ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Epidemiological and morphological data of ocular melanocytic lesions

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Abstract

Ocular melanocytic lesions comprise a spectrum of lesions ranging from benign nevi to invasive melanoma. Clinical and histopathological appearance of conjunctival lesions ranges from freckle to lentigo and to nevi. Between these types, conjunctival nevi and conjunctival melanosis are the most frequent. Conjunctival and uveal melanocytes are derived from the neural crest, as their cutaneous counterparts, whereas the pigment epithelial melanocytes are derived from the neuroepithelium or the layers of the optic cup. Melanomas can develop in one of several places within the eye, and can be divided in uveal melanomas and conjunctival melanomas. The purpose of the study was to investigate the epidemiological and morphological data of ocular melanocytic lesions, especially intraocular melanoma, through analysis of the ocular biopsies received in the Department of Pathology, Emergency City Hospital, Timisoara, Romania, for the period of five years. We did not observed any gender predilection neither in benign nor in malignant tumors. In our study, whatever the tumor location was, the most common type of melanomas was mixed with both, epithelioid and spindle cells. In some cases, immunohistochemical investigations are useful to appreciate the benign or malignant character of the tumor.

Keywords: ocular melanoma, ocular melanocytic lesions, ocular melanocytes, GNAQ.

Introduction

Ocular melanocytic lesions comprise a spectrum of lesions ranging from benign nevi to invasive melanoma. The origins of these lesions are conjunctival and uveal melanocytes, which, as their cutaneous counterpart, arise from pluripotent neural crest cells of the developing neural tube and overlying ectoderm. Benign ocular melanocytic lesions involve conjunctiva and different structures of uvea.

Clinical and histopathological appearance of conjunctival lesions ranges from freckle to lentigo and to nevi. Between these types, conjunctival nevi and conjunctival melanosis are the most frequent. Histopathological, the conjunctival nevi are grouped in subepithelial, junctional, compound nevi, blue and melanocytosis [1]. Most conjunctival nevi are considered acquired because they appear later in childhood, puberty or early adulthood [2, 3].

At uveal level, the nevi of the ciliary body and choroid are found in at least 30% of people, with no sex predilection. They are extremely rare in children. Iris nevi occur with increased incidence in people with neurofibromatosis.

There have been described conjunctival melanocytic lesions of intermediate character with precancerous features as primary acquired conjunctival melanosis.

Melanomas can develop in one of several places within the eye, and are divided in uveal melanomas and conjunctival melanomas.

Uveal melanomas are the most common type of ocular melanoma, comprising 95% of cases. As regarding their localization, uveal melanomas can be classified as anterior uveal melanomas (iris) and posterior uveal melanomas (choroid and ciliary body) and can simultaneously involve more than one uveal structure. Conjunctival melanomas are extremely rare, being diagnosed in 5% of cases of ocular melanomas and can occur in the conjunctiva or on the eyelid.

The purpose of the study was to investigate the epidemiological and morphological data of ocular melanocytic lesions, especially intraocular melanomas, through analysis of the ocular biopsies received in the Department of Pathology, Emergency City Hospital, Timişoara, Romania, for the period of five years.

Materials and Methods

The study was retrospective and included 32 cases of ocular biopsies from 2006 to 2011. The study was composed of both, benign and malignant tumors that were grouped according to their localization (conjunctiva and uvea) and to the histological type of the cells. The tissue specimens were obtained from an excisional biopsy and processed with routine histological technique. The specimens were fixed in 4% (v/v) formalin and embedded

in paraffin. Three micrometers thick serial sections were cut and classically stained with Hematoxylin–Eosin.

