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**CLINICAL, HISTOPATHOLOGICAL,
IMMUNOHISTOCHEMICAL AND GENETIC STUDY OF THE
EYELID CARCINOMAS**

ABSTRACT

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INTRODUCTION

Eyelid malignant tumors represent actually cutaneous malignant tumors and are completely curable, if they are early diagnosed [1]. Cutaneous malignant tumors can arise from the epidermis, dermis of eyelid annexes. These tumors may extremely rare represent methastasis from other tumors, arising from other sites and include histopathologically different tumors, arising from various cutaneous cells [2].

Malignant tumors are relatively frequent encountered around the eye, due to the fact that most of them are induced by sun-exposure damage or arise from benignant cutaneous tumors that are caused by sun exposure. Many of these tumors are small and grow slowly, fact that delays the presentation, and thus the diagnosis. Though most of the eyelid tumors do not metastasize, they may produce very severe local distructions, that is the reason why any periocular evolving lesion, especially if associated to bleeding, must be diagnosed via biopsy. Histopathological confirmation of malignancy represents a must before major resections or any other surgical procedures which may lead to aesthetically poor results [2].

CHAPTER I

Eyeball anatomy

The eyeball gains information from the surrounding environment as light beams and afterwards analyzes and processes it. Eye sight and visual perception processes involve a complex system of structures, every one of them being concepted for a precise aim. The organisation of every structure makes the function possible. The eye hosts elements that take the light beams and turn them into nervous influx. The eyeball is protected by its intraorbital localization [13].

The eyeball is composed of three layers:

- Outer layer or fibrous layer, which includes the cornea and the sclera
- Medium layer or vascular layer, including the iris, the cilliary body and the choroid
- Inner layer or retina, having optical and non-visual parts [14].

Outer layer of the eyeball

The fibrous layer of the eyeball may be compared with two joined spheres. The anterior one is smaller and transparent, represent $1/6$ of the outer layer and it is called cornea. This structure has a curvature of approximately 8mm. The posterior sphere is called the sclera, it is larger than the cornea, it is opaque, with solid structure and represents $5/6$ of the outer layer. The sclera represent the fibrous skeleton of the eyeball, thus offering shape and resistance, representing the spot for extraocular muscles insertion. The sclera has a curvature of approximately 12mm.

Middle layer of the eyeball

The middle layer of the eyeball or the uvea consists in three areas (from anterior to posterior): the iris, the ciliary body and the choroid. The uvea is also known as the vascular layer of the eyeball, because the largest of its structures, the choroid, consists mostly of blood vessels, that vascularize the retinal superficial layers [13].

Inner layer of the eyeball

The inner layer of the eyeball is actually a nervous layer, called the retina, located between the choroid and the vitreous body. The retina extends from the optic disc margins (the place where the nervous fibers joined in the optic nerve leave the eyeball) to the ora serrata, where it continues with the ciliary body epithelium, these two structures having a common embryological origin. The retina contains hundreds of millions cells and cell processes, though it appears as a thin, transparent membrane [13].

The lens

The lens is a transparent, elliptic, avascular structure that is situated in the posterior chamber of the eye, anteriorly to the vitreous body and posteriorly to the iris. The lens is suspended to the ciliary body via zonular fibres. The posterior part of the lens is attached to the anterior vitreous through the capsular hyaloid ligament, which represent an adhesion ring. In this ring there is a virtual space, an area without any adhesions between the lens and the vitreous body [13].

The chambers of the eyeball

The eyeball contains three chambers: the anterior chamber, the posterior chamber and the vitreous. The anterior and the posterior chambers contain aqueous humor, while the vitreous contains the vitreous gel [13].

CHAPTER II

Eyeball annexes

The eyeball annexes represent specialized structures located close to the eyeball. These structures are: the eyelids, the conjunctiva, the extraocular muscles and the lacrimal apparatus, which includes the secretion component (which produces the tears), and the excretion part (which drains the tears) [13].

CHAPTER III

Cutaneous carcinogenesis

Cancer is essentially a genetic disease which is characterized by genomic instability and by genetic and epigenetic defects accumulation. Genetic disorders that induce the cancerous disease may be inherited as oncogenes and tumor suppressor genes, leading to family cancer syndromes. Defects may appear in the process of DNA replication and repair, fact which may lead to neoplasia similarly.

