CONTRIBUTIONS AT HISTOLOGICAL STUDY IN PTERIGYUM
INTRODUCTION

Pterygium is an ocular surface lesion that can develop either in one eye, or bilateral. It is the form of a triangular strip of fibrovascular tissue on the epibulbar conjunctiva surface, in the area of palpebral fissure, with the basis oriented to the nose and the top pointing to cornea. The lesion may progress to the core. It has a variable evolution: slow or even can stop spontaneous to the rapid invasion of the cornea until the middle of it. Sometimes, the head of pterygium may undergo malignant epithelium metaplasia. Incidence is increased in certain regions of the world as in tropical and subtropical areas between 0 and 30° north and south. Can relapse, relapse often surpassing the original pterygium (Tan DT, Chee SP, Dear KB, Lim AS, 1997).

Pterygium is not simply a degenerative process of the conjunctiva, as it is ranged along pinuecula in ophthalmology treaties. Recent data suggest that is a growth disorder and it is an active, invasive, inflammatory process, associated with cell proliferation, tissue remodeling and angiogenesis (Coroneo MT, Di Girolamo N, Wakefield D, 1999, Kwok LS, Coroneo, MT, 1994). Pathogenesis of primary pterygium seeks to explain the role of extrinsic factors, particularly ultraviolet radiation and its location with basis at the nose. Pterygium formation is linked to cornea and conjunctiva micro-traumatisms and with exposure to ultraviolet radiation (Threlfall TJ, English DR, 1999). Risk factors for pterygium were evaluated in different parts of the world but UV radiations remain constantly included between this factors (Rojas JR, Malaga H, 1986, Khoo J, Saw SM, Banerjee K, Chia SE, Tandem 1998, norn MS 1982).

Motivation for choosing this theme is multiple: the relatively high frequency of pterygium, recurrence noted after surgical removal, clinical aspects that can take on, from local to mild dysplasia and invasive carcinoma in situ, even despite the fact that he is considered a relatively benign process. Is also important the fact that sometimes the lesion can extend on and can lead to decreased vision by irregular astigmatism induced by corneal stromal damage with coverage of the visual axis which affects retinal image quality that depends on transparency and refractive strength of the cornea – the first ocular diopter, holding a key role in the transmission, refraction and reflection of light.

The purpose of this study is to contribute to elucidate some aspects of disease etiopathogenesis which, despite the many advanced theories, statistical analysis, geographic studies, microscopic studies even immunohistochemical studies, is insufficiently explained. Also the possibility of explaining some aspects of recurrences after ablation and the contribution to the development of surgical and non-surgical therapeutic strategy to reduce recurrence, severity of inflammation, tissue invasion, proliferation and angiogenesis can be established. Considering the purpose mentioned above, the aim of the studies performed in this thesis was to explore histological and immunohistochemical characteristics of primary and recurrent pterygium sections and correlate the findings with clinical aspects is thus to be able to test the predictability of the development and recurrence of the disease on the basis of morphological criteria.

Methods: In general part of the thesis, which is detailed in the first two chapters, it was performed an update of relevant theories from the scientific literature published. The personal contribution, divided in the last two chapters of the thesis is directed to perform three studies (clinical study, a histological study and an immunohistochemical study) each offering its several objectives:

1. Clinical study of patients oriented to enlighten clinical particularities
2. Histological study conducted in order to establish histological specific modification in correlation with clinical particularities detailed in the above described clinical studies
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3. Immunohistochemical study of selected operatory pieces, focused on investigation of the main etiopathogenic theories involved in the genesis of pterygium

CHAPTER 1

EMBRYOLOGY AND HISTOPHYSIOLOGY DEVELOPMENT of the EXTERNAL TUNIC of the EYE

1.1. DEVELOPMENT of the EXTERNAL TUNIC of the eye

At the development of eyeball contributes and embryonic lineages: the ectoderm and mesoderm. Drawing the eye to reach maturity through three stages of development:

1. Embryo consisting of a simple segmentation of the zygote and runs until week 3 of fetal development;
2. Organogenesis resulting in the development of various ocular structures of the week 4 to week 8 of intrauterine life;
3. Differentiation of ocular tissues and annexes from the ninth week until the ninth month of intrauterine life

1.2. HISTOLOGY of the EXTERNAL TUNIC of the eye

Eyeball, the most important visual device, is composed of a wall and content. The wall is composed of three concentric tunics:

- External fibrous tunic composed of the sclera and cornea;
- Musculoskeletal middle vascular tunic called uvea that includes the following structures: the iris, ciliary body and choroid;
- Nervous internal tunic, retina

Content is the transparent media of the eye: lens placed behind the iris, aqueous humor located in the space between the lens and cornea, vitreous body situated behind the lens.

