Rom J Morphol Embryol 2021, 62(4):907–915 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.62.4.03

ORIGINAL PAPER

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

Clinical and immunohistopathological study of conjunctival melanocytic lesions in pediatric and adolescent patients. A case series

CAMELIA MARGARETA BOGDĂNICI[1,2], CLAUDIA FLORIDA COSTEA[1,3], GABRIELA FLORENŢA DUMITRESCU[4], ANCA SAVA[4,5], DELIA GABRIELA CIOBANU APOSTOL[5,6], DRAGOŞ VIOREL SCRIPCARIU[7], SIMONA DELIA NICOARĂ[8], DANIELA MARIA TĂNASE[9], ELENA CORINA ANDRIESCU[6], ALEXANDRU CĂRĂULEANU[10], MIHAI DANCIU[5,6], SILVIA CRISTINA SĂLĂVĂSTRU[1], SPERANŢA SCHMITZER[11], ANDREI IONUŢ CUCU[12], IRINA ANDREEA NIAGU[1]

[1] *Department of Surgery II, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[2] *1st Ophthalmology Clinic, St. Spiridon Emergency Clinical Hospital, Iaşi, Romania*

[3] *2nd Ophthalmology Clinic, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iaşi, Romania*

[4] *Department of Pathology, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iaşi, Romania*

[5] *Department of Morphofunctional Sciences I, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[6] *Department of Pathology, St. Spiridon Emergency Clinical Hospital, Iaşi, Romania*

[7] *Department of General Surgery, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[8] *Department of Ophthalmology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[9] *Department of Internal Medicine, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[10] *Department of Obstetrics and Gynecology, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania*

[11] *Department of Ophthalmology, Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[12] *2nd Neurosurgery Clinic, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iaşi, Romania*

Abstract

Background: Conjunctival pigmented neoplasia can be benign, premalignant or malignant tumors. Our study aims to establish the epidemiological, gross morphological and immunohistopathological features of the conjunctival pigmented lesions in pediatric and adolescent patients (<18 years), to establish an accurate diagnosis. *Patients, Material and Methods*: This is a retrospective case series study conducted within two Ophthalmology Clinics from Iaşi, Romania, on seven pediatric and adolescent patients. Using the Clinical Observation Chart and the Pathology Registers over a six-years period (January 2015–December 2021), we noted the patients' demographic data, clinical data, and ophthalmological investigations of the lesion, as well as the type of their treatment. All histological sections stained with Hematoxylin–Eosin (HE) and with five antibodies [pan-cytokeratin (pan-CK) AE1/AE3, S100 protein, Melan A, human melanoma black 45 (HMB45), and Ki67] were re-examined by four pathologists for each case, to identify the type of the conjunctival lesion and its histological and immunohistochemical features. *Results*: The mean age of all patients was 10.28 years, and the female/male ratio was 1.3. Right eye was more often affected (71.42%). 71.42% of cases presented an elevated lesion, 57.14% of cases showed a lightly pigmented lesion, but 14.28% of cases exhibited a pink lesion and this feature described the inflamed juvenile conjunctival nevus. In all cases (100%) the conjunctival pigmented tumor was removed with safety margins. The microscopic examination revealed a compound melanocytic nevus in 57.14% cases, a junctional conjunctival nevus in 14.28% cases, an inflamed juvenile nevus in 14.28% cases, and a conjunctival melanoma arising from a pre-existing nevus in 14.28% cases. In all cases of nevi, the nevoid melanocytes showed strong immunopositivity for Melan A and S100 protein, variable and weak immunopositivity for HMB45, and a mean Ki67 labeling index of 1.71%. Conjunctival melanoma revealed strong immunopositivity of tumor cells for HMB45, Melan A and S100 protein, and a Ki67 labeling index of 20%. In all cases, the conjunctival epithelium showed strong immunopositivity for pan-CK AE1/AE3. All our cases (100%) had a favorable outcome after the surgical removal of the tumor. *Conclusions*: Any excision of a conjunctival pigmented lesion must be subject to a systematic immunohistopathological examination, and there is a set of antibodies (anti-HMB45 and anti-Ki67) that are useful for differential diagnosis between a conjunctival nevus and a conjunctival melanoma.

Keywords: pediatric and adolescent patients, conjunctival pigmented tumor, nevus, melanoma, immunohistochemistry.

Introduction

The *World Health Organization* (WHO) Classification published in 1980 has been most widely used for almost 40 years and including three pathological categories for conjunctival pigmented lesions: conjunctival nevus, conjunctival melanosis, and malignant melanoma [1]. Also, conjunctival nevus was further classified into three histological subtypes, depending on the location of the tumor cells relative to the surface conjunctival epithelium:

compound, junctional, and subepithelial nevus. Recently, in 2018, a new *WHO* Classification of eye tumors of the eye was published, stating that melanocytic tumors of the conjunctiva are divided into the following histological categories: (*i*) conjunctival nevus (junctional, compound, and subepithelial); (*ii*) inflamed juvenile conjunctival nevus; (*iii*) blue nevus of the conjunctiva; (*iv*) Spitz nevus of the conjunctiva; (*v*) benign epithelial melanosis of the conjunctiva; (*vi*) conjunctival melanocytic intraepithelial neoplasia (C-MIN) that includes primary acquired melanosis (PAM), with and without atypia; (*vii*) conjunctival melanoma [2].

In a recent study, Shields *et al*. (2017) analyzed 806 patients younger than 21 years with conjunctival tumor and found out that nevi represented the most frequent pathology (61%), but melanoma was the rarest as only 2.2% of all patients were diagnosed with this malignant melanocytic neoplasia [3].

Most conjunctival nevi occur in childhood or puberty, when the nevus cell proliferation is determined by hormonal modifications [4, 5], while malignant conjunctival melanoma develops in middle aged and older patients [6]. The latest data reported that the incidence of conjunctival melanoma in the USA and Europe has increased by 295% over the last 27 years, and this fact requires increased attention for early diagnosis because this malignant tumor poses an important risk for metastasis and even death [7]. It is considered that this increasing of the incidence of the conjunctival melanoma was due to increased exposure to ultraviolet (UV) radiation [7].

However, even if conjunctival melanoma in children has a very low incidence, this type of neoplasm has a high level of malignancy, and its diagnosis is very important in terms of life expectancy.

