Acet;* ***References and Fundings:*** *Yakup Acet;* ***Materials:*** *Yakup Acet.*

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