1.2.1 CONJUNCTIVA

Conjunctiva is a mucous membrane lining the eyelids and back of both front of sclerotic. Topographic and clinic are described the following areas: eyelid conjunctiva, basement conjunctiva and bulbar conjunctiva. Conjunctiva is composed of epithelium and chorion (own blade) containing glands, blood vessels, lymph and nerves. It rests on a layer of loose connective tissue, subconjunctival tissue, allowing the subjacent layers sliding conjunctival mucosa. Palpebral conjunctiva epithelium which is related to the tarsus is bilayer, cylindrical. Bulbar conjunctiva is nechertalinizat squamous type epithelium. Chorion (the lamina propria) is located under the epithelium and is consisting of a superficial and one deep layer.

1.2.2. SCLERA

Sclera, part of the external tunic of the eyeball, holds the key structural role of the eye circles, being stronger and less extensible. Sclera is a microscopic structure of fibrous tissue rich in collagen fibers, with a provision of the less orderly than corneal. Contains fine elastic fibers. Between the conjunctive fibers are cellular components, fibrocytes and cromatofor cells located in small numbers, especially around the vascular openings. Sclera is less perfused. Nutritional intake is through adjacent tissue structures. Sclera is present within the structure nerves, evidence of its own innervation.

1.2.3. CORNEA

Cornea, the first eye diopter, because of its transparency and refractive power has a role in transmission, reflection and refraction of light, thereby cornea is determining retinal image quality. Microscopic structure - cornea is a heterogeneous tissue, consisting of:

- Previously epithelium, covered with a thin layer of liquid or precorneean tear film;
- Bowman membrane previously separated from the epithelium by a basement membrane;
- Corneal stroma or corneal tissue proper;
CHAPTER 2

ETIOPATHOGENIC ASPECTS OF DEVELOPMENT AND EVOLUTION OF THE PTERYGIUM

The pathogenesis of pterygium is still controversial and the explanation for its formation has been issued several theories.

2.1. The role of ultraviolet radiation

Ultraviolet radiation is electromagnetic radiation of wavelength 100-400nm. According to Draper's law, only a fraction of the energy absorbed by the tissues may be used or may cause injury. Largest amount of energy required is called the induction of biological effects threshold dose. Sources of ultraviolet radiation to which a person may be exposed to daily are multiple: incandescent and fluorescent lamps, lasers, arc voltaic, solar light, which is the main source. It was found that people who work indoors than receiving about 3% of the total ambient level of UV radiation compared with those more active outdoors and who receive more than 10% of ultraviolet radiation environment (Marc B, 2004). In this respect, a pterygium growth rate is five times higher in people living in rural areas compared to those living in cities (DJ Moran, FC hollows, 1984).

2.2. The role of apoptosis

Epithelial tissues maintain homeostasis by regulating cell proliferation and apoptosis closely cell apoptosis representing normal cell programmed death, cell which has reached the end of its life cycle (Donald TH Tan, Wen Ying Tang, Yan Ping Liu, Hak- Su Goh, Duncan R Smith, 2000). Some studies have shown that the rate of cell proliferation in pterygium samples is similar to those found on the surface of normal conjunctiva. This suggests that pterygium is a disorder of excessive cell proliferation but may be the result of a lack of suitable cell apoptosis.

2.3. Role of metalloproteinase and their tissue inhibitors:

The importance of epithelial cells of the pterygium was demonstrated, because they express high levels of matrix proteinases (MLSS). It was also observed an increase in MLSP of pterygium fibroblasts compared with fibroblasts of normal conjunctiva (Lee SB, Li DQ, Gunja-Smith Z, 1999). In addition, cytokines and growth factors were localized in pterigym cells. From here one can assume that both types of cells could act together to basement membrane degradation and other structural components of connective tissue (Mackenzie FD, Hirst LW, Battistutta D, Green A, 1992).