The classical method for a certain diagnosis of a conjunctival pigmented lesion is represented by its surgical removal followed by the examination of its microscopic characteristics. Even if pathologists can usually establish a correct diagnosis of a conjunctival pigmented lesion when they examine the ocular tissues in standard Hematoxylin–Eosin (HE) staining, in some instances the neoplasia could have borderline malignant characteristics and thus the pathological diagnosis become quite difficult even for experienced pathologists [8]. In these cases, immunohistochemical (IHC) staining is recommended for differential diagnosis [9].

As far as we know, there are only few articles in literature dealing with pigmented conjunctival lesions in pediatric and adolescent populations [3, 4, 10, 11], but the prognosis of some of these lesions, especially of melanoma, could be quite worrisome as it can be a threat to patient's sight and life [10].

Aim

The purpose of our study was to describe the epidemiology, clinical characteristics and pathological features, emphasizing the IHC aspects of melanocytic conjunctival lesions in pediatric and adolescent patients (<18 years), with the aim of improving the management of this ocular pathology in terms of diagnosis, but also of surgical treatment.

Patients, Materials and Methods

Patients

We conducted a retrospective study on the conjunctival pigmented lesions diagnosed in patients younger than 18 years and surgically excised in the Ophthalmology Clinics of two Hospitals from Iaşi, Romania (St. Spiridon Emergency Clinical Hospital and Prof. Dr. Nicolae Oblu Emergency Clinical Hospital) over a six-year period (January 2015–December 2021). Using the Clinical Observation Chart and the Pathology Registers, we noted the patients' demographic data (age and gender), clinical data (location and macroscopic characteristics of the lesions, such as contour, and color), ophthalmological investigations that have been made during the hospitalization [visual acuity (VA), intraocular pressure (IOP), ocular motility examination, anterior segment examination at slit-lamp, and fundus examination], as well as the type of their treatment. Surgical excision of conjunctival nevi was performed in all cases.

Tissue samples

All available surgical specimens were originally fixed in 10% neutral buffered formalin solution for 24 hours, dehydrated in acetone and xylene, and then embedded into paraffin. Finally, histological sections of 3 μm were stained with HE for microscopic examination.

All the excision specimens and histological slides were retrieved from the Pathology Departments archives. New histological sections with a thickness of 3 μm were obtained from the available paraffin blocks to realize the IHC staining. To identify the tumoral melanocytes we used the following antibodies: anti-S100 protein, anti-Melan A, anti-human melanoma black 45 (HMB45), but for the identification of the conjunctival epithelial cells we used anti-pan-CK AE1/AE3. Ki67 labeling index was used for assessing the proliferative activity of the pigmented conjunctival lesions (Table 1).

Table 1 – ***Immunohistochemical panel of antibodies used in our case series***

Antibody	Clone	Manufacturer	Dilution	Antigen retrieval	Control
Pan-CK AE1/AE3	Mouse monoclonal AE1/AE3	DAKO	1/50	Citrate, pH 6	Liver
Melan A	Mouse monoclonal A103	DAKO	1/50	pH 9	Skin
Melanosome	Mouse monoclonal HMB45	DAKO	1/50	Citrate, pH 6	Melanoma
Ki67	Mouse monoclonal MM1	Novocastra	1/200	Citrate, pH 6	Amygdala
S100 protein	Rabbit polyclonal	DAKO	RTU		Schwannoma

CK: Cytokeratin; HMB45: Human melanoma black 45; RTU: Ready-to-use.

We also used the following IHC technique:

- serial histological sections were placed on silanized-electrostatically charged slides for an efficient adhesion;
- the histological sections were deparaffinized in xylene, rehydrated in progressive decreasing concentrations of four alcohol baths (100%, 90%, 80% and 70%) and rinsed in distilled water;
- unmasking of the antigenic site was performed using

by the heat-induced epitope retrieval (HIER) method, with retrieval antigen solution with a pH of 6, or a pH of 9, depending on the antibody used;

▪ the slides were inserted in the antigen retrieval solution and then were placed in a microwave oven for 30 minutes; then the slides were allowed to cool slowly at room temperature for 20 minutes;

▪ endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 10 minutes;

▪ after inhibition of endogenous peroxidase, sections were incubated with primary antibodies at 4°C overnight;

▪ amplification of the immunoreactions was performed with the help of specific secondary and tertiary antibodies of the Labeled Streptavidin Biotin–Horseradish Peroxidase (LSAB–HRP) complex;

▪ the reaction was developed with 3,3'-Diaminobenzidine (DAB) tetrahydrochloride chromogen (DakoCytomation) for 10 minutes;

▪ cross-staining of the sections was performed with Mayer's Hematoxylin;

▪ simultaneously, positive and negative controls were performed against the correspondent tissue used as an internal positive control for the antibodies we have used for the present study.

The IHC positive reaction was considered in the presence of a brown cytoplasmic staining in tumoral nevoid cells for S100 protein, Melan A, and HMB45, a brown cytoplasmic staining in conjunctival epithelial cell for pan-CK AE1/AE3, and a brown nuclear staining of nevoid cells for Ki67.

The histological sections were re-examined on the optical microscope by four pathologists for each case, to identify the histological type of the conjunctival lesion, describing at the same time its histological and IHC features. The final diagnosis was established by consensus.

Statistical analysis

The obtained data were entered in a Microsoft Excel spreadsheet and analyzed by means of percentages.

Results

Between January 2015–December 2021, seven children and adolescents (<18 years old) were referred to our two Ophthalmology Clinics for evaluation of conjunctival pigmented lesions (Table 2).

Table 2 – ***Patients' demographic data, clinical data, ophthalmological investigations, and immunohistopathological features of the conjunctival pigmented lesions in our case series***

No.	G	A	Clinical diagnosis at the admission to hospital	Location of the lesion	VA	IOP	OM	SL	FE	Macroscopic features	Pathological diagnosis				
											HE staining	*IHC*			
												CEp	*Tumor melanocytic cells*		
												CK	*HMB45*	*S100*	*Ki67*
1.	F	10	LE conjunctival pigmented lesion	Interpalpebral bulbar conjunctiva	N	N	N	N	N	Lightly pigmented, elevated conjunctival lesion, with an irregular contour	Compound melanocytic conjunctival nevus	+++	+	+++	1.6%
2.	M	17	RE conjunctival nevus	Interpalpebral bulbar conjunctiva	N	N	N	N	N	Lightly pigmented, elevated conjunctival lesion, with microcysts on its surface	Compound melanocytic conjunctival nevus	+++	+	+++	1.8%
3.	F	6	LE conjunctival nevus	Juxta-limbal	N	N	N	N	N	Lightly pigmented, small, flat conjunctival lesion	Compound melanocytic conjunctival nevus	+++	+	+++	1.7%
4.	F	7	RE conjunctival nevus	Interpalpebral bulbar conjunctiva	N	N	N	N	N	Heavy pigmented, elevated, irregular conjunctival lesion	Compound melanocytic conjunctival nevus	+++	+	+++	1.7%
5.	F	12	LE conjunctival lesion	Juxta-limbal	N	N	N	N	N	Lightly pigmented, flat conjunctival lesion	Junctional conjunctival nevus	+++	+	+++	1.9%
6.	M	13	RE conjunctival lesion	Juxta-limbal	N	N	N	N	N	Pink, slightly elevated, conjunctival lesion	Inflamed juvenile conjunctival nevus	+++	+/-	+++	1.6%
7.	M	7	RE pigmented conjunctival lesion	Temporal conjunctiva	N	N	N	N	N	Heavy pigmented (brown), elevated conjunctival lesion	Conjunctival melanoma arising from a preexisting nevus	+++	+++	+++	20%