In one case, additional immunohistochemical reactions for HMB45, CD34 and Ki67 were made in order to establish the correct diagnosis. Sections of 3-µm thick were made and mounted onto Superfrost slides. The deparaffining was followed by rehydration using decreasing concentrations of alcohol. For CD34 reaction, we used enzymatic antigen retrieval. Antigen retrieval was done in Epitope Retrieval Solution pH 6 (10×) for Ki67 (Novocastra, code RE7113-CE) and in Epitope Retrieval Solution pH 9 (10×) for CD34 (Novocastra, code RE7119-CE), for 30 minutes using microwaves. The inhibition of the endogenous peroxidase and of other tissue protein was done using the solutions contained in Novolink Max Polymer Detection System (Novocastra) that was used also as detection system. The washing solution was represented by Bond Wash Solution, 10× (Leica). The antibodies used were HMB45 (Dako Monoclonal Mouse Anti-Human Melanosome, ready-to-use, clone HMB45), CD34 (Dako Flex Monoclonal Mouse Anti-Human Melanosome, ready-to-use, Class II, clone QBEnd10) and Ki67 (Novocastra, Liquid Concentrated Monoclonal Antibody, clone MM1). For dilution of Ki67 antibodies, we used Novocastra IHC Diluent, in a ratio of 1:150. The incubation time with primary antibody was 10 minutes for HMB45, 30 minutes for Ki67 and 15 minutes for CD34. The antigen–antibody complex was visualized with 3,3'-diaminobenzidine (from Novolink Max Polymer Detection System, Novocastra). The slides were washed in tap water and counterstained with Mayer's Hematoxylin and then dehydrated, cleared, and mounted. The signal was brown with cytoplasmic distribution for HMB45 and CD34 and nuclear for Ki67. In each determination, external control slides were included.

Histopathological evaluation was performed with Nikon Eclipse E600 microscope and images were acquired using Lucia G system.

Mean age and gender distribution was calculated.

Results

All the cases included in the study had the clinical diagnosis of melanocytic lesion. After histopathological examination, the cases were divided in six benign lesions and 26 malignant tumors of the uvea and the conjunctiva.

In the malignant lesions group, we observed no gender predilection, the male/female ratio being 1. Mean age for men was 53.76 years and 59.38 years for women.

Between ocular melanomas, the most common were those with uveal localization, 77% of the cases (18/23) (Figure 1). The most frequent localization of ocular melanomas was choroid, 54% (14/26), followed by ciliary body, 11.5% (3/26), iris, 4% (1/26) and conjunctive, 19% (5/26). In three cases, the exact localization of the tumor could not be established (11.5%). The sex distribution of uveal melanomas for each site of occurrence is summarized in Figure 2. The percent distribution of posterior uveal melanoma (choroidal and ciliary body) is represented in Figure 3. The age group and sex distributions of posterior uveal melanomas are summarized in Figure 4.

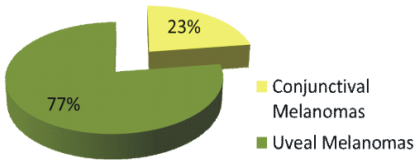

Figure 1 – ***Distribution of ocular melanomas regarding localization.***

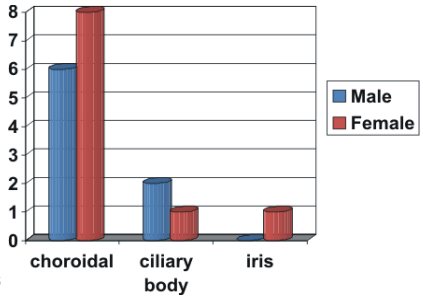

Figure 2 – ***Sex distribution of uveal melanomas.***

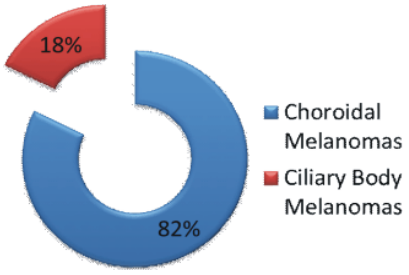

Figure 3 – ***Distribution of posterior uveal melanomas regarding localization.***

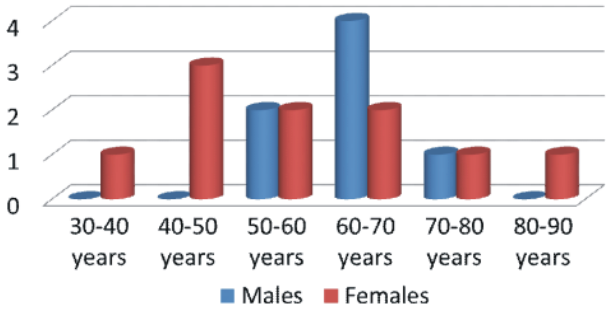

Figure 4 – ***Age group and sex distribution of posterior uveal melanomas.***

In our study, there was only one case of anterior uveal melanoma (iris melanoma) in an 83-year-old woman.