2.4. The role of angiogenic factors
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It was suggested that there may be angiogenic factors involved in the pathogenesis of pterygium that grows from repeated irritation from the leaf sclerocorneean: TNF-α (tumor necrosis factor alpha), TGF-b (basic fibroblast growth factor), PDGF (factor platelet-derived growth), TGF-β (and transforming growth factor β). It is possible that prolonged exposure to ultraviolet radiation to cause biological changes in the Bowman membrane and that altered protein to act as an angiogenic factor or pterigogenic (JP Elliott JA Eliason, 1987). Overexpression of angiogenic factors along with low expression of inhibitors of angiogenesis factors may provide a way for the pathogenic mechanism of pterygium, and the possibility of therapeutic intervention to prevent recurrence of pterygium.

2.5. The role of immunological factors

It was noted the presence of IgE and IgG deposits in the stroma of pterygium. In the same area with deposits of immunoglobulin were identified infiltrating lymphocytes and plasma cells, lymphocytes represent the pivotal cells of the immune reactions in the presence of antigens which are different to trigger a specific response, humoral or cellular. Using immunohistochemistry can assess the nature and intensity of immune response triggered immunity. Effective immune response involves cellular cooperation.

2.6. The role of heredity

It was considered hereditary factor are involved because a large number of patients with pterygium have a family history for this condition. Unable to verify pterygium is inherited as an independent feature or affected individuals have in common an increased susceptibility to oculo-dermal effects of sunlight.

2.7. The role of the tear film

Tear film is essential for transparency and quality of ocular surface optically. Tear film abnormalities have been proposed as an etiological factor for the growth of pterygium was observed by advancing his head when confronted with dry eye (Taylor HR, 1980, Holly FJ, 1985; Liotet S, Van Bijsterveld OP, Blétry A, 1987). It was suggested that tear film evaporation by wind, devitalizes tissues from 1/3 palpebral fissure, and actinic radiation affects the conjunctiva, corneal epithelium and the Bowman membrane (Coroneo, 1993).

2.8. Hypothetical model of how the formation of pterygium

Corroborating the results of several studies conducted by different specialized researchers have developed a hypothetical model for pterygium formation method. UV light could be the initial trigger leading to a decrease limbic stem cells which induces a severe language and active epithelial cells at or near the leaf to produce cytokines (such as IL-6 and IL-8) and growth factors such as FGF, Le Grow Platelets-Derived Factor, Transforming Growth Factor Le, tumor necrosis factor α (Nolan TM, Di Girolamo N, Minas TC, Wakefield D, 2004; Džunić B, P Jovanović, Veselinović D, Petrovic A, Stefanovic I, Kovacevic I, 2010). This multifunctional protein:
- Triggers a cascade of events including inflammation, proliferation, angiogenesis and antiapoptosis (Anand-Apte B, Pepper MS, Voest E, 1997).
- Cytokines are able to induce expression of MMPs and their tissue inhibitors (TIMPs), allowing them to induce tissue remodeling (Bowman basal membrane, stroma) and invasion of pterygium (Fariss RN, Apte SS, Olsen BR, 1997).
CHAPTER 3
HISTOLOGICAL STUDY OF PTERYGYUM

2.1. MATERIAL AND METHODS

The material investigated in the dissertation is:

• primary and recurrent pterygium fragments collected from a total of 125 patients who underwent surgical removal of pterygium in the Ophthalmology Clinic of the Emergency County Hospital Craiova between 2007-2010.

• normal conjunctival membrane (37 fragments) obtained from the upper bulbar conjunctiva, taken from a portion of patients who underwent surgical removal of pterygium.

Sampling was done in patients who had no other ocular disease (allergic conjunctivitis and atopic other conjunctival diseases, recent ocular surgery) and systemic disease category without collagen disease.

Methods

Fragments of pterygium and conjunctival mucosa were processed by histological techniques for inclusion in paraffin, resulting in a 3-5μ thick sections that were examined 55I Nikon research microscope equipped with digital camera and software exposure 5Mpi and retrieval Elements NISS microscopic images automatically. Sections were stained with hematoxylin-eosin stains, tricromic based green-light by the method Szekelly Goldner, Masson tricromic aniline blue and PAS-hematoxylin staining.