A: Age [years]; CEp: Conjunctival epithelium; CK: Cytokeratin; F: Female; FE: Fundus examination; G: Gender; HE: Hematoxylin–Eosin; HMB45: Human melanoma black 45; IHC: Immunohistochemistry; IOP: Intraocular pressure; LE: Left eye; M: Male; N: Normal; No.: Number; OM: Ocular motility examination; RE: Right eye; SL: Anterior segment examination at slit-lamp; VA: Visual acuity.

There were more females (4/7 cases; 57.14%) than males (3/7 cases; 42.85%) (Figure 1), and thus the female/male ratio was 1.33 in favor of female patients. The overall age of patients ranged from six to 17 years old. The mean age of all patients was 10.28 years, but when we analyzed only patients with benign lesions, we found out a mean age of 10.83 years and the only patient with conjunctival melanoma was seven years old. Right eyes were slightly more often affected (4/7 cases; 57.14%) than the left eyes (3/7 cases; 42.857%) (Figure 3, A–C). In 3/7 (42.85%) cases, the pigmented lesions developed in the interpalpebral bulbar conjunctiva, 3/7 (42.85%) cases were juxta-limbal (Figure 3, A–C), and in 1/7 (14.28%) cases, *i.e.*, conjunctival melanoma was identified in the temporal conjunctiva (Figure 3B). 5/7 (71.42%) cases presented an elevated conjunctival lesion, and 2/7 (28.57%) cases were flat. In 4/7 (57.14%) cases, we identified a lightly pigmented conjunctival lesion, which was correlated with a compound nevus (Figure 3A)

or a junctional nevus (Figure 3C), 2/7 (28.57%) cases presented a heavy pigmented (brown) lesion (Figure 3B), and 1/7 (14.28%) cases exhibited a pink color lesion, which described the inflamed juvenile conjunctival nevus.

In all cases (100%), the VA, IOP, ocular motility, anterior segment examination at slit-lamp, and fundus examination were normal. In all cases (100%), the conjunctival tumor was surgical removed with safety margins. The surgery was performed mostly to rule out a conjunctival melanoma (4/7 cases; 57.14%), due to a recent growth of the lesion (1/7 cases; 14.28%), or because of a cosmetic concern (1/7 cases; 14.28%).

In 4/7 (57.14%) cases, the microscopic examination revealed a compound melanocytic nevus (Figures 2, 3A and 4, A–F) with a lentiginous expansion along the epithelium of coverage, with reduced pigmentation and with important inflammatory infiltrate, made up of lymphocytes and plasmacytes, at the base of the lesion. Inside the nevus, there were some cystic structures made up of invaginated conjunctival epithelium which are surrounded by nevoid cell nests (Figure 4, A and B).

A junctional conjunctival nevus was diagnosed in 1/7 (14.28%) cases (Figure 2). The histopathological exam identified contiguous nests of nevoid melanocytes located near the basal cell region of the conjunctival epithelium.

1/7 (14.28%) cases exhibited an inflamed juvenile conjunctival nevus (Figures 2 and 5, A–G) made up of intraepithelial and subepithelial nevoid melanocytic nests organized around solid or cystic epithelial inclusions, the latter also including goblet cells. There was also a dense, diffuse, inflammatory infiltrate in tumor stroma, exhibiting lymphocytes, plasma cells and numerous eosinophils. At the base of the lesion, lymphocytes were organized into a lymphoid follicle with germinal center. Nevoid melanocytes also showed focal pagetoid spreading along the conjunctival epithelium (Figure 5, A–C).

In all these cases of nevi, the nevoid melanocytes showed strong immunopositivity for Melan A (Figures 4C and 5E) and S100 protein (Figure 5F), along with variable and weakly immunopositivity for HMB45 (Figure 4D), and a mean Ki67 proliferation index of 1.71% (Figure 5G). The conjunctival epithelium revealed strong immunopositivity for pan-CK AE1/AE3 (Figures 4B and 5D).

In 1/7 (14.28%) cases (Figure 3B), the clinical examination revealed a single, irregular, prominent, pigmented lesion, situated 3 mm from the limbus in the temporal part of the conjunctiva in the right eye. The treatment for this patient was total surgical removal of the tumor with at least 1 mm of macroscopic healthy tissue, removal of Tenon's capsule, and then cryotherapy over the underlying sclera and autologous conjunctival graft for the local defect. The pathological examination showed in this case a thin surface epithelium and a proliferation of atypical melanocytes with a bizarre polygonal epithelioid feature, eosinophilic cytoplasm, large, atypical nuclei, and prominent eosinophilic nucleoli. The tumor cells were arranged in a dispersed shape, but also infiltrated the conjunctival epithelium in a pagetoid manner. At the edge of the excision, we noted the presence of a compound nevus at the edge of the excision. IHC staining's revealed strong immunopositivity of the conjunctival epithelium for pan-CK AE1/AE3, strong immunopositivity of tumor cells for HMB45, Melan A and S100 protein, as well as a Ki67 proliferation index of 20%. The final diagnosis was conjunctival melanoma arising from a pre-existing nevus.

