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ЖУРНАЛ БИОМЕДИЦИНЫ И ПРАКТИКИ
JOURNAL OF BIOMEDICINE AND PRACTICE

ДАВРИЙЛИГИ: 2016-2026

ЖИЛД 11
СОН 3

2026



ЧОП
ЭТИЛГАН САНА:
20.06.2026

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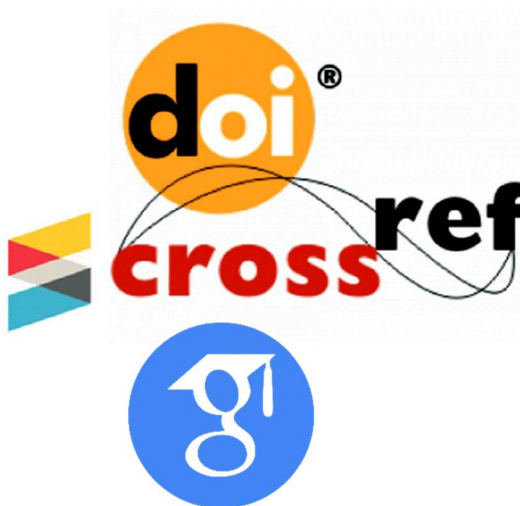
11 ЖИЛД, 3 СОН

ЖУРНАЛ БИМЕДИЦИНЫ И ПРАКТИКИ

ТОМ 11, НОМЕР 3

JOURNAL OF BIOMEDICINE AND PRACTICE

VOLUME 11, ISSUE 3



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БИОМЕДИЦИНА ВА АМАЛИЁТ ЖУРНАЛИ

ЖУРНАЛ БИОМЕДИЦИНЫ И ПРАКТИКИ | JOURNAL OF BIOMEDICINE AND PRACTICE

УДК: 617.77-007.281

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CLINICAL AND IMMUNOHISTOCHEMICAL CHANGES IN THE UPPER EYELID IN BLEPHAROCALASIS IN WOMEN OF DIFFERENT AGE AND HORMONAL GROUPS

For citation: Kadirova Aziza Muratovna, Khakimova Mavluda Shavkatjonovna. Clinical and immunohistochemical changes in the upper eyelid in blepharochalasis in women of different age and hormonal groups

<http://dx.doi.org/10.5281/zenodo.21313238>

ANNOTATION

Purpose of study: to evaluate the clinical and immunohistochemical characteristics of the upper eyelid skin in women with blepharochalasis from different age-hormonal groups based on the expression levels of estrogen receptors ER- α and ER- β . **Materials and methods:** after standart ophthalmological examination, 90 patients with blepharochalasis aged 24 to 67 years were divided into three groups according to age and hormonal status. Additionally, all patients underwent assessment of the condition of the upper eyelid skin, biopsy sampling, and immunohistochemical examination using monoclonal antibodies against estrogen receptors ER- α and ER- β . The level of receptor expression was evaluated using the H-score method. **Results:** the mean ER- α expression level in patients of reproductive age was 78.4 ± 4.6 , in the perimenopausal group 61.2 ± 3.9 , and in the postmenopausal group 44.7 ± 3.2 . A similar trend was observed for ER- β expression: 72.9 ± 4.1 , 58.6 ± 3.4 , and 39.5 ± 2.8 , respectively. Analysis of variance revealed statistically significant differences between the studied groups in the expression levels of ER- α ($p=0.0003$) and ER- β ($p=0.0001$). **Conclusion:** the obtained results indicate a decrease in estrogen receptor expression with increasing patient age and confirm the involvement of hormonal factors in the development of blepharochalasis.

Keywords: blepharochalasis, upper eyelid, immunohistochemistry, ER- α , ER- β , estrogen receptors, menopause.