Basal cell carcinoma

The discovery of *patched 1 (PTCH1)* gene mutations in the genome of Gorlin syndrome patients (also known as naevoid basal cell carcinoma syndrome) and in sporadic basal cell carcinoma lead to the discovery of Sonic Hedgehog (SHH) signaling pathway as playing an important role in human carcinogenesis [7,16]. PTCH1 gene mutations or the mutations of another component of this signaling pathway are involved in the occurrence of neoplasia such as: medulloblastoma, mammary carcinoma, meningioma, colorectal carcinoma [7,17], pancreatic adenocarcinoma and esophagus adenocarcinoma [7,18,19] and small cell lung cancer [17,20].

Squamous cell carcinoma

Squamous cell carcinoma development is actually a multistep process, which requires a series of mutations, which involve mostly anti – apoptotic pathways and cell proliferation pathways. The genes that suffer mutations in squamous cell carcinoma pathology are TP53, RAS and p16/CDKN2A [3,8].

Malignant melanoma

The transformation of a normal melanocyte in melanoma occurs via sequential accumulation of molecular and genetic alterations, which are for the moment only partially understood. At molecular level, the activation of the proliferative kinase signaling pathways which involve oncogenes such as BRAF, NRAS or KIT, followed by alterations in aging and apoptosis pathways that involve tumor suppressor genes as CDKN2A, TP53 or PTEN, represent events in the malignant melanoma multistep pathogenesis [3,15].

CHAPTER IV

CLINICAL STUDY OF THE EYELID CARCINOMAS

Basal cell carcinoma represents the most common human malignant tumor. In the United States, about 75 – 90% of the cutaneous neoplasia are basal cell carcinomas and over 90% of the eyelid tumors are also basal cell carcinomas [3,4,5,6]. About 99% of the basal cell carcinomas occur in caucasians [2].

In the United States, the second most common cutaneous malignant tumor is the squamous cell carcinoma, accounting for about 10 – 20% of the cutaneous malignant tumors and for about 5% of the eyelid malignant tumors [9,10]. Periocular squamous cell carcinoma affects mostly the inferior eyelid. Comparing to basal cell carcinoma, squamous cell carcinoma affects more often the superior eyelid and the lateral canthus [11,12].

For this study, we have analyzed several cases for 4 years (2010 – 2014) following the localization, frequency and histopathological type of the tumor, as well as the surgical reconstruction techniques. Our study counts 103 patients with eyelid carcinomas, 54 males and 49 females, aged between 47 and 92 years. The study was conducted in the mentioned period in the Ophthalmology Clinic of the Emergency County Hospital of Craiova, Romania.

CHAPTER V

HISTOPATHOLOGICAL STUDY OF THE EYELID MALIGNANT TUMORS

Eyelid basal cell carcinoma represent a group of cutaneous malignant tumors, characterized by the presence of many lobules, columns, strands or bands of basaloid cells („germinative cells”) [21].

The numerous variants of basal cell carcinoma have some common histological features: the presence of lobules, columns, bands or strands of basaloid cells, little cytoplasm and peripheral cell palisadation, surrounded by free fibromucous stroma. Sometimes areas of retraction between the tumor and the stroma may be present. The interaction between tumor and stroma is weak, due to the characteristic absence of the hemidesmosomi, which anchors normally the epidermis to the dermis. Apoptosis is usually apparent. The keratin eliberation in the strome, as apoptosis result, may lead to amyloid deposits formation. Mucous cystic degeneration and focal vacualisation with lipid differentiation may be also seen. In rare cases, sebocytes or follicular differentiation with squamous vortex may be seen, and gray – blue corneocytes. In some tumors melanocyte proliferations may be seen, which may lead to pigmentation via melanine production, which may be deposited in tumor cells or in surrounding macrophages [21].

Squamous cell carcinoma represent a malignant tumor of the epidermal keratinocytes, in which the cell component present different grades of squamous differentiation [21]. Squamous cell carcinoma is composed of nests, strands and strips of squamous differentiation cells, which arise from the epidermis and extend in the dermis on a variable range. Cells present eosinophilic cytoplasm and a big nucleus, frequently vesicular; prominent intercellular bridges may be seen. Variable central keratinisation may be seen and keratotic pearls formation are present, depending on the differentiation grade [21].