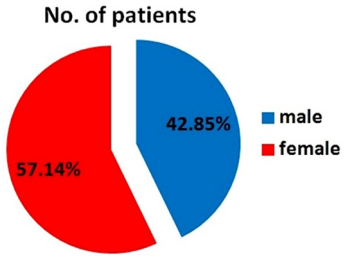

Figure 1 – ***Diagram of the distribution of cases according to patients' gender.***

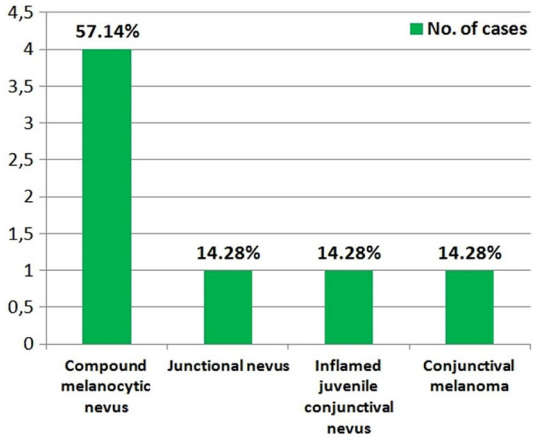

Figure 2 – ***Diagram of the distribution of cases according to immunohistopathological diagnosis of the lesion.***

Figure 3 – ***Anterior segment photographs showing clinical morphology of conjunctival pigmented lesions correlated with their histological classification: (A) Case No. 3 – girl, 6 years old, presented a juxta-limbal, irregular, small, flat, lightly pigmented conjunctival lesion, which was diagnosed as a compound conjunctival nevus on her left eye; (B) Case No. 7 – boy, 7 years old, showed a solitary, sharply demarcated, prominent, heavy pigmented lesion, located on the temporal conjunctiva of her right eye that was diagnosed as a conjunctival melanoma arising from a preexisting nevus; (C) Case No. 5 – girl, 12 years old, exhibited on her right eye a juxta-limbal, irregular, slightly elevated, lightly tan conjunctival lesion, with few microcysts on its surface, which was diagnosed as a junctional conjunctival nevus.***

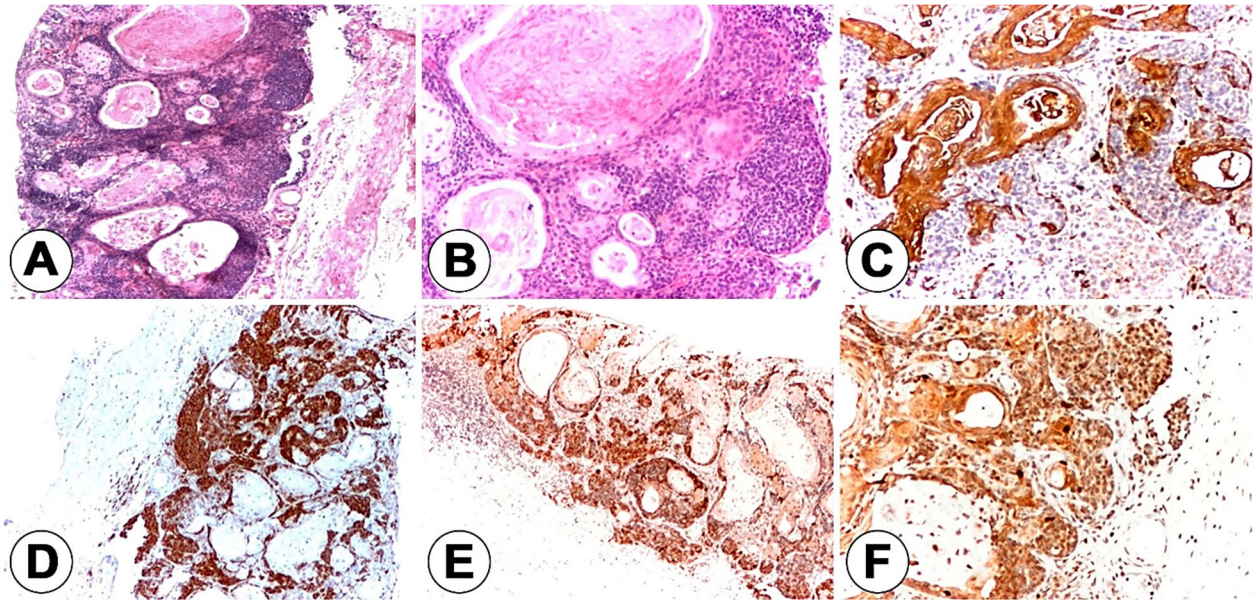

Figure 4 – ***Microphotographs of compound conjunctival nevus. Case No. 3 – girl patient, 6 years old: (A) Compound nevus with nevoid melanocytes presence along the covering epithelium, with low pigmentation and significant inflammatory lymphoplasmacytic infiltrate at the base of the lesion. Nests of nevoid melanocytes around and among multiple cystic spaces lined by conjunctival epithelium. Nevoid cell nests on the base of the lesion and around cystic structures. Multiple large epithelial cysts could be seen in the histological section. Conjunctival epithelium shows squamous metaplasia and hyperplasia, with deep invagination and formation of cystic structures that are surrounded by tumor cells; (B) Cytoplasmic immunopositivity of the conjunctival epithelium for CK AE1/AE3 revealed its hyperplasia, but nevocytic cell nests do not show any immunostaining; (C) Immunopositivity for Melan A in the cytoplasm of the nevoid melanocytic cells demonstrates their organization into small nests around cystic spaces and at the base of the lesion, but non-staining cells represented the conjunctival epithelium; (D) Slight immunopositivity in the cytoplasm of the nevoid cell nests for HMB45; (E) The conjunctival epithelium and inflammatory cells did not stained with anti-HMB45 antibody; (F) Less than 1% of the nuclei were labeled for Ki67. HE staining: (A) ×20. Anti-CK AE1/AE3 antibody immunomarking: (B) ×100. Anti-Melan A antibody immunomarking: (C) ×400. Anti-HMB45 antibody immunomarking: (D and E) ×40. Anti-Ki67 antibody immunomarking: (F) ×200. CK: Cytokeratin; HE: Hematoxylin–Eosin; HMB45: Human melanoma black 45.***

All our cases (100%) had a favorable outcome after the surgical removal of the tumor.

Discussions

In childhood (<14 years), melanocytic, choristomatous, vascular, epithelial, and lymphoid neoplasia can especially be diagnosed at the conjunctival level, but melanocytic lesions are the most common tumors, representing approx. 30% of all cases [12].

Recently, in 2021, a group of researchers at Moorfields Eye Hospital, London (UK), evaluated over a six-years period all children and adolescents under the age of 20 who were diagnosed with a conjunctival nevus. Their series included a total of 77 patients with a mean age of 12 years (range: four to 20 years) [13].

A few years ago, a group of Polish authors have analyzed 30 melanocytic conjunctival lesions diagnosed in a pediatric hospital and have found a mean age of 11.2 years for their patients (range 4.0–17.5 years) [14].