3.2. RESULTS

Between 01/01/2007 and 12/31/2010 were harvested Ophthalmology Clinic Emergency Hospital Craiova, by surgical excision, pterygium fragments from a total of 125 patients both female and male. In most cases, pterygium surgery was in an advanced stage when it led the visual presentation of patients due to discomfort created by covering the optical axis. Of the 125 operated cases of pterygium, 32 were recurrent pterygium. In most cases (95%) pterygium was located in the internal angle of the orbit relative to that located in the external angle. Of the 125 pieces examined, 80 (64%) were from women and 45 (36%) were from men. Unlike the literature, that indicates a higher incidence of pterygium in males, in our study, females predominated.

Patients, both those with primary pterygium and those with recurrent pterygium were examined clinically to identify areas of progression (Fuchs islands) and the clinical appearance which can argue for evolutionary or stationary pterygium (very meaty, well vascularized, preceded the infiltrative zone).

We examined microscopically primary and recurrent pterygium fragments from patients who underwent surgical removal, noting the changes both in the epithelium and stroma. We found morphological differed from one case to another and even from the same case sections were observed regional differences. However, these changes were related to the evolutionary stage of the disease. A very small number of sections of pterygium had a structural organization, both in the epithelium and the stroma, similar to the bulbar conjunctiva.

The epithelium of pterygium examined sections is stratified squamous noncheratic, consisting of three cell layers. I noticed that the epithelium is sometimes uneven in thickness, alternating with thicker zones thinner areas because of the variety of cell layers on the epithelium of pterygium presents a segment to another. On some sections, epithelial hyperplasia causes a thickening of the interlayer. In an extremely small
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Abstract

A number of sections has been observed that it was accompanied by a tendency to become a keratinic tissue. Epithelium can be normal looking, but I met areas of dysplastic epithelium or even epithelial erosion areas. These issues are probably due to greater aggressiveness of etiologic factors have outgrown the capacity of local cellular defense. Among the epithelial cells of mucus-secreting cells are present, caliciform cells, which we have observed that they are willing either isolated or grouped. In areas where the epithelium is entering deep in stroma, forming real crypts, caliciform cell count is much higher compared with surface epithelium producing a pseudo glandular appearance. Pseudo glandular epithelial invagination and appearance are caused by accelerated proliferation of cells in the basal layer which increases the number of cells polyhedral, cylindrical and caliciform, epithelium resulting inability to integrate them, leading to their targeting to the conjunctiva. I noticed that in certain areas or basal membrane is thinner, it is either fragmented or even missing. These aspects of the basement membrane indicating points of least resistance that allowed the epithelium to enter in the lamina propria.

The distribution and arrangement of fibers, how we met under different aspects, often do not have a specific orientation or training tend beam. Fibrocytes can achieve very intense collagen fibrilogenesis process, so in some sections microscopically examined, collagen fibers are abundant making large areas of true fibrosis. The number, size and type of blood vessels were very different on the examined sections. Numerous capillaries are located near the basal membrane, in close proximity to the epithelium actively providing input they need substances in epithelial cells increased mitotic activity. A large number of vessels we met around and within areas with high concentration of inflammatory cells are hence necessary blood supply and the multiplication operation. Some vessels have a tendency to thrombosis, others are thrombosed, while others have a weak wall, in some areas there is evidence of a hematic extravasated. In addition to typical capillaries, we met frequently enlarged capillary lumen with numerous anastomoses with neoformation vessels aspect, scattered throughout the chorion. Histopathological aspects vary depending on developmental stage, progression or stationary phase, and as is primary or recurrent pterygium. Of the 125 sections examined microscopically for pterygium, 53 (42.4%) were classified as type angiomatos, 41 (32.8) corresponding to the appearance of fiber and 31 cases (24.8%) were of mixed type. The percentages of the three histopathological types detected by us in case law taken in the study were similar to those cited in the literature. It was performed an analysis based on histopathological issues encountered, because based on this analysis we can establish the morphological parameters that characterize the primary pterygium, recurrent forms and its evolutionary stage. Intensity changes seen for each of these structural elements we quantify it on a scale of 0 to 3 points. In most of the sections examined, the intensity of inflammation was sporadic, perivascular (group 1), blood level was within a group and fibrinoid changes were classified as group 2. In the primary pterygium sections from patients with Fuchs spots we found an intense inflammatory process and a more pronounced vascularity compared with patients who showed no clinical Fuchs spots. In contrast, fibrilar component was well represented in patients who have not spotted Fuchs. This is a clear indicator that the spots presence can be a clinical indicator of pterygium activity, of its progression, while the absence of Fuchs spots can mean the existence of a stationary process.