The grade of anaplasia in the tumor cell nests is used for the tumor grading. A rather subjective classification is one that groups the squamous cell carcinomas in well, moderate and poor differentiated. Most of the squamous cell carcinomas arise from the actinic keratosis, and the proof is the presence of this lesion at the invasive tumor's margins. Occasionally, squamous

cell carcinoma produce infiltration among the neural sheath, blood vessels' adventitia, and lymphatics. The presence of the perineural lymphocytes is an element that makes perineural invasion possible in deep sections [21]. A low to moderate chronic inflammatory infiltrate may exist at the tumor periphery; the infiltrate may include eosinophils [21,22].

The histopathological study was performed on 103 cases of eyelid carcinomas, of which 80 basal cell carcinomas and 23 squamous cell carcinomas. The histopathological study aimed the evaluation of histopathological parameters, which have been correlated to the clinical and immunohistochemical parameters.

CHAPTER VI

IMMUNOHISTOCHEMICAL STUDY OF THE EYELID CARCINOMAS

The immunohistochemical analysis was performed on 43 cases of primary cutaneous eyelid carcinomas, diagnosed between 2010-2014 in the Pathological Anatomy Laboratory of the Emergency County Hospital of Craiova, Romania.

In this study several reactions were performed, using antibodies which have been classified as follows:

- Proliferation factors (ki67, PCNA)
- Apoptosis factors (p53, bcl-2)
- Growth factors (VEGF, EGFR)

In the immunohistochemical study we have evaluated the immunoexpression of ki67, PCNA, p53, bcl-2, EGFR and VEGF on 43 eyelid cutaneous carcinomas, depending on the tumor type, differentiation grade, tumor extension (T and N categories) and tumor stage. In this study we have identified no methastasis of the analyzed carcinomas (M0).

In our study, 23 cases of basal cell carcinoma were analyzed. We have also analyzed for comparison 20 cases of squamous cell carcinomas.

CHAPTER VII

GENETIC STUDY OF THE EYELID CARCINOMAS

Our study included 29 pairs of samples, containing tumor fragment (T) and resection margins (peritumoral tissue – PT), coming from patients whom have been hospitalized, diagnosed and operated in the Ophthalmology Clinic of the Emergency County Hospital of Craiova, Romania, between 2013 and 2014. All cases were diagnosed using standard diagnose procedures and histopathologically confirmed.

The 29 pairs of samples were studied in order to determine the genic expression of VEGF and EGFR. Out of these 29 pairs of samples, 17 pairs came from the patients diagnosed with basal cell carcinoma and 12 pairs came from the patients diagnosed with squamous cell carcinoma.

The genic expression of VEGF-A was analyzed on the 29 pairs of samples containing tumor and peritumoral tissue. The qRT-PCR analysis proved that VEGF-A was expressed both in the tumor and in the peritumoral tissue, but variably.

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CHAPTER VIII

GENERAL DISCUSSIONS

Cutaneous carcinomas of the eyelids represent the most frequent tumors of the periocular area. Basal cell carcinoma is the most frequent human malignant tumor. Squamous cell carcinoma of the eyelid is the second most common cutaneous malignant tumor.

Histopathologically, our study highlights a predominance of the basal cell carcinoma, comparing to the squamous cell carcinoma, with a ratio of approximately 3.5/1.

Regarding the cell proliferation markers, ki67 represents a high molecular weight nuclear protein and it is considered to be the most reliable proliferation marker. The PCNA proliferation

marker presented in our study a medium / high expression in both basal cell carcinomas and in squamous cell carcinomas.

From the factors involved in programmed cell death, bcl-2 is an important gene in the mechanism of apoptosis, codifying a protein that inhibits apoptosis. Abnormal expression of this gene may lead to control loss of the modified cells, thus creating predisposition to neoplasia [24,25]. P53 mutations are frequent in case of malignant tumors, and the immunohistochemical detection highlights the alterations at genic level, due to the fact that normal p53 protein is difficult to determine because of the short half – life and of the low expressed levels [24,26].

In our study EGFR and VEGF immunoexpression was more intense in squamous cell carcinomas than in basal cell carcinomas, facts that have been statistically significant.

In head and neck squamous cell carcinomas, the increased expression of VEGF is associated with a more aggressive tumor phenotype, both clinically and experimentally [23,27].

CHAPTER IX

CONCLUSIONS

Our study highlights an increasing incidence of the eyelid carcinomas in the recent past years, as well as an increasing trend from one year to the next..

Molecular analysis of these tumors may have therapeutical consequences, molecular targeted therapy representing an alternative to classic therapy, especially in patients who can not endure a surgical procedure or in patients with locally advanced or metastatic disease.

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