Before these authors, another group of researchers from Los Angeles (USA) analyzed conjunctival pigmented lesions in patients under the age of 20 who were admitted to their Clinic. They identified benign lesions in 95.77% of cases and reported an average age of 11.6 years for these patients (range: three to 20 years) [15].

Our group of patients had a mean age comparable to that identified by all of these studies, although they varied in both number and age range.

Some authors reported that conjunctival melanocyte lesions mainly affect girls [14], as we also recorded in the present study, but other authors stated that boys are more affected by this pathology [13]. Negretti *et al.* (2021) pointed out that 95% of all the conjunctival nevi that they have analyzed were located on the bulbar conjunctiva, most common (53%) in the temporal horizontal quadrant, and often involving the limbus (57%) [13]. Some other authors reported that nevi are located in the interpalpebral bulbar conjunctiva (67–72%), the lacrimal caruncle (15–22%), and tarsus (0.7%) [15, 16]. Cases from our series also presented a predisposition to develop juxta-limbal or in the interpalpebral conjunctiva, but we did not identify any conjunctival melanocytic lesions at the level of the lacrimal caruncle or *plica semilunaris*.

Pigmentation of conjunctival nevi is variable from light tan to brown or even amelanotic [17] and this color variability was also visible in our series. On slit-lamp examination, most nevi are well-defined and have a cystic aspect. Nevi can change with puberty or pregnancy, but otherwise should remain the same [7]. The clinical manifestations are variable and thus diagnostic difficulties can appear [18]. Signs of malignancy are represented by unusual site, like the palpebral or forniceal conjunctiva, the appearance of important feeder vessels, sudden growth, an increasing in pigmentation, and its development after the second decade of life [19].

Literature reported that melanocytic conjunctival lesions

are benign in 92% of cases, almost 8% are borderline lesions, and less than 3% are malignant tumors [4]. Negretti *et al.* (2021) reported a conjunctival melanoma in 8% of all cases in their series [13]. In our series, we found out almost a double percentage of conjunctival melanoma, but this fact is correlated with our small number of cases.

