# MOLECULAR MARKERS OF THE PROGRESSION OF CONJUNCTIVAL NEOPLASTIC EPITHELIAL LESIONS

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Conjunctival lesions can vary from benign, premalignant and malignant lesions [1]. In USA, 52% of conjunctival tumors are benign, 18% of the lesions are pre-malignant and malignant lesions develop in 30% of cases [1]. Pterigea is the most common benign lesion of the conjunctiva. It is considered as degenerative disease; However it bears some molecular features common to malignant tumors. Ljubojevic et al., found that 44% of pterigea cases are characterised with the mutation of p53 gene and 9% of the lesions are characterised with high proliferative activity, based on Ki67 labelling index [2]. Several studies also indicate the PCNA and Cyclin D1 activity in pterigea. In addition to proliferation markers, the presence of growth factors, tumor suppressor genes including p63 and apoptotic markers, including BCL2 has been also shown. According to Chui at al., 12% of pterigea cases are characterised with the co-occurrence of atypical epithelial and melanocytic foci [3]. Based on this data, one can assume that pterigea might be characterised with the malignant progression potential. Therefore the study of the molecular characteristics of this entity is very important [4]. In addition to pterigea, there are number of other common lesions in conjunctiva. Ocular surface squamous neoplasia, includes conjunctival intraepithelial neoplasia and squamous cell carcinoma, in addition to other epithelial changes from mild to moderate and severe atypia. According to the study of Gichuhi at al., the mentioned nosology is developed from conjunctival basal epithelial cells and the dysregulation of p53 plays an important role [5]. The progression of conjunctival intraepithelial neoplasia and carcinoma in situ to conjunctival squamous cell carcinoma is very common. Aoki at all., showed that proliferation nuclear antigen (PCNA) is more commonly expressed in squamous cell carcinoma and is relatively rare in dysplastic lesions, whilst the presence of p53 mutations is more common to dysplastic lesions. The results of this study suggest that the investigation of proliferation and differentiation markers might guide us to detect the early signs of malignancy and therefore to determine correct treatment and prognosis of the disease [6]. The aim of our study was to analyse the proliferation and apoptotic characteristics in conjunctival epithelial lesions, including actinic keratosis, pterigea, conjunctival intraepithelial lesions 1 to 3 (CoIN1-3) and in squamous cell carcinoma.

**Material and methods.** Study included formalin-fixed and paraffin-embedded (FFPE) tissue sections of 10 normal conjunctivas, 12 actinic keratosis, 25 pterigeas, 14 CoIN1, 12 CoIN2, 8 CoIN3 and 7 squamous cell carcinoma, altogether 88 cases. FFPE tissue blocks were retrieved from the teaching, research and diagnostic laboratory of Tbilisi State Medical University. H&E stained sections were revised and diagnosed by two independent pathologists (T.M., G.B.). Squamous-glandular index was evaluated in H&E stained specimens as the number of glands in 10HPF. Tissue sections were stained by standard immunohistochemical procedure, using antibodies against: Ki67, Cyclin D1, Bcl2, phosphohistone H3, p53, p63 and CK7. The evaluation of marker expression has been performed by two independent pathologists (T.M., G.B.) in two major compartments of the lesion, including the basal cell layer and superficial cell layer. The percentage of marker positive cells has been recorded and analysed with the following statistical methods: correlations were assessed using Spearman's rank test and comparisons between groups were evaluated using Mann-Whitney and Kruskal-Wallis test. The sensitivity and specificity of the test was assessed using 95% confidence interval. P value $<0.05$ was considered as statistically significant. All statistical tests were performed using SPSS statistical software V20.00.