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КЛИНИКО-ИММУНОГИСТОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ ВЕРХНЕГО ВЕКА ПРИ БЛЕФАРОХАЛАЗИСЕ У ЖЕНЩИН РАЗНЫХ ВОЗРАСТНО-ГОРМОНАЛЬНЫХ ГРУПП

АННОТАЦИЯ

Цель исследования: оценить клинико-иммуногистохимические особенности кожи верхнего века при блефарохалазисе у женщин различных возрастно-гормональных групп на основании уровня экспрессии рецепторов эстрогена ER- α и ER- β . **Материал и методы:** после стандартного офтальмологического исследования 90 пациентов с блефарохалазисом в возрасте от 24 до 67 лет были распределены на три группы в зависимости от возраста и гормонального статуса. Дополнительно, у всех пациентов проводились: оценка состояния кожи верхнего века, забор биопсийного материала, иммуногистохимическое исследование с использованием моноклональных антител к рецепторам эстрогена ER- α и ER- β , оценка уровня экспрессии методом H-score. **Результаты:** средние значения экспрессии ER- α у пациенток репродуктивного возраста составили $78,4 \pm 4,6$ балла, в перименопаузальной группе — $61,2 \pm 3,9$, в постменопаузальной — $44,7 \pm 3,2$. Аналогичная тенденция наблюдалась при оценке ER- β : $72,9 \pm 4,1$; $58,6 \pm 3,4$ и $39,5 \pm 2,8$ соответственно. Проведённый дисперсионный анализ выявил статистически значимые различия между исследуемыми группами по уровню экспрессии ER- α ($p=0,0003$) и ER- β ($p=0,0001$).

Заключение: полученные результаты свидетельствуют о снижении экспрессии рецепторов эстрогена с увеличением возраста пациенток и подтверждают участие гормонального фактора в развитии блефарохалазиса.

Ключевые слова: блефарохалазис, верхнее веко, иммуногистохимия, ER- α , ER- β , рецепторы эстрогена, менопауза.

Introduction. Blepharochalasis is a chronic progressive disease accompanied by thinning of the upper eyelid skin, a decrease in its elasticity, and the formation of redundant skin folds that lead to functional and cosmetic disturbances [11, 15, 18, 22]. Although the prevalence of this pathology is relatively low, its clinical significance is determined by the gradual deterioration of the morphofunctional condition of the tissues of the periorbital region and the formation of dermatochalasis, which may restrict the upper visual field [7, 11].

In recent years, increasing attention has been paid to the role of hormonal factors in the regulation of the structural organization of the skin, particularly the influence of estrogens on connective tissue remodeling processes [3, 9, 20]. It is known that the biological action of estrogens is mediated through intracellular estrogen receptors alpha (ER- α) and beta (ER- β), which participate in the regulation of fibroblast proliferation, extracellular matrix metabolism, and trophic processes in the dermis.

Age-associated decline in estrogen levels in women during the perimenopausal and postmenopausal periods may be accompanied by changes in the expression of ER- α and ER- β [1, 4, 9, 13, 19]. This, in turn, may lead to disturbances in regenerative processes and an increase in the degree of atrophic changes in the skin of the upper eyelid [6, 10, 14, 21]. However, the clinical and immunohistochemical features of estrogen receptor expression in the upper eyelid skin in women with blepharochalasis belonging to different age-hormonal groups remain insufficiently studied [2, 5, 8, 12, 13, 16, 17].

Therefore, the investigation of ER- α and ER- β expression in the upper eyelid skin in patients with blepharochalasis appears relevant for identifying a possible relationship between hormonal status and morphological changes in periorbital tissues [21].

Purpose of the study: to investigate the clinical and immunohistochemical features of the upper eyelid skin in women with blepharochalasis from different age-hormonal groups based on the expression levels of estrogen receptors ER- α and ER- β .

Materials and methods. The study included 78 women aged 24 to 67 years who were treated at the A.A. Yusupov Eye Treatment Center for excessive upper eyelid skin. The sample was formed taking into account the age and hormonal status of the patients, which made it possible to identify

three clinically comparable groups: women of reproductive age (24–35 years), perimenopausal patients (36–50 years), postmenopausal patients (51–67 years).