The dominating histological type was mixed, with both epithelioid and spindle cells, 76% (20/26). The rest of 24% was divided equally between tumors composed only with spindle or epithelioid cells, three cases each.

Most of posterior uveal melanomas had mixed cellularity with spindle B-cells in a fascicular pattern of growth between epithelioid cells. The spindle B-cells had oval nuclei with distinct nucleoli and no evident cell borders. In the cytoplasm, we observed variable amount of melanin (Figure 5).

The epithelioid cells contained large, round nuclei with prominent nucleoli and abundant eosinophilic cytoplasm with distinct cell borders. Many epithelioid cells were heavily pigmented (Figure 6).

In the same tumor, the pigmented cells had heterogeneous distribution, some areas being deeply pigmented (Figure 7) in the neighborhood of other acromic area (Figure 8).

The epithelioid cells showed increased pleomorphism compared with spindle B-cells (Figures 8 and 9). In two cases, the epithelioid cells had intermediate features with less cytoplasm and smaller nucleus than the classic epithelioid cells. These cases showed less pleomorphism than the classic type (Figure 10).

The iris melanoma was composed of heavily pigmented spindle cells (Figure 11).

Most of conjunctival melanomas were constituted of both epithelioid and spindle cells. The cells were pleomorphic with increased nuclear-to-cytoplasmic ratio. A small number of large, abnormal cells had round nuclei and big eosinophilic nucleoli. Similar to uveal melanoma, the pigmentation was present in all the cases with heterogeneous distribution. The pigmentation differed from slide to slide and even in the same microscopic field, with area of heavily pigmented cells in close relationship with completely acromic area (Figure 12). Mitotic figures were rare.

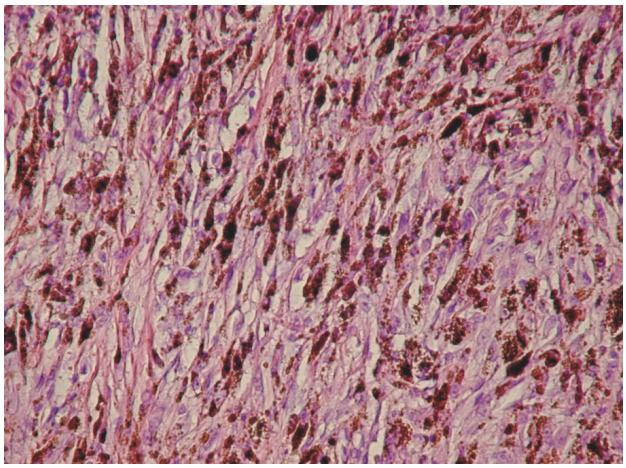

Figure 5 – ***Choroidal melanoma with spindle B-cells (HE stain, ob. 20×).***

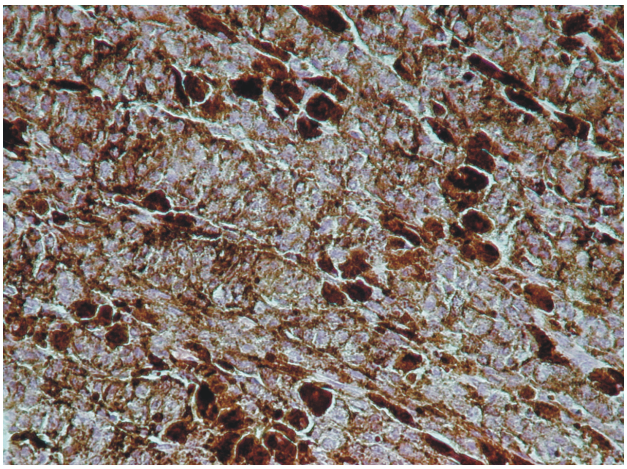

Figure 6 – ***Choroidal melanoma with heavily pigmented epithelioid cells (HE stain, ob. 40×).***