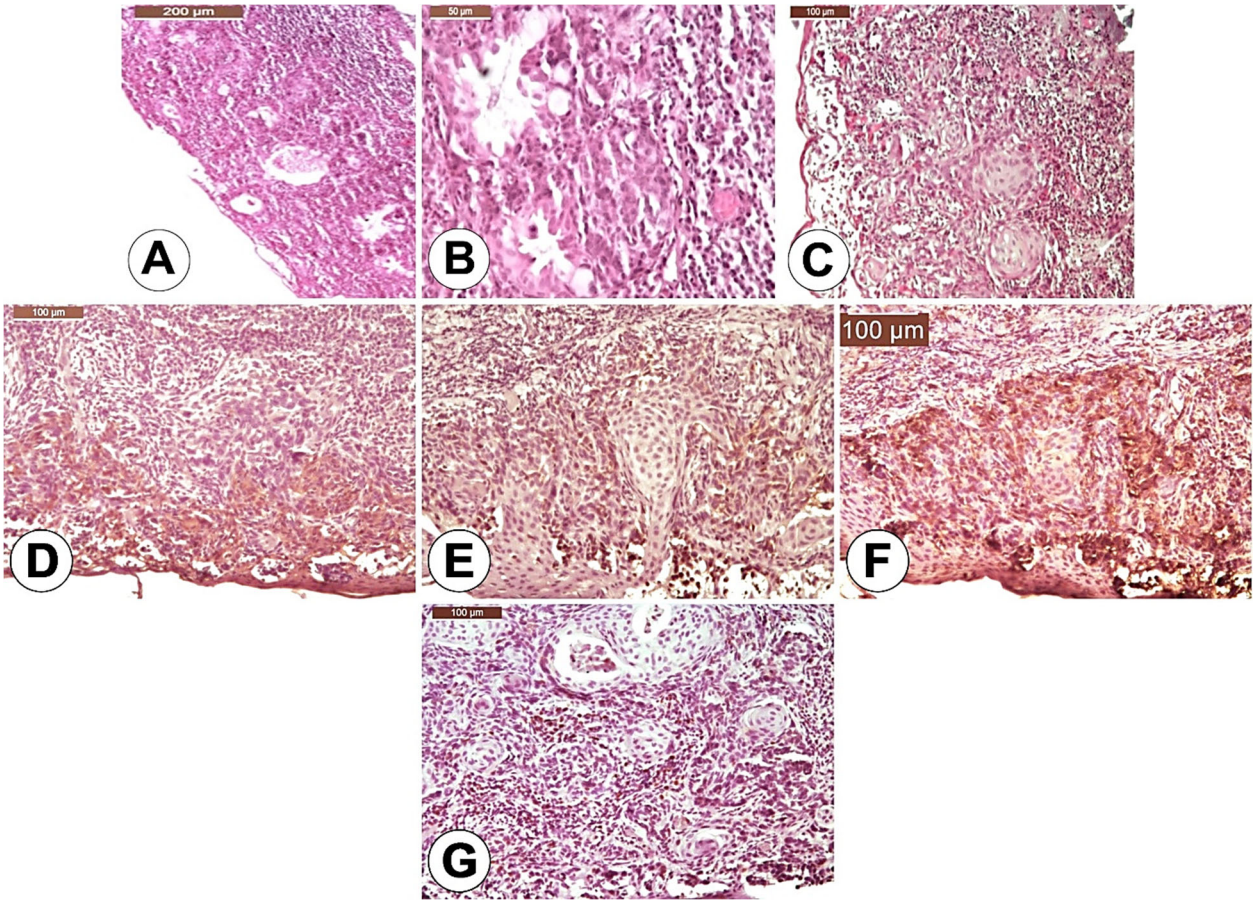

Figure 5 – ***Microphotographs of the inflamed juvenile conjunctival nevus. Case No. 6: boy patient, 13 years old: (A) The histological sections showed compound nevoid melanocytic proliferation made up of intraepithelial and subepithelial melanocytic nests organized around solid or cystic epithelial inclusions that also contain goblet cells. There was also a dense, diffuse, stromal inflammatory infiltrate with lymphocytes, plasma cells and eosinophils. At the base of the lesion, lymphocytes were organized into a lymphoid follicle with germinal center; (B) Cystic epithelial inclusions, with a predominance of goblet cells, surrounded by nests of nevoid melanocytes, along with dense stromal inflammatory infiltrate; (C) Nevoid melanocytic cells were located in conjunctival epithelium, but also in subepithelial connective tissue alongside with solid epithelial inclusions and eosinophils in a high number, which were diffusely dispersed among the nests of nevoid melanocytes; (D) Positive immunostaining for CK AE1/AE3 of cells organized into solid structures included into the tumor mass demonstrated their epithelial nature; (E) Positive immunostaining for Melan A in the cytoplasm of nevo-melanocyte cells revealed their focal pagetoid spread in the conjunctival epithelium, as well the presence of tumor nests in the subepithelial conjunctive tissue; (F) Immunopositivity for S100 protein also showed the intraepithelial as well as the stromal nevomelanocytic nests; (G) Ki67 mean labeling index was 1% in nevocytic cells. HE staining: (A) ×100; (B and C) ×200. Anti-CK AE1/AE3 antibody immunomarking: (D) ×200. Anti-Melan A antibody immunomarking: (E) ×200. Anti-S100 antibody immunomarking: (F) ×200. Anti-Ki67 antibody immunomarking: (G) ×200. CK: Cytokeratin; HE: Hematoxylin–Eosin.***

In terms of histological subtyping of the benign conjunctival nevi, McDonnell *et al.* (1989) recorded almost 72% compound nevi, 21% junctional nevi, and 3.5% subepithelial nevi. Spitz nevus and cellular blue nevus represented less than 2% each [15]. Analyzing a series of 30 cases of melanocytic conjunctival lesions, Urban *et al.* (2017) found out that compound nevi represented about 74% of all nevi, junctional nevi were observed in almost 24% of cases, while subepithelial nevi represent only 9% of all conjunctival nevi [14]. In the present study, compound nevi were also the most frequent type of conjunctival nevi, but we did not find any subepithelial nevus, Spitz nevus or cellular blue nevus.

In our series, from a histopathological point of view, compound nevi showed nests of nevoid melanocytes, more often with low pigmentation, which located intraepithelial and subepithelial, along with epithelial cystic inclusions and prominent inflammation in the tumor stroma. Even though the patients declared growth in size, during the past few months of their pigmented conjunctival nevus the clinical appearance did not show any signs of malignancy. The moderate growth of the lesion before the surgical removal could be associated with epithelial cysts increasing in size because of goblet cells mucin secretions associated with a large quantity of inflammatory cells that are located inside the tumoral stroma.

Inflamed juvenile conjunctival nevus is a benign lesion that is located most often on the juxta-limbal conjunctiva and occurs in children and adolescents (average age of surgery 11–13 years) [20–22]. This lesion is associated with systemic allergy, allergic conjunctivitis, and vernal conjunctivitis [4, 6] and for this reason a strong inflammation could be seen in nevus stroma. In such a case, we identified lymphoid follicles with germinal center, but also heavy stromal infiltrate with eosinophils. In children, inflamed juvenile conjunctival nevus is usually a lightly pigmented or amelanotic lesion, located juxta-limbal that become pigmented at puberty or pregnancy [20, 21].

The IHC staining's could be essential for the diagnosis of conjunctival melanocytic lesions. IHC markers like pan-CK AE1/AE3, Melan A, S100 protein, HMB45, and Ki67 labeling index are helpful to establish the final diagnosis. Analyzing 16 cases of conjunctival pigmented lesions (compound nevi, subepithelial nevi, acquired melanoses, and melanomas) stained with HMB45, Glasgow *et al.* (1990) stated that both benign and malignant lesions showed diffuse cytoplasmic staining, but compound nevi and subepithelial nevi showed less intense staining compared with conjunctival melanomas. These authors concluded that, even though skin nevi do not show any staining with anti-HMB45 antibody, in the case of conjunctival nevi there is an immunoreactivity for this antibody in the nevoid melanocytes located in the subepithelial tissue. However, anti-HMB45 antibody is not useful for the differential diagnosis between a conjunctival nevus and a conjunctival melanoma [23]. Our study revealed that both conjunctival melanoma and conjunctival nevi, except for inflamed juvenile conjunctival nevus, presented an immunopositivity for HMB45, but in the case of nevi the immunoreactivity to this antibody was less intense.

Jakobiec *et al.* (2010) realized a retrospective immunohistopathological study of 35 conjunctival nevi and invasive melanomas and found out that S100 protein is not useful in separating benign from malignant neoplasias, as both types of tumors immunostained moderately to strongly with this antibody. More valuable tool for the differential diagnosis in this case seems to be the Ki67 labeling index because its value was less than 2% in conjunctival nevi, but conjunctival melanomas express a much higher value (17.3%) [24]. Also, in our case series, we found out a mean Ki67 labeling index of 1.71% in conjunctival nevi, but in conjunctival melanoma it had a value of 20%. We encountered some difficulties to establish the value of Ki67 labeling index in inflamed juvenile conjunctival nevus due to the numerous lymphocytes in the tumoral stroma as their nuclei strongly immunostained with anti-Ki67 antibody.

Shields *et al.* (2004) reported that only 10% of the conjunctival pigmented lesions from their series were excised for cosmetic reasons [25], but Negretti *et al.* (2021) affirmed that 75% of their patients requested the excision of the lesion probably because they were mainly adolescents and were preoccupied for their facial appearance [13].

While nevus is the most common conjunctival tumor in childhood, conjunctival melanoma is very rare in this age group. It usually develops from a pre-existing nevus when it changes in size and pigmentation, but this situation can occur in less than 1% of all conjunctival nevi [3, 26, 27]. Yangzes *et al.* (2018) reported the case of a 16-year-old boy who was hospitalized with a large brownish lesion that measure 20×12 mm in its diameters and was located on the temporal conjunctiva in his right eye. After a conjunctival excisional biopsy of the lesion, the histopathological exam established the diagnosis of a conjunctival melanoma with immunopositivity for HMB45, Melan A and a high Ki67 proliferation index [28].

Recently, a group of Australian researchers conducted a systematic review searching for cases of conjunctival melanoma occurring in patients younger than 18 years of age. They found 17 studies with only 32 patients. These patients have a mean age of 11 years (range: four to 18 years old). A slight predominance of boys was identified among those patients (56.25%). The correct diagnosis was established only by histological examination, especially because in several cases conjunctival melanoma developed on a pre-existing nevus. The management was surgical, and all of them had a favorable outcome. Only two patients died due to the tumor, thus leading to the conclusion that conjunctival melanoma in children has a more favorable prognosis compared to its adult counterpart [10].

