PTERYGIUM-HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL ASPECTS, IN CORRELATION WITH EXOGENOUS AND ENDOGENOUS FACTORS

PHD THESIS-SUMMARY

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Key Words: pterygium, virtual eye model, kinematic method, Finite Element Method, Histological Study, Immunohistochemical study
INTRODUCTION

Pterygium is one of the most common eye diseases [1], being a fibrovascular growth of subepithelial tissues, originating on the bulbar conjunctiva, which advances on the corneal surface in the form of a wing.

The objective of the PhD thesis was to approach through a interdisciplinary research, the clinical study correlated with paraclinical diagnostic methods.

The first part of the doctoral thesis "Current state of knowledge" is an update and a synthesis of data from the literature with reference to the histology and histophysiology of the outer tunic of the eyeball and the etiopathogenic and clinical aspects of the pterygium, a multifactorial disease.

The second part of the thesis refers to the “Personal contribution” and is structured on several chapters in which the personal studies undertaken are being presented.

CURRENT STATE OF KNOWLEDGE

CHAPTER 1

Histophysiology of the outer tunic of the eyeball

Conjunctiva is a richly vascularized transparent mucous membrane that lines the inner surface of the eyelids and is reflected on the anterior face of the eyeball to the level of the cornea-scleral tongue, continuing with the cornea.

Sclera is the external tunic of the eye, which begins at the level of the limbus and ends at the optic nerve, surrounding the posterior 5/6 of the eye.

Cornea is the main refractive surface of the eyeball and provides protection to intraocular structures. It is a smooth, transparent membrane, measuring 11-12 mm horizontally and 9-11 mm vertically [2].

Histophysiology of the conjunctiva. The conjunctiva represents both a mechanical obstacle and an immune barrier, through its rich content of immune cells capable of capturing and destroying aggressor agents.

CHAPTER 2

Pterygium-etiopathological aspects

Pterygium is an inflammatory, invasive and strongly vascularized growth, considered to develop through limbic epithelial stem cells activation. The etiopathogenesis of the pterygium is incompletely known. Current literature considers the following risk factors: prolonged UV exposure [3, 4, 5], smoking
[3], residency area (urban or rural) directly correlated with UV exposure and work conditions. Higher age and sex are also considered as risk factors [1, 6], and even repeated microtraumas, through dust exposure, chronic conjunctival inflammation, genetic predisposition and dry eye syndrome, which would indicate a multifactorial pathogenesis.

PERSONAL CONTRIBUTION

CHAPTER 3

Pterygium – Clinical-statistical study

The research was performed on 118 patients with pterygium who were hospitalized for surgical treatment in the Ophthalmology Clinic of the Craiova County Emergency Clinical Hospital, during 01.03.2017-01.03.2019.

Patients included in the clinical-statistical study were clinically evaluated and divided into several groups, depending on the following parameters: age, sex, environment of origin, size of the lesion, location of the lesion, exposure to UV radiation, type of surgical treatment, and other associated pathologies (Fig 3.1., Fig 3.2., Table 3.1.).

Analyzing the size of the lesion correlated with exposure to UV radiation, it resulted that the largest lesions were found in patients with prolonged exposure to the sun.

The size of the pterygium correlates with the presence of all three symptoms evaluated (tearing, foreign body sensation, decreased visual acuity), most likely amplifying its perception for both men and women.

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**Fig 3.1.** Distribution of the study lot according to age decade

**Fig 3.2.** Distribution according to age decade and pterygium grade
### Table 3.1

Distribution of the study lot according to environment of origin, sex and the main variables analyzed in the study

<table>
<thead>
<tr>
<th>Sex / Environment of origin</th>
<th>Urban</th>
<th>Rural</th>
<th>Sex / Environment of origin</th>
<th>Urban</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>$P$</td>
<td></td>
</tr>
<tr>
<td>Sex / Environment of origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>$P$</td>
<td></td>
</tr>
<tr>
<td>Right Eye / Left Eye</td>
<td>13 / 7</td>
<td>2 / 4</td>
<td>19 / 21</td>
<td>34 / 18</td>
<td>0.94</td>
</tr>
<tr>
<td>Unilateral / Bilateral</td>
<td>15 / 5</td>
<td>3 / 3</td>
<td>23 / 17</td>
<td>37 / 15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Internal angle / Internal+external angle</td>
<td>19 / 1</td>
<td>6 / 0</td>
<td>35 / 5</td>
<td>51 / 1</td>
<td>0.31</td>
</tr>
<tr>
<td>UV Exposure</td>
<td>6 / 14</td>
<td>1 / 5</td>
<td>37 / 3</td>
<td>50 / 2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chemical burn</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0.76</td>
</tr>
<tr>
<td>Trauma</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>Smoking</td>
<td>11</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Tearing</td>
<td>14</td>
<td>6</td>
<td>31</td>
<td>43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Foreign body sensation</td>
<td>6</td>
<td>4</td>
<td>18</td>
<td>22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Decreased visual acuity</td>
<td>7</td>
<td>4</td>
<td>25</td>
<td>28</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**CHAPTER 4**

**Kinematical simulation of a virtual eye model behaviour in healthy eye and pterygium**

In order to obtain the virtual model, tomographic images of a 54-year-old male patient were used. InVesalius 3.0 program, as well as the Geomagic for Solidworks program were used to convert the tomography tissues into three-dimensional geometry.