**Results and discussion.** The evaluation of squamous index in conjunctival epithelial lesions showed that the squamous index in normal conjunctiva was 67±5.2, in actinic keratosis it was 93.2±6.7, in pterigea squamous index was 69±8.6 in CoIN1 squamous index was 76±6.7, in CoIN2 squamous index was 81±10.8, in CoIN3 squamous index was 95.3±6.7 and in CSCC squamous index was 98.7±1.1. The evaluation of glandular index in conjunctival epithelial lesions showed that, the glandular index in normal conjunctiva was 33±4.9, in actinic keratosis the glandular index was 8±4.4, in pterigea the glandular index was 31±4.3, in CoIN1 the glandular index was 24±6.1, in CoIN2 the glandular index was 19±5.6, in CoIN3 the glandular index was 5.7±2.3 and in CSCC the glandular index was 1.3±0.4. The calculation of squamous-glandular index (SGI) in conjunctival epithelial lesions showed the following results: the SGI in normal conjunctiva was 2±1.1, SGI in actinic keratosis was 11.25±1.5, SGI in pterigea was 3.1±2, SGI in CoIN1 was 3.2±1, SGI in CoIN2 was 4.3±1.9, SGI in CoIN3 was 16.7±2.9 and the SGI in CSCC was 75.9±2.8.

The evaluation of proliferation markers in conjunctival epithelial lesions showed the following results: Ki67 labelling index in basal layer of normal conjunctiva was 5.4±2.1 and in superficial layer it was 0±0, in actinic keratosis Ki67 labelling index was 19.7±3.6 in basal layer and 3.8±1.2 in superficial layer. In pterigea Ki67 labelling index was 8.1±2.4 in basal layer and 2.2±0.9 in superficial layer. In CoIN1 Ki67 labelling index was 19.6±6.2 in basal layer and 0±0 in superficial layer. In CoIN2 Ki67 labelling index was 21.3±7.8 in basal layer and 2.5±1 in superficial layer. In CoIN3 Ki67 labelling index was 25.3±4.2 in basal layer and 23.7±5.2 in superficial layer in CSCC Ki67 labelling index was 40.6±4.6 in basal layer and 36.7±7.3 in superficial layer.

The PHH3 labelling index in normal conjunctiva was 1±0.4 in basal layer, in actinic keratosis PHH3 labelling index was 7±2.1 in basal layer and 6.1±2.3 in superficial layer, in pterigea PHH3 labelling index was 5±1.9 in basal layer, in CoIN1 PHH3 labelling index was 8±2.3 in basal layer, in CoIN2 PHH3 labelling index was 10±3.7 in basal layer. The PHH3 labelling index in the superficial layer of normal conjunctiva, pterigea, CoIN1 and CoIN2 was 0±0. The PHH3 labelling index in CoIN3 was 12±4.2 in basal layer and 10.7±2.9 in superficial layer. The PHH3 labelling index in CSCC was 17±5.5 in basal layer and 15.2±3.3 in superficial layer.

*Table 1. The distribution of squamous, glandular and squamous-glandular indexes in conjunctival epithelial lesions*

| | Squamous Index | Glandular index | Squamous-Glandular Index |
|---|---|---|---|
| **Normal Conjunctiva** | 67±5.2 | 33±4.9 | 2 |
| **Actinic Keratosis** | 93.2±6.7 | 8±4.4 | 11.25 |
| **Pterigea** | 69±8.6 | 31±4.3 | 3.1 |
| **CoIN1** | 76±6.7 | 24±6.1 | 3.2 |
| **CoIN2** | 81±10.8 | 19±5.6 | 4.3 |
| **CoIN3** | 95.3±6.7 | 5.7±2.3 | 16.7 |
| **CSCC** | 98.7±1.1 | 1.3±0.4 | 75.9 |

*Table 2. The distribution of proliferation index based on Ki67 and PHH3 labelling indexes in basal and superficial layers of conjunctival squamous lesions*

| | Ki67 | | PHH3 | |
|---|---|---|---|---|
| | **Basal** | **Superficial** | **Basal** | **Superficial** |
| **Normal Conjunctiva** | 5.4±2.1 | 0 | 1±0.4 | 0 |
| **Actinic Keratosis** | 19.7±3.6 | 3.8±1.2 | 7±2.1 | 6.1±2.3 |
| **Pterigea** | 8.1±2.4 | 2.2±0.9 | 5±1.9 | 0 |
| **CoIN1** | 19.6±6.2 | 0 | 8±2.3 | 0 |
| **CoIN2** | 21.3±7.8 | 2.5±1 | 10±3.7 | 0 |
| **CoIN3** | 25.3±4.2 | 23.7±5.2 | 12±4.2 | 10.7±2.9 |
| **CSCC** | 40.6±4.6 | 36.7±7.3 | 17±5.5 | 15.2±3.3 |