Slit-lamp biomicroscopy and color photographs are indicated for monitoring these patients. If the nevus increases in size, changes its color, or if there is an important vascularization, then its promptly evaluation is recommended [7, 29, 30].

Treatment is usually represented by complete surgical excision of the tumor, and it can be associated with cryotherapy of the conjunctival edges and bare sclera [31], with at least 3 mm to 4 mm healthy edges [32]. Conjunctivoplasty or transplantation of amniotic membrane can be associated as well, depending on the size of the remaining defect [33].

Diffuse conjunctival melanoma treatment is represented by total surgical excision of the lesion associated with cryotherapy or Mitomycin C. Local resection associated with radiotherapy is indicated for orbital relapses and lymph nodes impairment. Conjunctival melanomas are X-rays resistant but are sensitive to Cobalt or Radium [34].

Recurrences can develop due to inadequate excision, corneal involvement or multifocal disease.

Conclusions

The diagnosis and treatment of conjunctival pigmented lesions is difficult and time-consuming. In most cases, the clinical features of these lesions may lead to suspicion of malignancy. For young people, surgery is indicated only when the lesion grows in size or when functional problems appear. However, any excision of a conjunctival pigmented lesion must be subject to a systematic immunohistopathological examination, and there is a set of antibodies (anti-S100 protein, anti-Melan A, anti-HMB45) and Ki67 labeling index that are useful for differential diagnosis between a conjunctival nevus and a conjunctival melanoma.

Conflict of interests

The authors declare that they have no conflict of interests.

Ethics Statement

The study protocol was approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania, and adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from at least one parent or legal tutor of each child, after explaining the study design to both parents and children.

References

[1] Zimmerman LE, Sobin LH (eds). World Health Organization (WHO) histological typing of tumours of the eye and its adnexa. International Histological Classification of Tumours No. 24, WHO, Geneva, Switzerland, 1980. https://apps.who.int/iris/handle/10665/41599

[2] Grossniklaus HE, Eberhart CG, Kivelä TT (eds). World Health Organization (WHO) classification of tumours of the eye. 4th edition, vol. 12, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2018. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-The-Eye-2018

[3] Shields CL, Sioufi K, Alset AE, Boal NS, Casey MG, Knapp AN, Sugarman JA, Schoen MA, Gordon PS, Say EAT, Shields JA. Clinical features differentiating benign from malignant conjunctival tumors in children. JAMA Ophthalmol, 2017, 135(3):215–224. https://doi.org/10.1001/jamaophthalmol.2016.5544 PMID: 28125759

[4] Shields CL, Shields JA. Conjunctival tumors in children. Curr Opin Ophthalmol, 2007, 18(5):351–360. https://doi.org/10.1097/ICU.0b013e32823ecfbb PMID: 17700226

[5] Rakusiewicz K, Kanigowska K, Grajkowska W, Hautz W. Surgical treatment of conjunctival nevi in pediatric patients. Klinika Oczna (Acta Ophthalmol Pol), 2019, 121(1):29–33. https://doi.org/10.5114/ko.2019.85213 http://www.klinikaoczna.pl/Surgical-treatment-of-conjunctival-nevi-in-pediatric-patients,124,36712,0,1.html

[6] Shields CL, Shields JA, Gündüz K, Cater J, Mercado GV, Gross N, Lally B. Conjunctival melanoma: risk factors for recurrence, exenteration, metastasis and death in 150 consecutive patients. Arch Ophthalmol, 2000, 118(11):1497–1507. https://doi.org/10.1001/archopht.118.11.1497 PMID: 11074806

[7] Oellers P, Karp CL. Management of pigmented conjunctival lesions. Ocul Surf, 2012, 10(4):251–263. https://doi.org/10.1016/j.jtos.2012.08.002 PMID: 23084146

[8] Margo CE, Roper DL, Hidayat AA. Borderline melanocytic tumor of the conjunctiva: diagnostic and therapeutic considerations. J Pediatr Ophthalmol Strabismus, 1991, 28(5):268–270. https://doi.org/10.3928/0191-3913-19910901-08 PMID: 1955962

[9] Jacobiec FA, Colby K, Bajart AM, Saragas SJ. Moulin A. Immunohistochemical studies of atypical conjunctival melanocytic nevi. Arch Ophthalmol, 2009, 127(8):970–980. https://doi.org/10.1001/archophthalmol.2009.171 PMID: 19667333

[10] Balzer BWR, Cherepanoff S, Joshua AM, Giblin M, Conway RM, Anazodo AC. Conjunctival melanoma in childhood and adolescence: a systematic review. Ocul Oncol Pathol, 2019, 5(6):387–395. https://doi.org/10.1159/000497813 PMID: 31768361 PMCID: PMC6873081

[11] Shields CL, Demirci H, Karatza E, Shields JA. Clinical survey of 1643 melanocytic and nonmelanocytic conjunctival tumors. Ophthalmology, 2004, 111(9):1747–1754. https://doi.org/10.1016/j.ophtha.2004.02.013 PMID: 15350332

[12] Zimmermann-Paiz MA, García de la Riva JC. Conjunctival tumors in children: histopathologic diagnosis in 165 cases. Arq Bras Oftalmol, 2015, 78(6):337–339. https://doi.org/10.5935/0004-2749.20150089 PMID: 26677032

[13] Negretti GS, Roelofs KA, Damato B, Sagoo M, Parvizi S, Cohen VML. The natural history of conjunctival naevi in children and adolescents. Eye (Lond), 2021, 35(9):2579–2584. https://doi.org/10.1038/s41433-020-01273-4 PMID: 33199866 PMCID: PMC7668404

[14] Urban B, Bakunowicz-Łazarczyk A, Reszeć J, Michalczuk M. Histological and clinical evaluation of conjunctival pigmented lesions in children and adolescents. Klinika Oczna (Acta Ophthalmol Pol), 2017, 119(3):141–144. https://doi.org/10.5114/ko.2017.76193 http://www.klinikaoczna.pl/Histological-and-clinical-evaluation-of-conjunctival-pigmented-lesions-in-children-and-adolescents,124,32936,0,1.html

[15] McDonnell JM, Carpenter JD, Jacobs P, Wan WL, Gilmore JE. Conjunctival melanocytic lesions in children. Ophthalmology, 1989, 96(7):986–993. https://doi.org/10.1016/s0161-6420(89)32772-2 PMID: 2771364