3.3. DISCUSSION

In 1997, Tan DT, Chee SP, Dear KB, Lim AS classified pterygium morphology into three categories: atrophic, hypertrophic, intermediate and suggested that the morphology they represent is a risk factor for relapse. According to another classification, there are three histological forms of pterygium: angiomatos, the stroma contains a significant number of vessels with edema intravascular, fibrous, with many fibers and little evidence of vascular and mixed, with both elements (KP García Carmona, Romero Guadarrama MB Rodríguez Florido MA, Tenorio G, 2006). We have realized a new classification according to two criteria refer to the vascular component and the fibrilar ones. The most common type of pterygium morphology we met was at angiomatos (42.4%), which corresponds to the bibliography, which cites this as a percentage to 40%. And for the other two forms, the results were close wledged those of other authors: the type of
fiber in our study represented a percentage of 32.8%, compared with 31.5% and mixed 24.8%, compared by 28.5%.

The presence of the inflammatory lymphocytes and macrophage-plasmacytoma in the lamina propria shows that a process is conducted is of type composed by macrophage defense and immune type. There are both cellular cooperation between macrophages (nonspecific defense pivotal cell) and lymphocytes (cell-specific key defense, immune), between the various lymphocyte subpopulations and between macrophages and neutrophil microfages in order to achieve an effective immune response on intensity and corelated with antigenic stimulus.

CHAPTER 4
IMUNOHISTOCHEMISTRY OF PTERYGIUM STUDY

4.1. MATERIAL AND METHODS

The study was focused on investigating the main etiopathogenic theories involved in the genesis of pterygium:
• Angiogenesis of the pterygium was investigated with markers:
a CD31, which allows identification of vascular endothelial and vascular microdensity default setting; VEGF has allowed one to identify the main sources of the pterygium angiogenesis factors;
• Involvement of growth factors in the pathogenesis of pterygium markers investigated:
an EGF, EGFR and TGFb involved in intense proliferation of pterygium tissue, which led to consideration of this pathological entity as a pseudotumour;
• Involvement of tumor suppressor genes investigated by marker:
p53 that would be responsible for the uncontrolled proliferation of the pterygium.

4.2. RESULTS
4.2.1. ANGIOGENESIS IMPLICATIONS IN PATHOGENESIS OF PTERYGIUM

Morphology, distribution and blood vessels microdensity
The most intense reactivity to this marker in the pterygium was highlighted particularly in the subepitelial area. This peculiar distribution of blood vessels is due to the need for increased intake of nutrients for proliferation in the epithelium of pterygium. We also noted the presence of conjunctival vessels in particular small and colabated. It was, also noticed the presence of elongated vessels. The morphology of these vessels is conclusive for the existence of an active process of angiogenesis subepitelial the conjunctiva pterygium.

Mean MVD, measured at a magnitude X 400 lens were 19.58 ± 6.7 in the pterygium compared to normal conjunctiva, the mean value was 7.6 ± 4.5

Involvement of vascular endothelial growth factor (VEGF) in angiogenesis and respectively in the pathogenesis of the pterygium
VEGF was present in all cases and was higher than in the conjunctive membrane. Qualitatively, the intensity of immunoreactivity was a heterogeneous reaction varied from case to case. In the pterygium, we have recorded a strong response to VEGF for: epithelial cells, with the exception of mucous cells, vascular endothelial cells, stromal fibroblasts and inflammatory cells.

4.2.2. IN VolVEMENT OF GROWTH FACTORS IN PTERYGIUM PATHOGENESIS
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- **involvement epidermal growth factor (EGF) and its receptor (EGFR) in the pathogenesis of pterygium**
  EGF was present in all investigated samples of pterygium. The reaction was predominantly an intense, but the reaction was also weak. Immunoreactivity to this marker from normal conjunctiva was a weak and even absent. Reactivity for EGF was evident in endothelial cells. Immunoreactivity to this marker from normal conjunctiva was a weak and even absent. EGFR was expressed in 13 out of 21 cases. Reaction was generally strong and is comparable to the level of expression of this receptor in endothelial cells of the conjunctival pterygium.