*Table 3. The distribution of apoptotic index, based on Bcl2 and p53 in conjunctival epithelial lesions*

| | BCL2 | | P53 | |
|---|---|---|---|---|
| | **Basal** | **Superficial** | **Basal** | **Superficial** |
| **Normal Conjunctiva** | 94±5.6 | 92.2±9.3 | 0 | 0 |
| **Actinic Keratosis** | 6.7±1.9 | 12.9±4.5 | 24±4.3 | 16±2.6 |
| **Pterigea** | 23.2±3.7 | 91.9±7.2 | 12±3.8 | 0 |
| **CoIN1** | 60.3±10.9 | 87.6±6.3 | 11±3.6 | 0 |
| **CoIN2** | 20.5±5.1 | 90.2±10.7 | 15±2.2 | 0 |
| **CoIN3** | 5.4±2.1 | 10.7±2.6 | 27±4.3 | 13.7±3.3 |
| **CSCC** | 2.5±0.8 | 4.2±1.1 | 36±5.4 | 16.9±5.2 |

The evaluation of apoptotic index showed the following results: the Bcl2 labelling index in normal conjunctiva was 94±5.6 in basal layer and 92.2±9.3 in superficial layer; in actinic keratosis the Bcl2 labelling index was 6.7±1.9 in basal layer and 12.9±4.5 in superficial layer; in pterigea the Bcl2 labelling index was 23.2±3.7 in basal layer and 91.9±7.2 in superficial layer; in CoIN1 the Bcl2 labelling index was 60.3±10.9 in basal layer and 87.6±6.3 in superficial layer; in CoIN2 the Bcl2 labelling index was 20.5±5.1 in basal layer and 90.2±10.7 in superficial layer; in CoIN3 the Bcl2 labelling index was 5.4±2.1 in basal layer and 10.7±2.6 in superficial layer; in CSCC Bcl2 labelling index was 2.5±0.8 in basal layer and 4.2±1.1 in superficial layer.

The p53 mutations which were detected as complete loss of p53 or strong overexpression of p53 protein was not seen in normal conjunctiva; in actinic keratosis p53 mutations were detected in 24±4.3 cells in basal layer and 16±2.6 in superficial layer; in pterigea p53 mutations were detected in 12±3.8 cells in basal layer, in CoIN1 it was detected in 11±3.6 in basal layer and in CoIN2 it was detected in 15±2.2 in basal layer; p53 mutations were not detected in superficial layer of pterigea, CoIN1 and CoIN2; p53 mutations were detected in 27±4.3 in basal layer and 13.7±3.3 cells in superficial layer in CoIN3; p53 mutations were detected in 36±5.4 in basal layer and 16.9±5.2 in superficial layer in CSCC.

The study of epithelial squamous and glandular epithelial markers, such as p63 and CK7 respectively showed the following results: the distribution of p63 in normal conjunctiva was 45±5.2 in basal cell layer and 50.5±5.5 in superficial layer; p63 distribution in actinic keratosis was 76±4.9 in basal layer and 82.5±5.6 in superficial layer; p63 distribution in pterigea was 52±2.4 in basal layer and 79.3±9.1 in superficial layer; in CoIN p63 distribution was 65±3.7 in basal layer and 67.2±8.2 in superficial layer; in CoIN3 the p63 distribution was 80±6.1 in basal layer and 86.8±10.2 in superficial layer; in CSCC the p63 distribution was 90±4.3 in basal layer and 95.2±12.3 in superficial layer.

*Table 4. The distribution of P63, CK7 and P63/CK7 index in conjunctival epithelial lesions*

| P63 | | CK7 | | P63/CK7 Index | |
|---|---|---|---|---|---|
| **Basal** | **Superficial** | **Basal** | **Superficial** | **Basal** | **Superficial** |
| 45±5.2 | 50.5±5.5 | 10±2.9 | 48±10.3 | 4.5 | 1.0 |
| 76±4.9 | 82±5.6 | 2.7±1.7 | 4.3±1.3 | 28.1 | 19.0 |
| 52±2.4 | 79.3±9.1 | 9±1.8 | 42±9.9 | 5.7 | 1.9 |
| 65±3.7 | 67.2±8.2 | 7.9±3.4 | 34±8.3 | 8.2 | 2.0 |
| 74±4.8 | 76.1±7.1 | 7±3.1 | 25±6.7 | 10.6 | 3.0 |
| 80±6.1 | 86.8±10.2 | 2.1±0.9 | 2.3±1.2 | 38 | 37.7 |
| 90±4.3 | 95.2±12.3 | 1.7±0.2 | 1.9±0.7 | 52.9 | 50.1 |