Several simulation steps were completed: three-dimensional modeling of the bone component of the human eye, three-dimensional modeling of the components of the eyeball (Fig 4.1., Fig 4.2.).

![Fig 4.1. Final model of the globe.](image1)

![Fig 4.2. Complete biomechanical model](image2)
In order to analyze the structures of the normal human eye and pterygium eye using the finite element method, the maps of stresses, displacements and deformations for the analyzed movements were obtained. (Table 4.1.)

<table>
<thead>
<tr>
<th>Normal Eye</th>
<th>Abduction</th>
<th>Adduction</th>
<th>Down Gaze</th>
<th>Up Gaze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td>232700</td>
<td>538400</td>
<td>365700</td>
<td>291000</td>
</tr>
<tr>
<td>Displacement</td>
<td>0.06963</td>
<td>0.1378</td>
<td>0.07331</td>
<td>0.07273</td>
</tr>
<tr>
<td>Deformation</td>
<td>0.09091</td>
<td>0.1799</td>
<td>0.1187</td>
<td>0.1031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pterygium Eye</th>
<th>Abduction</th>
<th>Adduction</th>
<th>Down Gaze</th>
<th>Up Gaze</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td>343700</td>
<td>562300</td>
<td>472300</td>
<td>366700</td>
</tr>
<tr>
<td>Displacement</td>
<td>0.3192</td>
<td>0.5733</td>
<td>0.3598</td>
<td>0.3208</td>
</tr>
<tr>
<td>Deformation</td>
<td>0.1085</td>
<td>0.1693</td>
<td>0.1187</td>
<td>0.1097</td>
</tr>
</tbody>
</table>

**Table 4.1.**
Numerical values stresses, displacements, deformations in normal and pterygium eyes

**CHAPTER 5**
Histological study

The studied material was represented by fragments of primary and relapsed pterygium, collected from 118 patients admitted to the Ophthalmology Clinic of the Craiova County Emergency Clinical Hospital, during 01.03.2017-01.03.2019, which underwent surgical treatment. The processing of the material was performed in the Histology Laboratory of UMF Craiova by the histological technique of inclusion in paraffin.

Microscopically, we identified several aspects, both in the epithelium and in the conjunctiva (Fig 5.1.). The epithelium showed secondary changes such as keratinization, acanthosis and dyskeratosis, cell atypia, epithelial cell hyperplasia (Fig. 5.2.), erosion (Fig. 5.3.), dysplasia (Fig. 5.4.).

On sections there was an inflammatory process with cells involved in the specific, immune defense reaction (lymphocytes, mast cells, plasma cells). Vascularization was intense, thrombosed vessels were detected, with perivascular or capillary blood extravasation with wide lumen and anastomoses (Fig. 5.5.), indicating the existence of a process of angiogenesis (Fig. 5.6.).

These lesional aspects of the conjunctiva are heterogeneous in some sections, all these types of lesions coexisting (inflammatory process, areas with intense vascularization, well-represented and fascicularly organized fibrillar component or fragmented collagen fibers), along with normal-looking stromal areas.
Fig 5.1. Pterygium - General Aspect
Col. HE X 10

Fig 5.2. Pterygium – Epithelial hyperplasia.
Col. tricr Masson X 20

Fig 5.3. Pterygium – Epithelial erosion.
Col tricr. Masson X 20

Fig 5.4. Pterygium – Epithelial dysplasia.
Col. HE X 10

Fig 5.5. Thrombotic vessel
Col tricr. Masson X 40

Fig 5.6. Angiogenesis
Col. tricr. Masson X 40
CHAPTER 6

Immunohistochemical study (IHC)

Of the 118 paraffin blocks obtained for histopathological examination, 40 blocks were selected for IHC study. The sections obtained were grouped histologically into three types: angiomatous, fibrous (7) and mixed (8). A set of 6 antibodies was used. (Table 6.1).