- **involvement of transforming growth factor epidermal beta (TGFβ) in the pathogenesis of pterygium**
  All investigated cases were positive for TGFβ. Intensity ranged from moderate to intense TGF-b was more evident in the epithelium of pterygium with a cytoplasmic pattern. Compared with normal conjunctiva, pterygium the immunoreactivity was more intense. We also noted a TGF-b reactivity in the cytoplasm of endothelial cells, basal membrane of vessels and epithelium, fibroblasts and inflammatory cells.

**4.2.3. MATRIX METALLOPROTEINASES AND INVOLVEMENT THEIR SPECIFIC INHIBITORS IN THE PATHOGENESIS OF PTERYGIUM**

- **MMP1 involvement in the pathogenesis of pterygium**
  MMP1 was detected in all investigated cases of pterygium. The pattern was a diffuse cytoplasmic and in terms of quality reaction was mainly one moderately. With a variable intensity, immunoreactivity was present throughout the epithelium of pterygium, and conjunctival fibroblasts.

- **TIMP1 involvement in the pathogenesis of pterygium**
  The TIMP1 immunoreactivity was detected in all cases investigated and qualitatively, the vast majority had an intense reaction. However, overall the pterygium TIMP1 was almost similar to MMP1. TIMP1-positive immunoreactivity was detected in the entire thickness of the epithelium. A positive reaction to this marker was highlighted in the fibroblasts and collagen bundles in the body of pterygium.

- **MMP9 involvement in the pathogenesis of pterygium**
  MMP9 was detected in all cases investigated and qualitatively, the vast majority had an intense reaction. For MMP-9 have reported a reduction of expression in the epithelium of pterygium. Vascular endothelium, fibroblasts and inflammatory cells in pterygium were immunoreactive to MMP-9. Intense positive cytoplasmic MMP9 were neutrophils, as well as those of luminal extravasated blood vessels.

**4.2.4. INVOLVEMENT TUMOR SUPPRESSOR GENES IN THE PATHOGENESIS OF PTERYGIUM**

- **p53 involvement in the pathogenesis of pterygium**
The p53 positivity was present in 14 of the 21 cases investigated. We detected immunoreactivity in conjunctive normal control groups examined. In the epithelium of pterygium, immunoreactivity was more evident in the basal layer. Occasionally, we noted the presence of nuclear reaction in the upper layers.

4.3. DISCUSSION

4.3.1 THE ANGIOGENESIS INVOLVEMENT IN PTERIGYUM PATHOGENESIS

The study conducted by us revealed the existence of a richer angiogenesis in the pterygium than in normal conjunctiva. In addition, these vessels were best represented subepitelial the conjunctiva, which is justified by the need to increased epithelial proliferation. Also, M Tsanou E Gorezis S, Ioachim E, Skyrlas A, et al., 2007, found that VEGF was overexpressed in endothelial cells and stromal cells of the pterygium, but not in epithelial cells compared with normal conjunctival tissue.

4.3.2 DISCUSSION ON THE INVOLVEMENT OF GROWTH FACTOR EGF, EGFR and TGFb IN THE PTERIGYM PATHOGENESIS

We have shown the involvement of EGF and its receptor (EGFR) in epithelial growth and angiogenesis of the pterygium, as a result of two factors overexpression in epithelial and endothelial cells. Several immunohistochemical studies have demonstrated the presence of EGFR pterygium (Liu Z, Xei Y, Zhang M, 2002, Hands R, Collison DJ, Maidment JM, Davies PD, Wormstone MI, 2002). EGFR with ErbB2, ErbB3 and erbB4 are four family members growth factor receptor I (Gullick WJ, 1986, Ciardiello F, Tortora G, 1998), which plays an important role in the proliferation, migration and cell differentiation (Li DQ, Tseng SC, 1996, Rajkumar T, Gullick WJ, 1994).

Our investigation showed overexpression of TGFb in all proliferative compartments pterygium, proving his involvement in the pathogenesis of this disease.