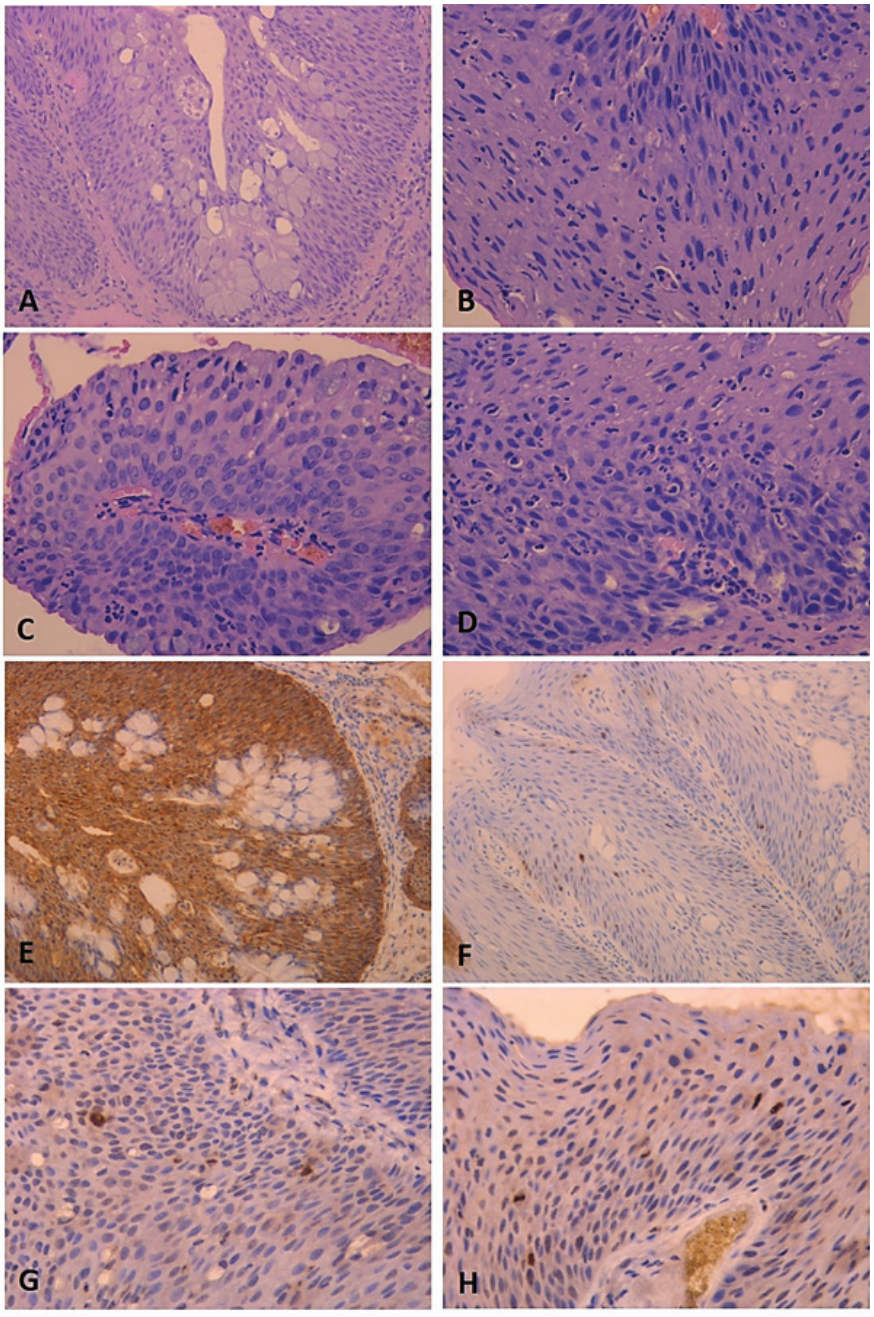
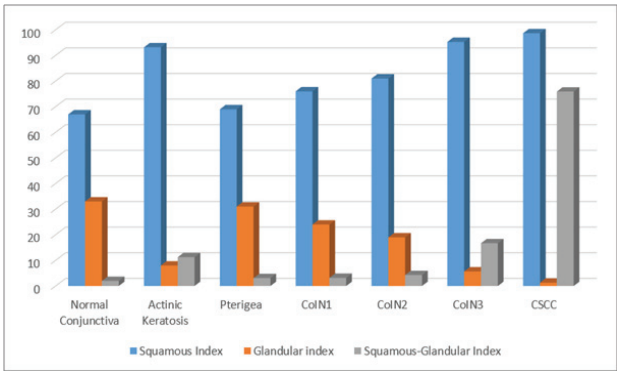