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Epitop / marker</th>
<th>Clona</th>
<th>Producer Cod</th>
<th>Antigenic Demasking</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Lymphocyte T</td>
<td>F7.2.38</td>
<td>DAKO A0452</td>
<td>Citrat pH=6</td>
<td>1:50</td>
</tr>
<tr>
<td>CD20</td>
<td>Lymphocyte B</td>
<td>1F8</td>
<td>DAKO M0784</td>
<td>Citrat pH=6</td>
<td>1:50</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophage</td>
<td>KP1</td>
<td>DAKO M0814</td>
<td>Citrat pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>Triptaza</td>
<td>Mast cells</td>
<td>AA1</td>
<td>DAKO M7052</td>
<td>Citrat pH=6</td>
<td>1:500</td>
</tr>
<tr>
<td>α-SMA</td>
<td>Myofibroblast</td>
<td>1A4.</td>
<td>DAKO M0851</td>
<td>Citrat pH=6</td>
<td>1:50</td>
</tr>
<tr>
<td>CD34</td>
<td>Vessels</td>
<td>QBE nd 10</td>
<td>DAKO M7165</td>
<td>Citrat pH=6</td>
<td>1:50</td>
</tr>
</tbody>
</table>

Through the IHC study, we aimed to establish the possible contribution of an immune mechanism in the pathogenesis of the pterygium. On the examined sections we identified important changes in the subepithelial conjunctiva.

An inflammatory process and an important vascularization were present in the sections. Therefore, we aimed to identify the type of cells participating in the inflammatory process: lymphocytes, plasma cells, mast cells, macrophages, their presence indicating an inflammatory pathogenesis of this disease.

The presence of lymphocytes and plasma cells in the pterygium stroma indicates the involvement of an immune process in the pathogenesis of the lesion. Moreover, the presence of lymphocytes and plasma cells suggests that pterygium formation involves a chronic inflammatory process associated with mononuclear cell infiltrate in the stroma. Conjunctiva can be considered a mucosa with an inductive role, but also an effector, the immunopathogenic mechanism being responsible for the pathogenesis of the pterygium.

CD3-positive T lymphocytes (Fig. 6.1.) and CD68-positive macrophages (Fig. 6.2.) were identified on some sections and in the structure of the covering epithelium. At the level of lamina propria we identified mast cells, using tryptase (Fig. 6.3., Fig. 6.4.). Our research revealed that at the level of the pterygium lesion there is an intense vascularization that also associates an intense process of angiogenesis (Fig 6.5.). Angiogenesis has been frequently associated with inflammatory foci (Fig. 6.6.).
Fig 6.1. Inflammatory infiltrate with T lymphocytes. Immunomarking CD3 X 20

Fig 6.2. Diffuse subepithelial macrophages. Immunomarking CD68 X 20

Fig 6.3. Diffuse subepithelial mast cells. Immunomarking with tryptase X 20

Fig 6.4. Epithelial erosion with mast cells. Immunomarking with tryptase X 20

Fig 6.5. Intense subepithelial angiogenesis. Immunomarking with CD34 X 20

Fig 6.6. Angiogenesis at inflammatory foci. Immunomarking with CD34 X 20
CHAPTER 7
General discussions

Pterygium is a common ocular condition whose prevalence varies from 2.2% for the Chinese population [9] to 41.8% for the Ethiopian population [10]. Pterygium is associated with the rural environment [9] or with sun-UV exposure [1,5,6]. Regarding the virtual eye model, the method used was also described for other virtual models [11].

The histological changes presented by pterygium are in close accordance with the clinical manifestations, which is why the histological aspects and clinical features can provide a better understanding of the pathogenic mechanism which may contribute to the establishment of surgical and non-surgical therapeutic strategies that may reduce recurrence, severity of inflammation, tissue invasion, proliferation and vascularization [12].

These aspects, in relation with the understanding of the morphology of the pterygium in a clinical context, have led to recent therapeutic options that reduce the recurrence rate and allow the use of a less invasive treatment than surgery.

The pathogenesis of the pterygium is not fully elucidated. Epidemiological studies have shown that UV radiation is the most important causal factor [13].

CHAPTER 8
General conclusions

Analyzing the size of the lesion correlated with exposure to UV radiation, it was observed that the largest lesions were found in patients with prolonged exposure to the sun and correlated with the presence of all three symptoms evaluated (tearing, foreign body sensation, decreased visual acuity).

The simulation of the behavior of a virtual model of healthy eyes and with pterygium was performed using the kinematic method and the finite element method, stating the originality of the study by the fact that a 3D Solid Works Model starting from a real case was not used in such studies. Histologically, the pterygium is a proliferative disorder of the epithelium and bulbar conjunctiva, starting from the corneal-scleral limbus. Alterations were discovered both at the epithelial level (keratinization, acanthosis, dyskeratosis, cell atypia, epithelial or goblet cell hyperplasia, erosion) and at the level of the conjunctiva (specific and nonspecific inflammatory process, intense vascularization and angiogenesis).

The study of morphological parameters (vascularization, inflammatory process) offers the possibility of recurrence predictability and evolutionary character of the pterygium.
Histopathological and immunohistochemical aspects together with clinical data provide a better understanding of the pathogenic mechanisms of pterygium, which may contribute to a more effective non-surgical therapeutic approach or associated with surgical therapy: anti-inflammatory, immunomodulatory, antiangiogenetic medication to reduce inflammation, cell proliferation, vascularization and tissue invasion.

**BIBLIOGRAPHY**