4.3.3 DISCUSSION ON Matrix metalloproteinases (MMP1 AND MMP9) AND THEIR TISSUE SPECIFIC inhibitors (TIMP) INVOLVEMENT in the pathogenesis of pterygium

Matrix metalloproteinases (MMP) are a family of neutral proteolytic enzymes able to degrade most extracellular matrix components (Stetler-Stevenson WG, 1996).

Our study revealed the expression of MMP1, MMP9 and TIMP1 in the pterygium epithelium, predominantly in the basal layer, the fibroblasts and collagen fiber bundles and in vascular endothelial cells and inflammatory cells. Such a pattern suggests the involvement of these immunohistochemical markers in the process of remodeling the extracellular material and degenerative processes taking place in the pterygium. In addition, they appear to be involved in the processes of angiogenesis and proliferation through the release of the extracellular matrix of growth factors.

A number of studies have demonstrated active involvement of MMP and TIMP in turnover and extensive infiltration of the matrix characterizing pterygium. A number of studies have shown low intensity of TIMP1 expression in all epithelial layers of pterygium, whereas TIMP3 was expressed more intensely, but the expression was restricted to the basal layers (Fariss RN, Apte SS, Olsen BR, Iwata K, Milam AH, 1997, Kenney MC, Chwa M, Alba A, Saghizadeh M, Huang ZS, Brown DJ, 1998; Karnacki KA, Goebel DJ, Poosch MS, Hazlett LD, 1998, Leco KJ, Khokha R, Pavloff N, Hawkes SP, Edwards DR, 1994; Vranka JA, Johnson E, Zhu X, Shepardson A, Alexander JP, et al., 1997).

4.3.4 Discussions about the involvement in the pathogenesis of pterygium of P53

Under the influence of different factors in tumor genesis, such as ultraviolet radiation, gamma radiation, certain chemicals, nuclear level of p53 increases the transcription factors involved in promoting

CHAPTER V
GENERAL CONCLUSIONS

1. Both the epithelium and stroma of pterygium have structural changes that differ depending on the evolutionary stage of the disease, as there are different structural aspects in the primary compared to recurrent pterygium.

2. Epithelium may be uneven in thickness due to a process of hyperplasia, may show areas of erosion probably due to greater aggressiveness of the etiologic factors that outgrown the capacity of local cellular defense.

3. Stroma presents areas with inflammatory infiltrate, located mainly around vessels and periglandular or disseminated, multifocal form of areas of different sizes, as there are sections that have not submitted inflammatory infiltrate.

4. Fibrillary component presented different aspects may not have a certain orientation, as some areas are surrounded by an inflammatory infiltrate rich fibrillary component developed as a localized fibrosis, which tends to encircle and delimitation of the inflammatory process or present a process of degeneration.

5. Fuchs spots are clinical indicator of pterygium activity, of its progression, but also of recurrence, while the absence of Fuchs stains can mean that there is a stationary process.

6. The expression of growth factors EGF and TGFb in all proliferative sections of pterygium, and highlights the progressive nature of this invasive entity, justifying its classification in pseudotumoral group.

7. The pattern of expression of MMP1, MMP9 and TIMP1 suggests their involvement in the remodeling of extracellular material and degenerative processes in the pterygium, which would allow classification of diseases thereof within collagenosis group.

8. Overexpression of p53 in basal pterygium epithelium certifies that on the one hand the involvement of UV radiation in the pathogenesis of this disease and on the other hand, proliferative character.
9. Such reactive structures on the one hand prove pterygium proliferative-invasive nature of this disease, like a tumor, and on the other hand, the process of remodeling and degradation of connective tissue, like a collagen compartment.

10. Morphological parameters (vascular aspect, changes in fibrin component, and the intensity of the inflammatory process) and immunohistochemical correlated with clinical features of each case offers the possibility of recurrence and predictability of the evolving nature of pterygium. For this, histological analysis of the characteristics of pterygium has become increasingly more useful in current practice.

11. Study of etiopathogenic aspects, histological and immunohistochemical, the ophthalmologist can understand pathogenic mechanisms of the development of pterygium in order to address a more effective therapeutic behavior: development of synthetic inhibitors of GFs and MPPs, can decrease the rate of relapse, the severity of inflammation, tissue invasion, angiogenesis and proliferation of pterygium.