*Fig. 1. A. Pterigea, H&E, x100; B. CoIN2, H&E, x200; C. CoIN3, H&E, x200; D.CSCC, H&E, x200; E. Bcl2 expression in pterigea, IHC, x100; F. Ki67 expression in CoIN1, IHC, x100; G. PHH3 expression in CoIN1, IHC, x200; H. PHH3 expression in CoIN2, x200*

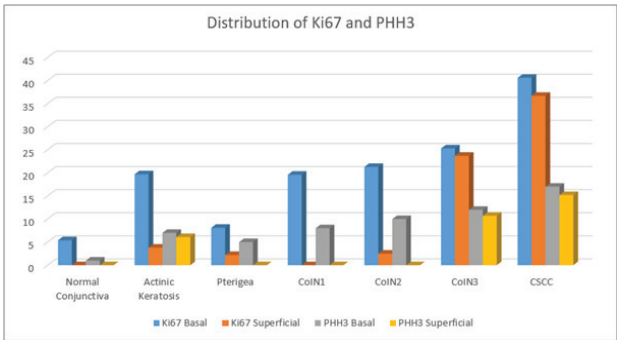
The distribution of CK7 showed the following results: in normal conjunctiva 10±2.9 in basal layer and 48±10.3 in superficial layer; in actinic keratosis 2.7±1.7 in basal layer and 4.3±1.3 in superficial layer; in pterigea 9±1.8 in basal layer and 42±9.9 in superficial layer; in CoIN1 7.9±3.4 in basal layer and 34±8.3 in superficial layer; in CoIN2 7±3.1 in basal layer and 25±6.7 in superficial layer; in CoIN3 2.1±0.9 in basal layer and 2.3±1.2 in superficial layer; in CSCC 1.7±0.2 in basal layer and 1.9±0.7 in superficial layer.

The study of P63/CK7 index showed the following results: in normal conjunctiva 4.5±1.8 in basal layer and 1±0.5 in superficial layer; in actinic keratosis 28.1±3 in basal layer and 19±4.3 in superficial layer; in pterigea 5.7±1.3 in basal layer and 1.9±0.9 in superficial layer; in CoIN1 8.2±1 in basal layer and 2±1 in superficial layer; in CoIN2 10.6±1.5 in basal layer and 3±1 in superficial layer; in CoIN3 2.1±0.9 in basal layer and 2.3±1.2 in superficial layer; in CSCC 52.9±21.5 in basal layer and 50.1±17.6 in superficial layer.

The analysis of the results of squamous index, glandular index and squamous-glandular index have shown that the squamous index is lowest in normal conjunctiva and it is significantly increased in line with the increase of atypia in squamous epithelial lesions. The highest proportion of squamous index has been found in CSCC. Whilst, the opposite trend has been seen with regards to glandular index. Hence, squamous-glandular index is also increased in line with the increase of atypia, with the dramatic increase in CSCC. In actinic keratosis the changes of squamous index, glandular index and squamous glandular index is similar to CoIN3.