The inclusion criteria were the presence of clinical signs of blepharochalasis, absence of previous surgical interventions on the upper eyelid, and voluntary informed consent to participate in the study and to perform morphological analysis of surgical material. Patients with inflammatory eyelid skin diseases, decompensated endocrine disorders, systemic connective tissue diseases, as well as patients receiving hormone replacement therapy during the previous 12 months were excluded from the study.

At the preoperative stage, all patients underwent a standard ophthalmological examination, including: visometry, biomicroscopy of the anterior segment of the eye, assessment of the anatomical condition of the upper eyelid skin. Additionally, photographic documentation of the periorbital region was performed under standardized lighting conditions using frontal and lateral projections. The obtained images were used to objectively assess the severity of dermatochalasis and to compare clinical findings with the results of morphological analysis.

Biopsy samples were obtained intraoperatively during upper blepharoplasty. Upper eyelid skin specimens were fixed in 10% neutral formalin solution for 24 hours, followed by routine processing through alcohols of increasing concentration and embedding in paraffin blocks. From the obtained blocks, serial sections 4–5 μm thick were prepared and mounted on adhesive-coated glass slides. After deparaffinization and rehydration, antigen retrieval was performed using a buffer solution under controlled temperature conditions.

Immunohistochemical analysis was performed using monoclonal antibodies against estrogen receptors alpha (ER- α) and beta (ER- β). Expression of estrogen receptors was evaluated in the nuclei of dermal cells of the upper eyelid skin. For quantitative assessment of the immunohistochemical reaction, the semi-quantitative H-score method was used, based on a combined evaluation of staining intensity and the proportion of immunopositive cells.

Staining intensity was evaluated using a three-point scale: weak moderate strong the percentage of positively stained cells was determined during microscopic examination in several fields of view at $\times 400$ magnification. The H-score was calculated according to the formula: $\text{H-score} = (1 \times \% \text{ weak expression}) + (2 \times \% \text{ moderate expression}) + (3 \times \% \text{ strong expression})$

The obtained values ranged from 0 to 300 points, allowing an objective assessment of ER- α and ER- β expression levels in patients from different age-hormonal groups.

To increase the reproducibility of results, immunohistochemical slides were evaluated by two independent morphologists in a blinded analysis without access to the clinical data of the patients.

Statistical analysis of the obtained results was performed using the SPSS software package (version 26.0). Normality of quantitative data distribution was assessed using the Shapiro–Wilk test. Intergroup comparisons of mean values were performed using one-way analysis of variance (ANOVA).

When statistically significant differences were identified, post-hoc analysis using the Tukey test was applied. The correlation between patient age and expression levels of ER- α and ER- β receptors was evaluated using Pearson correlation analysis. Differences were considered statistically significant at $p < 0.05$.

Results and discussion. Immunohistochemical examination of biopsy specimens of the upper eyelid skin in patients with blepharochalasis revealed pronounced differences in the expression levels of estrogen receptors ER- α and ER- β , depending on the age-hormonal status of the examined patients.

Morphological analysis of tissues obtained from patients of reproductive age demonstrated that the dermal layer of the upper eyelid skin retained a relatively preserved structural organization. The connective tissue component showed moderate cellular density, and the fibroblast population was represented by a sufficient number of morphologically intact cells with pronounced nuclear immunoreactivity when stained for ER- α .

Immunopositive fibroblast nuclei were evenly distributed, predominantly within the papillary and reticular layers of the dermis. This finding indicates preserved hormonal sensitivity of the tissues of the periorbital region.

Quantitative analysis demonstrated that the mean level of ER- α expression in this group was 78.4 ± 4.6 points according to the H-score scale, reflecting a relatively high level of receptor activity. A similar pattern was observed when evaluating ER- β expression, the mean value of which reached 72.9 ± 4.1 .

In patients of the perimenopausal age group, microscopic examination revealed initial signs of structural remodeling of the dermal layer. A tendency toward decreased cellular density within the connective tissue was observed, along with areas of uneven fibroblast distribution. Immunohistochemical staining for ER- α in this group demonstrated a reduction in the number of positively stained cell nuclei as well as a decrease in staining intensity.

