Correlation between conjunctival scraping cytology and other clinical dry eye metrics in determination of dry eye related inflammation

Korelacija između citološkog nalaza epitela konjunktive dobijenog skrajpingom i drugih kliničkih testova pri utvrđivanju inflamacije suvog oka

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Abstract

Background/Aim. New and improved definition of dry eye disease (DED) emphasized that hiperosmolarity and inflammation with initial tear film instability play etiological role. The aim of this study was to explore relation of some commonly used clinical tests to dry eye disease (DED) related inflammation measured by conjunctival scraping cytology.

Methods. We examined 100 subjects, 80 of them having DED. We performed Schirmer without anesthesia (Schirmer I), Fluorescein Tear Break Up Time (FTBUT), Rose Bengal (RB), Lid Parallel Conjunctival Folds (LIPCOF), Tear Meniscus Height (TMH) and Tear Ferning (TF) and compared the values to scraping scores of tarsal conjunctiva.

Results. FTBUT had the best sensitivity (93.6%). The highest specificity was found with RB (93.2%), but it was also high with Schirmer I, TF and FTBUT (respectively 89.8%, 84.5%, 78.0%). RB and FTBUT had the highest correlation with conjunctival scraping score (r = 0.707, p < 0.001; r = -0.507, p < 0.001).

Conclusion. In our study, FTBUT, though often used in many combinations of the DED tests, showed a remarkably high sensitivity and specificity on its own, as well as good correlation with DED related inflammation detected with conjunctival scraping cytology.

Key words: dry eye syndromes; conjunctiva; cytological techniques; sensitivity and specificity; diagnostic tests, routine; diagnosis, differential.

Apstrakt

Uvod/Cilj. Nova unapređena definicija bolesti suvog oka ističe hiperosmolarnost i upalu sa inicijalnom nestabilnošću suznog omotača kao najčešće etiološke faktore. Cilj rada bio je da se ispita korelacija između nekih često korишćenih kliničkih testova za suvo oko i citološkog nalaza epitelia konjukтивне добијеног скрежимом при утврђивању инфламације сувог ока.

Mетоде. Od ispitanih 100 bolesnika, dijagnozu suvo oko smo postavili kod 80. Učinjeno je merenje sekrecije suza bez anestezije u 5 minuta Schirmer trakom (Schirmer I), bojenje površine oka vitalnom bojom Rose Bengal (RB), merenje visine meniškusa suza (Tear Meniscus Height – TMH) i urađen test grana-
Introduction

While investigation on complex mechanism of dry eye disease (DED) has still been ongoing, hiperosmolarity and inflammation that are underlined since the first Dry Eye Workshop (DEWS) report 1 as well as by the OCEAN group 2 are something that all of us are focused on. Advance in the diagnostic tools, but also in therapy are based on these two crucial steps in that vicious circle 3, 4. New and improved definition of DED, published within the Tear Film and Ocular Surface Society (TFOS) DEWS II Definition and Classification Report 5, emphasizes that hiperosmotality and inflammation, together with the initial tear film instability and neurosensory abnormalities play etiological roles.

Measuring osmolarity, especially after the introduction of portable in situ osmometer (TearLab™, OcuSence, Tear-Lab Corp, San Diego, CA, USA) into clinical practice, seems to be a good way to recognize DED in its early stage 2, 6. However, there is a question of overlapping of normal subjects and mild form of dry eye (DE) 7. The rapid point-of-care diagnostic, 9-level test (InflammaDry; Rapid Pathogen Screening, Inc, Sarasota, FL) to detect elevated matrix metalloproteinase, was reported by Sambursky et al. 7 as a diagnostic tool with high sensitivity and specificity when detecting DE related inflammation. Messmer et al. 8 identified the presence of ocular surface inflammation in 40% of confirmed DE patients with this diagnostic test. Although the time-consuming laboratory test, the conjunctival scraping was introduced by Versura et al. 9 as a reliable method to diagnose and score ocular surface inflammation in DE.