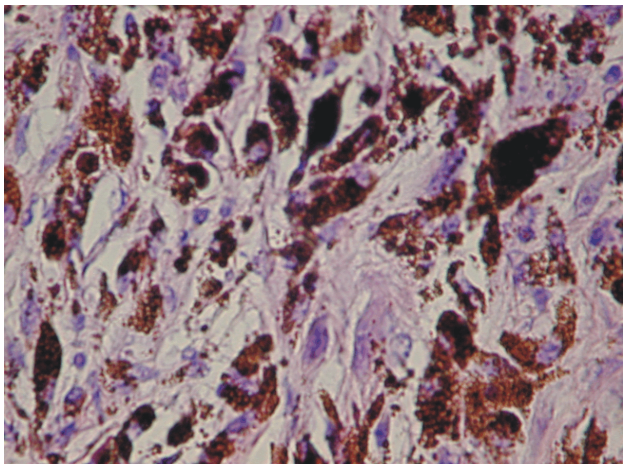

Figure 7 – ***Choroidal melanoma with pigmented spindle cells (HE stain, ob. 40×).***

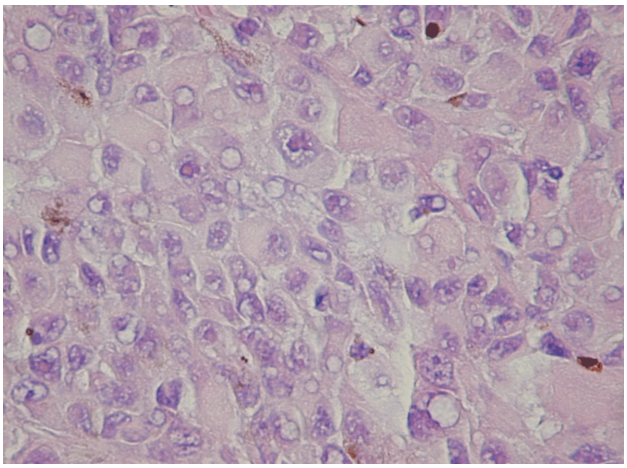

Figure 8 – ***Choroidal melanoma with acromic pleomorphic epithelioid cells (HE stain, ob. 40×).***

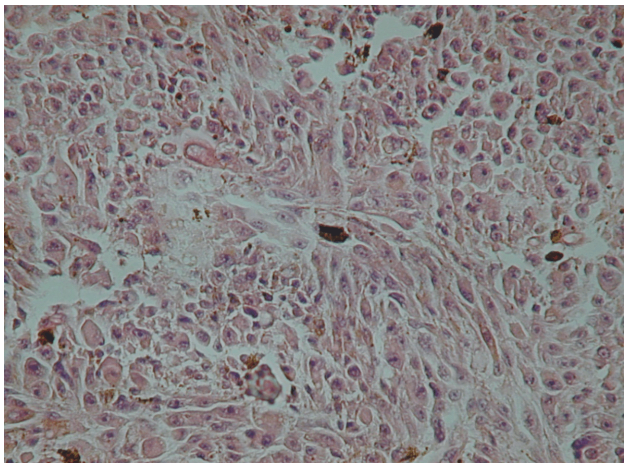

Figure 9 – ***Choroidal melanoma with pleomorphic distinct epithelioid cells (HE stain, ob. 20×).***

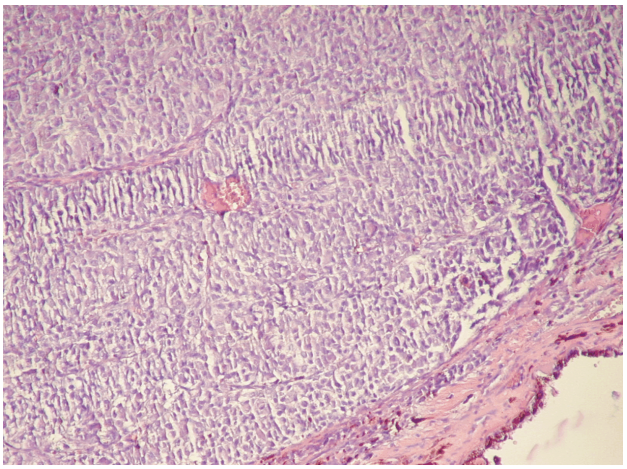

Figure 10 – ***Choroidal melanoma with intermediate epithelioid cells (HE stain, ob. 10×).***