[16] Novais GA, Fernandes BF, Belfort RN, Castiglione E, Cheema DP, Burnier MN Jr. Incidence of melanocytic lesions of the conjunctiva in a review of 10 675 ophthalmic specimens. Int J Surg Pathol, 2010, 18(1):60–63. https://doi.org/10.1177/1066896908319775 PMID: 18611943

[17] Weisenthal RW, Afshari NA, Bouchard CS, Colby KA, Rootman DS, Tu EY. Section 08: External disease and cornea. 2016–2017 Basic and Clinical Science Course (BCSC), American Academy of Ophthalmology, San Francisco, USA, 2016, 188–189. http://www.ophthalmologyebooks.store/product/2905/2016-2017-basic-and-clinical-science-course-bcsc-section-08-external-disease-and-cornea.html

[18] Vizvári E, Skribek Á, Polgár N, Vörös A, Sziklai P, Tóth-Molnár E. Conjunctival melanocytic naevus: diagnostic value of anterior segment optical coherence tomography and ultrasound biomicroscopy. PLoS One, 2018, 13(2):e0192908. https://doi.org/10.1371/journal.pone.0192908 PMID: 29444155 PMCID: PMC5812659

[19] Kanski JJ, Bowling B. Kanski's clinical ophthalmology: a systematic approach. 8th edition, Elsevier–Saunders, Australia, 2016, 468–469. https://www.uk.elsevierhealth.com/kanskis-clinical-ophthalmology-e-book-9780702055744.html

[20] Thiagalingam S, Johnson MM, Colby KA, Zembowicz A. Juvenile conjunctival nevus: clinicopathologic analysis of 33 cases. Am J Surg Pathol, 2008, 32(3):399–406. https://doi.org/10.1097/PAS.0b013e31815143f3 PMID: 18300811

[21] Zamir E, Mechoulam H, Micera A, Levi-Schaffer F, Pe'er J. Inflamed juvenile conjunctival naevus: clinicopathological characterization. Br J Ophthalmol, 2002, 86(1):28–30. https://doi.org/10.1136/bjo.86.1.28 PMID: 11801498 PMCID: PMC1770960

[22] Costea CF, Turliuc MD, Dimitriu G, Bogdănici CM, Moţoc A, Chihaia MA, Dancă C, Cucu A, Cărăuleanu A, Dumitrescu N, Indrei L, Turliuc Ş. Inflammatory juvenile compound conjunctival nevi. A clinicopathological study and literature review. Rom J Morphol Embryol, 2017, 58(3):739–747. PMID: 29250649

[23] Glasgow BJ, McCall LC, Foos RY. HMB-45 antibody reactivity in pigmented lesions of the conjunctiva. Am J Ophthalmol, 1990, 109(6):696–700. https://doi.org/10.1016/s0002-9394(14)72438-9 PMID: 2189315

[24] Jakobiec FA, Bhat P, Colby KA. Immunohistochemical studies of conjunctival nevi and melanomas. Arch Ophthalmol, 2010, 128(2):174–183. https://doi.org/10.1001/archophthalmol.2009.394 PMID: 20142539

[25] Shields CL, Fasiuddin AF, Mashayekhi A, Shields JA. Conjunctival nevi: clinical features and natural course in 410 consecutive patients. Arch Ophthalmol, 2004, 122(2):167–175. https://doi.org/10.1001/archopht.122.2.167 PMID: 14769591

[26] Burgués-Ceballos A, Saornil MA, García-Alvarez C, García Lagarto E. Pigmented conjunctival growing lesion in a teenager: nevus or melanoma? Can J Ophthalmol, 2013, 48(6):e154–e156. https://doi.org/10.1016/j.jcjo.2013.08.010 PMID: 24314430

[27] Brownstein S, Faraji H, Jackson WB, Font RL. Conjunctival melanoma in children: a clinicopathologic study of 2 cases. Arch Ophthalmol, 2006, 124(8):1190–1193. https://doi.org/10.1001/archopht.124.8.1190 PMID: 16908824

[28] Yangzes S, Gupta PC, Vaiphei K, Sharma VK, Ram J. Conjunctival melanoma in a child: a clinicopathological report. Saudi J Ophthalmol, 2018, 32(2):156–159. https://doi.org/10.1016/j.sjopt.2017.09.006 PMID: 29942187 PMCID: PMC6010591

[29] Ciuntu RE, Martinescu G, Anton N, Danciu M. Conjunctival melanocytic tumors in children – a challenge in diagnosis and treatment. Rom J Morphol Embryol, 2018, 59(1):317–322. PMID: 29940644

[30] Shields CL, Markowitz JS, Belinsky I, Schwartzstein H, George NS, Lally SE, Mashayekhi A, Shields JA. Conjunctival melanoma: outcomes based on tumor origin in 382 consecutive cases. Ophthalmology, 2011, 118(2):389–395.e1–2. https://doi.org/10.1016/j.ophtha.2010.06.021 PMID: 20723990

[31] Shields CL, Shields JA. Tumors of the conjunctiva and cornea. Surv Ophthalmol, 2004, 49(1):3–24. https://doi.org/10.1016/j.survophthal.2003.10.008 PMID: 14711437
[32] Costea CF, Anghel K, Dimitriu G, Dumitrescu GF, Faiyad Z, Dumitrescu AM, Sava A. Anatomoclinical aspects of conjunctival malignant metastatic melanoma. Rom J Morphol Embryol, 2014, 55(3):933–937. PMID: 25329123
[33] Şapte E, Costea CF, Cărăuleanu A, Dancă C, Dumitrescu GF, Dimitriu G, Chihaia MA, Buzdugă CM, Cucu A, Turliuc MD. Histological, immunohistochemical and clinical considerations on amniotic membrane transplant for ocular surface reconstruction. Rom J Morphol Embryol, 2017, 58(2):363–369. PMID: 28730219
[34] Shields CL, Naseripour M, Shields JA, Eagle RC Jr. Topical mitomycin-C for pagetoid invasion of the conjunctiva by eyelid sebaceous gland carcinoma. Ophthalmology, 2002, 109(11): 2129–2133. https://doi.org/10.1016/s0161-6420(02)01239-3 PMID: 12414427

Corresponding authors

Claudia Florida Costea, Associate Professor, MD, PhD, Discipline of Ophthalmology, Department of Surgery II, Grigore T. Popa University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Mobile +40744–972 648, e-mail: costea10@yahoo.com

Anca Sava, Professor, MD, PhD, Discipline of Anatomy and Embryology, Department of Morphofunctional Sciences I, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Mobile +40744–303 678, e-mail: dr.anca.sava.68@gmail.com

Received: January 25, 2022

Accepted: April 28, 2022