We were interested in exploring relation of some commonly used clinical tests available to ophthalmologists in our country with DED-related inflammation measured by conjunctival scraping cytology, in order to make the decision easier as to start an anti-inflammatory treatment.

Methods

We examined 100 subjects (200 eyes), 88 woman and 12 men. Mean age ± standard deviation (SD) was 50.17 ± 16.74 years. Eighty of them were referred to us by rheumatologists and general practitioners either during evaluation for the Sjögren syndrome (SS – 30 patients), or because of dry eye symptoms (50 patients). The control group was made of 20 patients in a period of one year, contact lens wear, topical eye drops and general practitioners either during evaluation for the Sjögren syndrome (SS – 30 patients), or because of dry eye symptoms (50 patients). The exclusion criteria were any ocular surgery that was performed in the period of one year, contact lens wear, topical eye therapy (if only tear substitutes, they had to be suspended at least 8 hours before the examination), entropion, ectropion, or other lid closure problems as well as ocular allergies, or presence of anterior blepharitis. The patients suspected to have the SS were not yet under any kind of systemic anti-inflammatory therapy. The study was approved by the Ethics Committee, of the Faculty of Medicine, Belgrade University. All patients signed an informed consent form.

We performed the following clinical tests: Schirmer without anesthesia (Schirmer I), fluorescein tear break up time (FTBUT), Rose Bengal (RB), Lid Parallel Conjunctival Folds (LIPCOF), Tear Meniscus Height (TMH) and Tear Ferning (TF). Eyelids were inspected for meibomian gland dysfunction (MGD). We also performed scraping of tarsal conjunctiva in order to evaluate ocular surface inflammation. Symptoms were evaluated on the basis of the Ocular Surface Disease Index (OSDI) and McNemmar’s questionnaires.

To confirm the DE diagnosis in our study, we considered results from a group of three clinical tests. These three tests, the Schirmer I, FTBUT and RB, represented the ophthalmological part of testing for SS according to the Copenhagen criteria, but proved useful in diagnosing DE out of SS context, also 10. Eighty patients, as we expected, had the dry eye disease, since one, or both eyes were positive in 2 of 3 clinical tests. Twenty patients from this symptomatic group had some form of MGD. In the control group, no eye met these criteria. One patient from the control group had MGD, without the signs, or symptoms of DED. Bearing in mind that we analyzed separately both eyes, we found that 139 eyes were positive and 61 negative for DED. We also graded the DE severity from 0 to 4 according to the DEWS report score system 1. Numbers of eyes within different grades are presented in Table 1.

Table 1: Distribution of eyes according to dry eye severity with the 0–4 score system from the DEWS report

<table>
<thead>
<tr>
<th>Dry eye severity</th>
<th>Number of eyes</th>
<th>%</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37</td>
<td>18.5</td>
<td>18.5</td>
</tr>
<tr>
<td>1</td>
<td>54</td>
<td>27.0</td>
<td>45.5</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>37.5</td>
<td>83.0</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>11.5</td>
<td>94.5</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>5.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

DEWS – Dry Eye Workshop Severity.

All tests were performed during one examination in the morning by two examiners. First, we examined the patients’ TMH and LIPCOF. TMH was measured by slit-lamp. We registered the values as 0.3 mm, 0.2 mm, 0.1 mm, less than 0.1 mm, using the slit–lamp microscope with objective lens graticule in 0.1 units. For the LIPCOF test, we registered in the temporal zone the values as no folds, ½ of fold in the temporal zone, one fold less that 0.2 mm height, two folds 0.2 mm height, 3 folds or more over 0.2 mm. Although similar, these stages, are not completely analogous to those most commonly used, described by Höh et al. 11 Instead of using a term normal meniscus tear height, we used the value of 0.2 mm as a cut-off value between the stages. Other authors also used this value as a normal one 12, and considered pathologi cal if below 13. We also divided the Stage 1 by Höh into two stages with present folds, in order to form four grades as the DEWS dry eye severity score system has. Then we performed the Schirmer I, FTBUT and RB test as outlined in the DEWS report 14. After folding the Schirmer paper strip at the notch, we placed the shorter part under the temporal one-third of the lower lid of both eyes. The patients were asked to close their eyes. We measured the length of wetting from the...