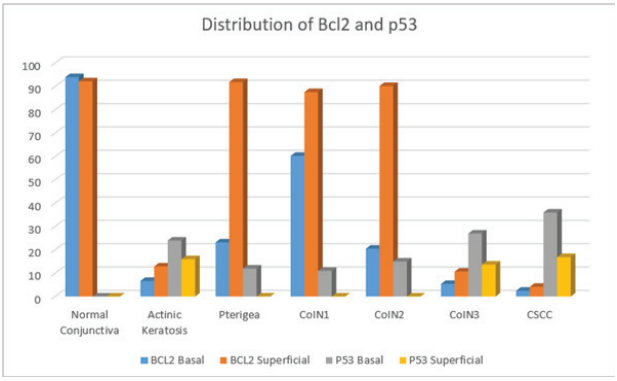

*Graph 1. The distribution of squamous index, glandular index and squamous-glandular index in conjunctival epithelial lesions*



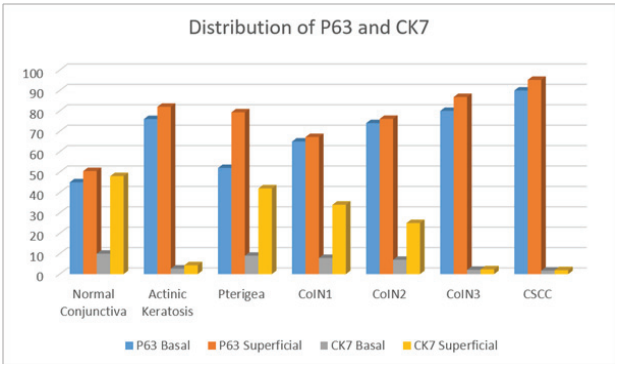

*Graph 2. The distribution of proliferation index, based on Ki67 and PHH3 in conjunctival epithelial lesions*

The analysis of the distribution of proliferation markers Ki67 and PHH3 in conjunctival epithelial lesions showed that the lowest proliferation index is seen in pterigea, followed by actinic keratosis, CoIN1 and CoIN2. The maximal proliferation index was detected in CSCC. The Ki67 labelling index was always higher compared to PHH3 labelling index in all cases and all lesions. PHH3 positivity was not detected in superficial layer of normal conjunctiva, pterigea, CoIN1 and CoIN2. In rest of the lesions, basal cell layer was characterised with higher proliferation index, compared to superficial layer in all cases.

The analysis of apoptotic index, based on Bcl2 labelling, showed that the highest apoptotic index is characteristic to normal conjunctiva and it is significantly decreased in conjunctival epithelial lesions. The lowest apoptotic index was seen in CSCC. In addition, Bcl2 labelling was always higher in superficial layer to all cases, compared to basal layer. With regards to p53 mutations, detected as the complete absence of p53 or the strong expression of p53 protein, it was not seen in normal conjunctiva. Lowest rates of p53 mutations was detected in basal layer of actinic pterigea, CoIN1 and CoIN2, whilst superficial layer did not show any sign of p53 mutations. P53 mutations were detectable in both layers of actinic keratosis, CoIN3 and CSCC.




*Graph 3. The distribution of Bcl2 and p53 in conjunctival epithelial lesions*

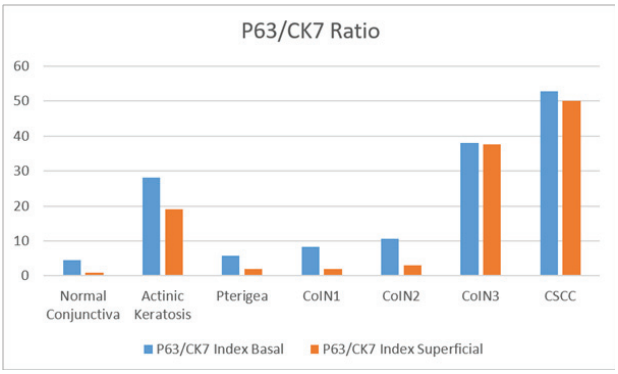



*Graph 4. The distribution of epithelial markers in conjunctival epithelial lesions*

The analysis of squamous epithelial marker P63 and glandular epithelial marker CK7 showed that the distribution of P63 is nearly equal to the distribution of CK7 in normal conjunctiva. Whilst the expression of P63 is significantly increased in conjunctival epithelial lesions and CK7 is significantly decreased. The CSCC epithelium is almost virtually represented by squamous epithelium marked by P63.

The analysis of squamous-glandular index, based on the ratio of P63 and CK7, showed that the highest P63/CK7 ratio is detected in actinic keratosis, CoIN3 and CSCC. In addition, this index is significantly higher in basal layers of normal konjunc-

tiva, actinic keratosis, pterigea, CoIN1 and CoIN2, whilst it is almost equal in basal and superficial layers of CoIN3 and CSCC.