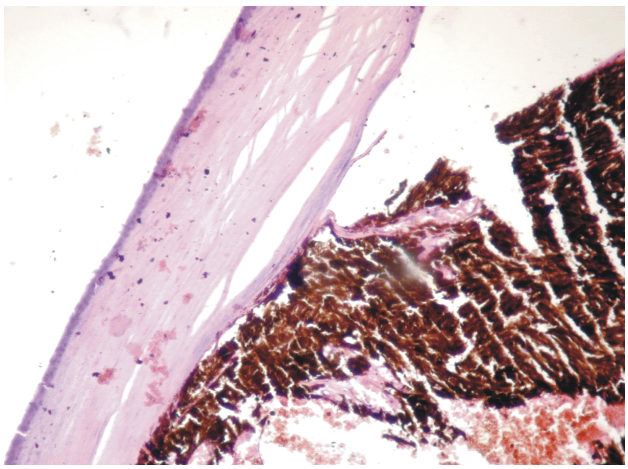

Figure 11 – ***Iris melanoma with heavily pigmented cells (HE stain, ob. 10×).***

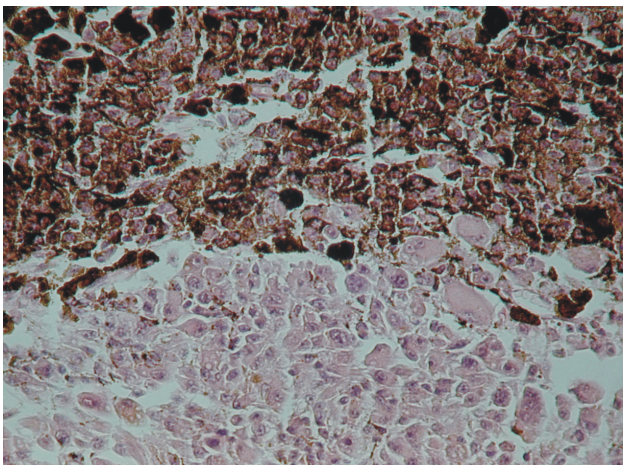

Figure 12 – ***Conjunctival melanoma with heterogeneous distribution of pigmented epithelioid cells (HE stain, ob. 20×).***

The benign tumors consisted of six conjunctival nevi (two compound nevi, two subepithelial nevi and an inflamed conjunctival nevus of puberty) and a conjunctival primary acquired melanosis, without atypia.

The gender distribution for this group was the same like in malignant tumors with no sex predilection. The mean age was 20.66 years for males and 35 years for females.

Histopathological, the two cases of compound nevi presented nests of melanocytes within the squamous stratified epithelium of the conjunctiva and between loosely arranged subepithelial collagen fibers of lamina propria. In these cases, we observed cystic epithelial inclusions in the connective tissue of lamina propria. In one case, the cysts were large and dominated the histological appearance, nearly obscuring the nevus presence.

The histopathologic appearance of subepithelial nevi showed islands of melanocytes only in the lamina propria. The nests of melanocytes were cohesive and found in intimate association with the adjacent epithelium.

We consider important to highlight a case of 12-year-old boy, who presented in 2011 for a conjunctival tumor. An excisional biopsy was performed and a histopathological diagnosis of compound conjunctival nevus was established. After six months, he returned for a recurrence. At this time, the clinical suspicion was of conjunctival melanosis. On the second biopsy, there was a prominent infiltrate of lymphocytes, plasma cells and eosinophils within a compound nevus between groups of melanocytes situated within the surface epithelium and in lamina propria, in the wall of some cystic inclusions. The subepithelial cysts were lined by surface non-keratinizing squamous stratified epithelium with goblet cells (Figure 13).

Because of clinical suspicion of conjunctival melanosis, there were performed immunohistochemical stains, the melanocytic cells being positive for HMB45 and negative for CD34 (Figures 14 and 15). The proliferative index highlighted by Ki67 antibodies was lower in the nuclei of nevus cells than the one of the surface epithelium, which leaded the diagnosis of a benign lesion. In this case, after histopathological and immunohistochemical evaluation, the final diagnosis was inflamed juvenile conjunctival nevus of puberty.