The average value of scraping scores for the group of eyes diagnosed as dry according to the Copenhagen criteria was 5.33 ± 1.99 (95% CI 5.00–5.66), while the average value for the group of non-dry eyes was 2.75 ± 2.04 (95% CI 2.23–3.28). The difference in the average scraping score between the two groups was found to be highly statistically significant by the Student’s t-test ($t = 8.368, p < 0.001$).

Of all clinical tests that we used, as compared to the conjunctival scraping, the FTBUT as a single test had the best sensitivity (93.6%). The LIPCOF and TMH also had a high sensitivity (92.2% and 80.9%, respectively). The highest specificity was found with RB (93.2%), but it was also high with Schirmer I, TF and FTBUT (89.8%, 84.5%, 78.0%, respectively) (Table 2).

The average scraping scores for the different groups of eyes were graded according to the DEWS report and presented in Figure 1. Most overlapping occurs between normal eyes and eyes with a mild form of dry eye. The difference between all other DEWS groups was statistically significant ($F = 43.197; p < 0.001$).

**Fig. 1 – The mean scraping scores in different dry eye severity groups.**

The average scraping scores for the different groups of eyes graded in the DEWS report show that the most of the overlapping we have between normal eyes and the ones that have a mild form of dry eye. The difference among all the other DEWS groups is statistically significant ($F = 43.197, p < 0.001$).

DEWS – Dry Eye Workshop Severity.

Of all clinical tests that we used, as compared to the conjunctival scraping, the FTBUT as a single test had the best sensitivity (93.6%). The LIPCOF and TMH also had a high sensitivity (92.2% and 80.9%, respectively). The highest specificity was found with RB (93.2%), but it was also high with Schirmer I, TF and FTBUT (89.8%, 84.5%, 78.0%, respectively) (Table 2).

All the tests were in a statistically significant correlation with the conjunctival scraping and among themselves. RB and FTBUT had the highest correlation factor with conjunctival scraping ($r = 0.707, p < 0.001$; $r = -0.507, p < 0.001$). Among the clinical tests, the best correlation was found between FTBUT and RB ($r = -0.620, p < 0.001$), and FTBUT and TF ($r = -0.535, p < 0.001$) (Table 3).

**Table 2**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FTBUT</th>
<th>RB</th>
<th>Sch I</th>
<th>LIPCOF</th>
<th>TMH</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se (%)</td>
<td>93.6</td>
<td>45.4</td>
<td>41.1</td>
<td>92.2</td>
<td>80.9</td>
<td>59.9</td>
</tr>
<tr>
<td>Sp (%)</td>
<td>77.9</td>
<td>93.2</td>
<td>89.8</td>
<td>33.9</td>
<td>44.1</td>
<td>84.5</td>
</tr>
<tr>
<td>PPV</td>
<td>0.91</td>
<td>0.94</td>
<td>0.91</td>
<td>0.77</td>
<td>0.78</td>
<td>0.89</td>
</tr>
<tr>
<td>NPV</td>
<td>0.85</td>
<td>0.42</td>
<td>0.39</td>
<td>0.65</td>
<td>0.49</td>
<td>0.48</td>
</tr>
</tbody>
</table>

PPV – positive predictive value; NPV – negative predictive value; FTBUT – Fluorescein Tear Break Up Time; RB – Rose Bengal; Sch I – Schirmer I; LIPCOF – Lid Parallel Conjunctival Folds; TMH – Tear Meniscus Height; TF – Tear Ferning.

We analyzed the results of McMonnies and OSDI questionnaires and they were in a positive correlation \((r = 0.644; p < 0.001)\). When we compared them with the inflammatory cell scores acquired with scraping of tarsal conjunctiva of worse eye, we found that the correlation coefficient was \(r = 0.315\) for the McMonnies questionnaire which was highly significant \((p = 0.001)\), and correlation with the OSDI questionnaire was significantly positive as well \((r = 0.290; p = 0.003)\). The patients with a higher score of inflammatory cells in tarsal conjunctiva had a higher score on both questionnaires.