*Graph 5. The distribution of P63/CK7 ratio in conjunctival epithelial lesions*

To the best of our knowledge we are first who analysed the squamous-glandular index in conjunctival epithelial lesions, by the evaluation of standard H&E specimens and as the ratio of P63/CK7 by immunohistochemistry. We have found that this ratio is significantly increased with the progression of conjunctival intraepithelial neoplasia and reaches its maximum in squamous cell carcinoma. Therefore, we suggest that squamous-glandular index may be used as an objective measure of CoIN progression. Moreover, one of the previous study of P63 did not show any relationship of P63 expression with the progression of CoIN, although it was significantly higher compared to normal conjunctiva [7]. With regards to proliferation and apoptosis markers, they are not also very well studied in conjunctival intraepithelial lesions. The study from Ohara et al., showed that Ki67 labelling index is significantly increased during the progression of CoIN [8] and this finding is in line to our results. To the best of our knowledge, we are also first who demonstrated the expression of PHH3 in conjunctival intraepithelial lesions. Based on our study results, this marker could be also used for the defining the malignant progression risk of conjunctival epithelial lesions.

**Conclusions.** Squamous-glandular index, based on the evaluation of H&E stained specimens as well as P63/CK7 ratio, represents the objective measure of the progression of conjunctival epithelial lesions. During this process the glandular epithelium is gradually, almost virtually, replaced by squamous epithelium. Based on proliferation, apoptotic and epithelial characteristics, CoIN2 is more similar to CoIN1, whilst there is a dramatic difference between CoIN1/2 and CoIN3. Therefore, we suggest that CoIN1 and 2 should be considered as low grade dysplasia, whilst CoIN3 should be considered as high grade dysplasia. Based on our study results, pterigea represents the benign entity. However, the presence of p53 mutations in pterigea indicates its potential malignant progression potential. Actinic keratosis, represents the intermediate entity between low grade dysplasia and high grade dysplasia of the conjunctival epithelium, which can also be progressed in high grade dysplasia.

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## SUMMARY

### MOLECULAR MARKERS OF THE PROGRESSION OF CONJUNCTIVAL NEOPLASTIC EPITHELIAL LESIONS

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Conjunctival epithelial lesions vary from benign to borderline malignancy to malignant, therefore it is extremely important to detect the molecular markers of the malignant progression of conjunctival intraepithelial lesions. The aim of our study was to analyse the molecular markers of the progression of conjunctival intraepithelial lesions. We have analysed Ki67, PHH3, Bcl2, P53, P63 and CK7 using standard immunohistochemistry. In addition, we have calculated the squamous-glandular index based on the evaluation of H&E stained specimens and as the ratio of P63/CK7. The results of our study indicated that the presence of squamous epithelium is significantly increased during the progression of conjunctival intraepithelial lesions, and therefore the squamous-glandular index is also increased. In addition, it is possible to divide conjunctival intraepithelial lesions as low grade and high grade lesions based on the distribution of proliferation and apoptosis markers.

**Keywords:** conjunctival neoplasia, molecular markers.

## РЕЗЮМЕ

### МОЛЕКУЛЯРНЫЕ МАРКЕРЫ ПРОГРЕССИИ ЭПИТЕЛИАЛЬНЫХ НЕОПЛАСТИЧЕСКИХ ПРОЦЕССОВ КОНЪЮНКТИВЫ

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К эпителиальным поражениям конъюнктивы относятся как доброкачественные, так и пограничной злокачествен-

ности и злокачественные процессы. Следовательно, весьма значимо выявление молекулярных маркеров прогрессии этих поражений.

Целью исследования явилось изучение молекулярных маркеров, определяющих риск прогрессии эпителиальных неопластических процессов конъюнктивы.

Стандартным иммуногистохимическим методом изучены молекулярные маркеры: Ki67, PHH3, Bcl2, P53, P63 и CK7. Рассчитан плоскоклеточно-железистый индекс по оценке препаратов стандартной гематоксилин-эозиновой окраски и соотношением P63/CK7. Результаты исследования показали, что в процессе прогрессии интраэпителиальных неоплазий конъюнктивы значительно увеличивается количество плоскоклеточного эпителия, следовательно, увеличивается плоскоклеточно-железистый индекс. По пролиферативным и апоптозным показателям возможно разделение интраэпителиальных поражений конъюнктивы на 2 группы - интраэпителиальные неоплазии низкой степени и интраэпителиальные неоплазии высокой степени.