In our study, we diagnosed only one case of conjunctival primary acquired melanosis, without atypia that histopathological was composed of nests of hyperplasic bland melanocytes in the lamina propria of conjunctiva with slight pigmentation (Figure 16).

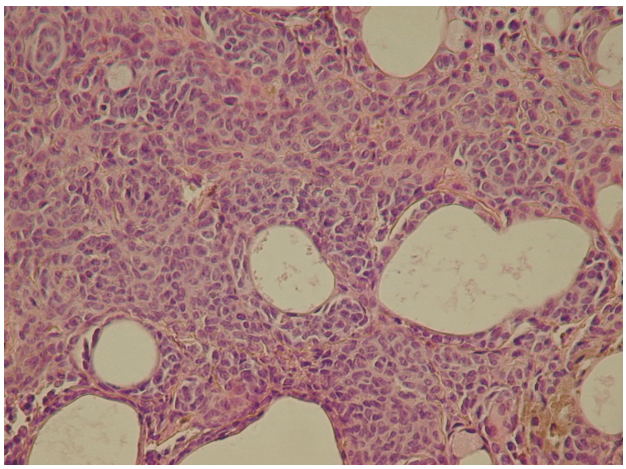

Figure 13 – ***Inflamed conjunctival nevus of puberty, nests of melanocytes between inclusions cysts (HE stain, ob. 10×).***

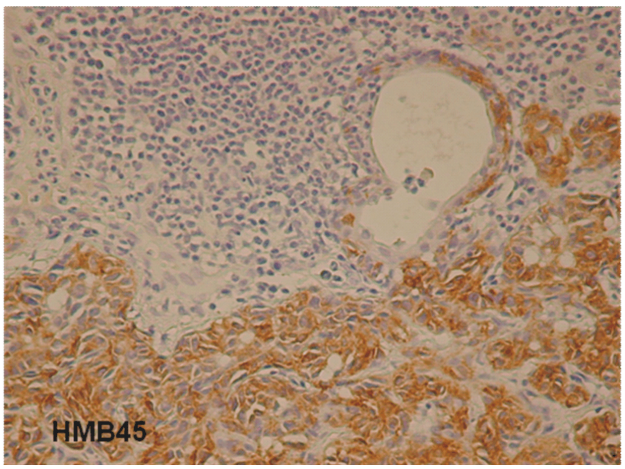

Figure 14 – ***Nevus cells positive for HMB45 with cytoplasmic distribution (ob. 10×).***

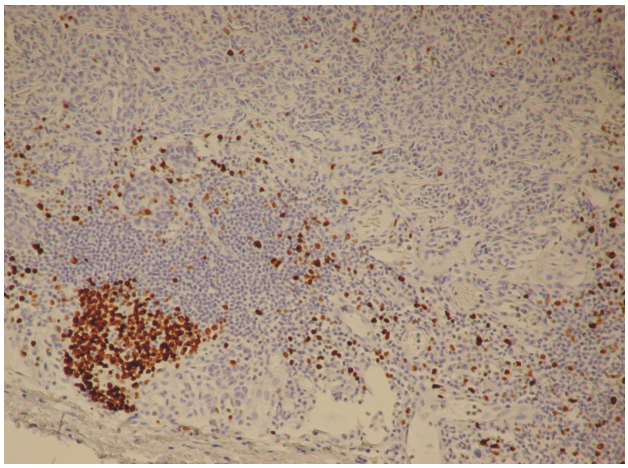

Figure 15 – ***Proliferative index highlighted by Ki67, in the nuclei of nevus cells and lymphocytes of germinal center (ob. 10×).***