When comparing the results of conjunctival scraping of patients younger than 60, we found a positive correlation \((r = 0.644; p < 0.001)\). When we compared them with the inflammatory cell scores acquired with scraping of tarsal conjunctiva of worse eye, we found that the correlation coefficient was \(r = 0.315\) for the McMonnies questionnaire which was highly significant \((p = 0.001)\), and correlation with the OSDI questionnaire was significantly positive as well \((r = 0.290; p = 0.003)\). The patients with a higher score of inflammatory cells in tarsal conjunctiva had a higher score on both questionnaires.

The relation between the inflammation and some of the inflammatory parameters, including the McMonnies questionnaire, was highly significant. The McMonnies questionnaire \((r = 0.349; p = 0.002)\), OSDI \((r = 0.341; p = 0.003)\), and TMH \((r = 0.684; p < 0.001)\) were all significantly correlated with the inflammatory parameters. The correlation coefficients were all above 0.3, indicating a strong positive relationship.

The average score of conjunctival scraping was higher in the group over 60 years of age than in the group of subjects aged under 60 years, and the difference was statistically significant \((t = -1.991, p = 0.049)\). For the over 60 years of age group, the average scraping score was 5.77, and for the under 60 years of age group, it was 4.74.

There was no difference between two age groups in the average scores of both questionnaires \((t = -1.495; p = 0.138)\). Of all examined patients, 88% of them were women (88 out of 100 patients). In the group diagnosed with DED, 91.2% were women, which was statistically higher \((\chi^2 = 4.001; p = 0.045)\) than in the control group (75%).

**Discussion**

In the vicious circle of DED, the inflammation is something that comes after tear film instability and hyperosmolarity, which might explain why, in our study, the conjunctival scraping could not show the clear distinction between the normal and mild dry eyes. Other authors state that in moderately severe dry eyes, there is an often subclinical inflammatory reaction of the ocular surface and the lacrimal gland, and so was confirmed in our study. That suggests that an anti-inflammatory treatment is needed in all except mild stage. Still, confirmation of presence of inflammation should make our decision easier to add this treatment to already existing artificial tears.

The relation between the inflammation and some of the clinical symptoms and signs of DED was suggested before. The diagnostic value of the clinical DE tests has been evaluated many times since 

FTBUT compared to the conjunctival scraping as a measure of DE-related inflammation in our study showed the best balance between sensitivity (93.6%) and specificity (77.9%). There was a strong correlation between the FTBUT and conjunctival scraping as well as with the RB and TF.
Alves et al. 20 also reported that the FTBUT sensitivity was 72.3% while specificity was 100%, and they correlated the best with other clinical tests they applied in diagnosing the dry eye in different diseases.

Versura et al. 4 found a strong correlation between FTBUT and tear osmolarity, although in their study, this correlation did not increase in its strength as dry eye severity did.

Discrepancy between the symptoms and signs is a reason why we cannot rely on questionnaires only when it comes to the DE diagnosis and staging of disease 21. The new definition of DED, published within the DEWS II report, addresses this problem of discrepancy between the signs and symptoms in some patients through the recognition of role for the neurophysiology in the sensory aspect of the disease 22. In our study, a poor correlation was evident in the group of patients over 60 years of age, where the average scraping score was higher. In their study, Vehof et al. 23 found that the increased age was a predictor of fewer symptoms than signs. This should make us more careful when ruling out, or staging DED in the older population.

Conclusion

FTBUT, though often used in many combinations of the DED tests, showed in our study a remarkably high sensitivity and specificity on its own, when correlated with the DED-related inflammation. RB and FTBUT had the highest correlation factor with the conjunctival scraping. A poor correlation was found between the symptoms and DE-related inflammation in the patients over 60 years of age. We share the opinion that it is the overall clinical judgment of a clinician that should still be the final judge of DE diagnosis and treatment, but we also believe that it is helpful to have a harder scientific evidence to guide our decision on an anti-inflammatory therapy inclusion in DE treatment.

REFERENCES