რეზიუმე

კონიუნქტივის ეპითელური ნეოპლაზიური პროცესების პროგრესიის მოლეკულური მარკერები

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კონიუნქტივის ეპითელური დაზიანებები მოიცავს როგორც კეთილთვისებიან, ისე გარდამავალი ავთვისებიანობის და ავთვისებიან დაზიანებებს. შესაბამისად, მნიშვნელოვანია ამ დაზიანებათა ავთვისებიანი პროგრესიის მოლეკულური მარკერების გამოვლენა.

კვლევის მიზანს წარმოადგენდა კონიუნქტივის ეპითელური ნეოპლაზიური პროცესების პროგრესიის რისკის განმსაზღვრელი მოლეკულური მარკერების შესწავლა.

სტანდარტული იმუნოჰისტოქიმიური მეთოდით გამოვლენილია შემდეგი მარკერები: Ki67, PHH3, Bcl2, P53, P63 და CK7. გამოანგარიშებულია ბრტყელუჯრედოვან-ეპითელური ინდექსი სტანდარტული ჰემატოქსილინ-ეოზინით შეღებილი ანათლების შეფასებით და P63/CK7 შეფარდებით.

კვლევის შედეგებმა აჩვენა, რომ კონიუნქტივის ინტრაეპითელური ნეოპლაზიების პროგრესიის პროცესში მნიშვნელოვნად მატულობს ბრტყელუჯრედოვანი ეპითელიუმის შემცველობა და, შესაბამისად, ბრტყელუჯრედოვან-ჯირკვლოვანი ინდექსი. გარდა ამისა, პროლიფერაციული და აპოპტოზური მახასიათებლების მიხედვით შესაძლებელია კონიუნქტივის ინტრაეპითელური დაზიანებების ორ ჯგუფად დაყოფა, კერძოდ, დაბალი ხარისხის ინტრაეპითელურ ნეოპლაზიად და მაღალი ხარისხის ინტრაეპითელურ ნეოპლაზიად.

## ИЗУЧЕНИЕ ЦИТОТОКСИЧЕСКОЙ АКТИВНОСТИ ИНДОЛЬНЫХ АЛКАЛОИДОВ ИЗ НАДЗЕМНЫХ ОРГАНОВ VINCA ROSEA L., ИНТРОДУЦИРОВАННОЙ В ЗАПАДНОЙ ГРУЗИИ

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Среди интродуцированных в Грузии алкалоидосодержащих кустарников, полукустарников, деревьев и травянистых растений особое место занимают полукустарники видов рода *Catharanthus G.D*. Из них на побережье Черного моря Аджарии интродуцированы: *Catharanthus rosea (L.) G.D. (Vinca rosea L.)* и *Catharanthus rosea f. Albus (Sweet) G.D.,* который изучен в Институте фармакохимии им. И. Кутателадзе Тбилисского государственного медицинского университета на содержание биологически активных алкалоидов [1,2,13].

Помимо указанных видов, ранее были исследованы: *C. ovalis Mgf.; C. longifolius Pichon; C. lanceus (Bojex A.Dc.) Pichon; C. pussilus (Murr.)* [7,8]. *Vinca rosea,* как лекарственное растение, издавна использовалась народами Южной Африки, Италии, Австралии, Южного Вьетнама, Филлипин и Англии. Листья употреблялись в виде чая в качестве диабетического средства, а в Южной Африке и Англии имелись патентованные препараты „Covinca“ и „Vinculine“, являющиеся настоем листьев розовой винки [7,10].

Особый интерес к виду *V. rosea* вызван выделением в 1958-1959 гг. алкалоида винкалейкобластина, который после фармакологического и клинического испытаний рекомендован для лечения болезни Ходжкина и хориокарциномы как средство, вызывающее С-митотические изменения в клетках и нарушение деления клеток в метафазе [7-9]. Из того же вида, несколько позднее, выделен димерный алкало-