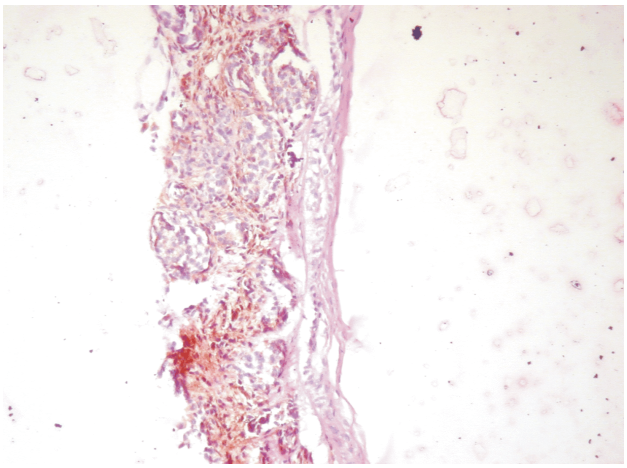

Figure 16 – ***Conjunctival primary acquired melanosis, without atypia, composed of hyperplasic bland melanocytes (HE stain, ob. 10×).***

Discussion

Melanomas of the uveal tract, are very rare, but in the same time represent the most frequent primary malignancy of the eye [4]. In contrast, conjunctival melanomas account for 1–2% of all ocular melanomas. In our study, the conjunctival melanomas represented 19% of all ocular melanomas.

Even if for skin melanomas the incidence raised in the last years, the frequency of ocular melanoma remained stable [4, 5]. The same remark can be made about the age of diagnosis. While cutaneous melanomas are diagnosed at younger ages as in 1970s, for the ocular melanomas this age stayed constant in older ages. The peak of incidence for uveal melanomas is in the seven decade of life, and for conjunctival melanomas at the middle age [6]. In our study, the mean age at diagnosis for uveal melanomas was 59 years, younger than in the literature. The mean age at diagnosis for conjunctival melanomas respected the published data.

Some of predisposing factors for uveal melanomas are Caucasian race, light eye color, fair complexion and the ability to tan [5, 7].

Many studies demonstrated that there is no association between sunlight exposure and risk of uveal melanoma [6]. An exception is represented by iris and conjunctival melanomas.

Similarly, there is no consistent evidence that occupational exposure to different agents is a risk factor for uveal melanoma [6, 8].

Ocular melanomas can arise *de novo* or from pre-existing melanocytic lesion (primary acquired melanosis or ocular nevus). For example, 75% of conjunctival melanomas arise from a primary acquired melanosis. For this reason, all these entities should be carefully observed.

One the other hand, it seems to be a strong relationship between familial atypical mole and melanoma (dysplastic nevus syndrome) and ocular melanocytic lesions. This autosomal dominant syndrome is characterized by numerous cutaneous dysplastic nevi and an increased incidence of skin and ocular melanomas.

Even if skin and ocular melanomas has the same origin, they differ significantly in their epidemiological, clinical, and cytogenetic features [9].

The chromosomal regions frequently observed to be deleted in most of ocular melanomas are on chromosome 3. Monosomy 3 is observed in around 50% of uveal melanomas compared with only 25% of cutaneous melanoma. Many authors consider this a powerful independent predictor of metastasis and negative outcome [10].

In addition, the gene expression status is modified in ocular melanomas. While cutaneous melanomas frequently harbor an activating mutation in either *NRAS* or *BRAF*, that promote melanoma development through the MAP kinase pathway, uveal melanoma lacks mutations in *BRAF*, *NRAS* or *KIT* [11–13], but has different other mutation.

Like in other parts of the body, the development of uveal melanomas involves early oncogenic mutations implicated in the regulation of the cell cycle or in the control of cell apoptosis.

The regulation of the cell cycle is disrupted at different levels. First, the inactivation of the retinoblastoma proteins by hyperphosphorylation that allows cells to reenter the cell cycle. Second, the mutation of the gene GNAQ (found in 49% of the uveal melanomas) and GNA11 (demonstrated in 31.9% of uveal melanomas) involve the RAF/MEK/ERK pathway that is important for melanocytes homeostasis. Activation of GNAQ mimics growth factor signaling leading to transcriptional activation of CCND1 and overexpression of cyclin D_1 [14, 15].

The control of cell apoptosis is disturbed by inactivation of the p53 pathway [16], defects in the Bcl-2 pathway [17], and activation of the pro-survival PI3-K pathway (loss of PTEN and activation of AKT) [18].

Recently, somatic mutations of *BAP1* (BRCA1 associated protein-1/ubiquitin carboxy-terminal hydrolase) were identified in many cases of ocular melanomas which exhibited also monosomy 3 [19].