The mean level of ER- α expression in the second group was 61.2 ± 3.9 points, which significantly differed from the corresponding value observed in patients of reproductive age. Similarly, the level of ER- β expression in the perimenopausal group also showed a decrease and amounted to 58.6 ± 3.4 .

Pathomorphological analysis of the upper eyelid tissues in postmenopausal patients revealed the most pronounced structural alterations. In the dermal layer, a significant reduction in the number of cellular elements was observed, accompanied by decreased intensity of the immunohistochemical reaction when staining for ER- α and ER- β .

A reduction in the proportion of immunopositive cells with nuclear localization of receptors was also noted, indicating a decrease in the functional activity of hormone-dependent connective tissue structures. The mean H-score value for ER- α in this group was 44.7 ± 3.2 , whereas the expression level of ER- β reached 39.5 ± 2.8 . These indicators were statistically significantly lower than the corresponding values observed in the first and second groups.

One-way analysis of variance confirmed the presence of statistically significant differences between the study groups in the expression levels of ER- α ($F = 18.6$; $p = 0.0003$) and ER- β ($F = 21.4$; $p = 0.0001$).

Additional correlation analysis revealed a pronounced negative relationship between patient age and expression levels of ER- α ($r = -0.71$; $p < 0.01$) and also ER- β ($r = -0.69$; $p < 0.01$).

The decrease in estrogen receptor expression was accompanied by pronounced structural remodeling of the dermal layer of the upper eyelid skin, manifested by a reduction in the number of cellular elements and decreased intensity of the immunohistochemical reaction.

The obtained data suggest that the age-related decline in hormonal sensitivity of the tissues of the periorbital region may represent one of the factors contributing to the development of atrophic changes in the upper eyelid skin in blepharochalasis.

Thus, the identified morphological changes in the upper eyelid skin correlate with the decrease in the expression levels of ER- α and ER- β , confirming the involvement of hormonal factors in the pathogenesis of this disease. The results obtained in the present study indicate the presence of a pronounced relationship between the age-hormonal status of patients and the expression levels of estrogen receptors ER- α and ER- β in the upper eyelid skin in blepharochalasis.

Immunohistochemical analysis demonstrated that women of reproductive age maintain relatively high levels of ER- α and ER- β expression, which may be explained by a stable hormonal background and sufficient estrogen stimulation of connective tissue. It is known that estrogens play an important role in regulating the metabolic activity of dermal cells, particularly fibroblasts, and in maintaining the structural integrity of the extracellular matrix. The reduction in the expression levels of ER- α and ER- β observed in perimenopausal and postmenopausal patients likely reflects decreased hormonal sensitivity of periorbital tissues associated with age-related estrogen deficiency.

Under conditions of insufficient receptor activity, the cellular regulatory mechanisms responsible for maintaining the normal architecture of the dermal layer become impaired. The morphological changes in the upper eyelid skin observed in patients of older age groups may therefore be considered a consequence of decreased hormone-mediated trophic processes. A reduction in the number of cells expressing ER- α and ER- β may lead to impaired regenerative processes and a decrease in the adaptive potential of connective tissue.

The gradual decrease in estrogen receptor levels observed in this study may contribute to reduced functional activity of dermal cellular elements, ultimately leading to the development of atrophic changes in the upper eyelid skin.

The presence of a negative correlation between patient age and expression levels of ER- α and ER- β further supports the hypothesis regarding the significant role of hormonal factors in the pathogenesis of blepharochalasis.

Thus, decreased expression of estrogen receptors in the upper eyelid skin may be considered one of the morphological mechanisms involved in the development of age-related connective tissue changes in the periorbital region.

The results obtained are consistent with current concepts regarding hormonal regulation of metabolic processes in the dermal layer of the skin and indicate the potential for further investigation of estrogen receptors as markers of structural changes in the tissues of the upper

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