Conjunctival melanomas molecular pathogenesis seems to implicate mutations of BRAF gene, like in cutaneous counterpart.

Uveal melanomas can arise in the anterior (iris) or the posterior (ciliary body or choroid) uveal tract. Most of uveal tract melanomas originate in the choroid. The ciliary body is less commonly site of origin, and the iris is the least common. In our study, the most common localization was choroid, followed by ciliary body and iris, respecting the data from the literature. In contrast, we did not observe the same ratio between choroid and ciliary body melanomas. While in the literature this ratio is 10:1, in our study was 5:1.

Initially, most uveal melanomas are completely asymptomatic. A growing tumor may cause distortion of the pupil (iris melanoma), blurred vision (ciliary body melanoma), and retinal detachment (choroidal melanoma). The retinal detachment leads to decreased visual acuity and angle-closure glaucoma.

In the early stages, the conjunctival melanomas may be asymptomatic or present blurred vision and tenderness of the eye. Among the later symptoms is loss of vision. Later stages can cause retinal detachment.

In most of the cases, the diagnosis of ocular melanomas is established only on clinical features. Clinically, the differential diagnosis of uveal melanomas includes nevi, hemangiomas and metastatic carcinoma [20]. Because small ocular melanoma is almost impossible to distinguish from a nevus and because the nevus can be considered a pre-existing lesion, all the growing melanocytic entities should be suspected for malignancy. The tumor thickness more than 2 mm, the presence of subretinal fluid, visual symptoms, orange pigment on the tumor surface and a tumor margin touching the optic disc may help to identify clinically a melanoma [21, 22].

There are some other tests, which help the clinician to diagnose an ocular melanoma, including Fluorescein angiography and ultrasonography.

The common origin of different type of melanomas (cutaneous and ocular) is observed on histopathologic slides of tumor material, both tumors being constituted of cells with the same morphological features [23].

Almost 80 years ago, Callender [24] described the histopathologic features of ocular melanoma and reported that their histopathology can predict survival. This classification was modified and refined [20, 25], at this time four distinct cellular types being recognized in intraocular melanoma:

1. Spindle A-cells: spindle-shaped cells with slender nuclei and lacking visible nucleoli;

2. Spindle B-cells: spindle-shaped cells with larger nuclei and distinct nucleoli;

3. Epithelioid cells: larger polygonal cells with one or more prominent nucleoli;

4. Intermediate cells: similar to but smaller than epithelioid cells.

Most primary intraocular melanomas contain variable proportions of epithelioid, spindle A, and spindle B-cells (mixed cell melanomas). Pure epithelioid cell primary melanomas are infrequent (approximately 3% of cases) [26]. In the *Collaborative Ocular Melanoma Study*, mixed cell type melanomas predominated (86% of cases) [20]. Until now, there is no consensus regarding the percent of epithelioid cells that classify a tumor in a mixed pattern [27].

One of the most important predictor of tumor behavior is cell type. Spindle A-cells confer the best prognosis, while epithelioid cells, the worst [20, 25, 28].

Because of lack of lymphatic vessels in the eye structures, ocular melanomas without extraocular extension have a strong tendency to metastasize hematogenously in the liver [29].

In our study, the dominating histological type was mixed, with both epithelioid and spindle cells, respecting the data of literature.

In addition to cell type, a number of other factors was demonstrated that influence prognosis. The most important include tumor size (dimension), mitotic activity, lymphocytic infiltration, and scleral and extraocular extension. Some authors consider also the fibrovascular loops as prognosis factor [30, 31].

Iris melanomas have the best prognosis, whereas melanomas of the ciliary body have the least favorable [27].

Conclusions

We did not observed any gender predilection neither in benign nor in malignant tumors. In our study, whatever the tumor location was, the most common type of melanomas was mixed with both, epithelioid and spindle cells. In some cases, immunohistochemical investigations are useful to appreciate the benign or malignant character of the lesion. Because of the association between ocular melanomas and dysplastic nevus syndrome, an interdisciplinary approach is necessary in order to diagnose earlier these rare tumors.

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Received: November 1st, 2012

Accepted: January 30th, 2013