CASE REPORT

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

Oral mucosa self-graft in a patient with invasive conjunctival melanoma: a case report

MARIA RODICA MĂNESCU[1)], MARIA BĂLĂŞOIU[2)], MONICA MIHAELA CRĂIŢOIU[3)], ŞTEFANIA CRĂIŢOIU[1)], CRISTIANA EUGENIA SIMIONESCU[1)], VERONICA SFREDEL[2)], SORIN NICOLAE DINESCU[4)], MIHAELA NICULESCU[1)], MARIAN BURCEA[5)], ANDREI-THEODOR BĂLĂŞOIU[6)], CARMEN LUMINIŢA MOCANU[7)]

[1)]*Department of Morphology, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Physiological Sciences, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Prosthetic Dentistry, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Epidemiology, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Ophthalmology, "Carol Davila" University of Medicine and Pharmacy of Bucharest, Romania*
[6)]*PhD Student, Department of Morphology, University of Medicine and Pharmacy of Craiova, Romania*
[7)]*Department of Ophthalmology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Conjunctival melanoma is a rare tumor, while malignant melanoma of the uveal tract is the most common primary intraocular tumor in adults. The authors highlighted the case of a 68-year-old male patient presented in June 2011 in the Ophthalmology Clinic of the Emergency County Hospital of Craiova, Romania, accusing foreign body sensation and the appearance of a tumor in the lower fornix of his right eye. The patient was clinically and paraclinically investigated, and clinically diagnosed with *de novo* malignant melanoma. The tumor was surgically excised and the lower fornix reconstructed with oral mucosa self-graft. Histopathological diagnosis was *de novo*, invasive, ulcerated malignant melanoma of the conjunctiva. Ki-67 immunohistochemical staining was also performed. After surgery, the patient was sent to the Oncology Department for specific treatment. Despite the histological poor prognosis factors present in our patient, two years after surgery and chemotherapy, the patient had no clinical sign of local or distance recurrent disease. Continuous ophthalmologic and oncological surveillance is necessary.

Keywords: malignant melanoma, Ki-67, oral mucosa self graft.

Introduction

Conjunctival melanoma is a rare tumor, accounting for about 2% of all ocular malignancies, while malignant melanoma of the uveal tract is the most common primary intraocular tumor in adults [1]. Malignant melanoma represents a malignant tumor arising from proliferating melanocytes, cells derived from the neural crest.

Conjunctival melanoma is a relatively rare condition, compared to cutaneous melanoma. The annual age-adjusted incidence rate (per million) varies for conjunctival melanoma from 0.15 in Asians to 0.5 in non-Hispanic Caucasians [2–4].

Due to high rates of recurrence and metastasis, malignant melanoma of the conjunctiva is associated with significant morbidity and mortality [2, 5, 6]. The tumoral dissemination is possible due to regional lymph nodes invasion with subsequent distant metastasis [2, 7].

Some features have been recognized as prognosis factors, such as location, expansion, multifocal location, involvement of resection margins or tumor depth. These features are prognosis factors for metastatic disease [2, 8, 9]. Histopathological characteristics do not seem to be significantly associated with the clinical outcome [2, 9]. The overall mortality of conjunctival melanoma is 25% in 10 years; the prognosis is worse with tumors thicker than 0.76 mm [1, 10].

Risk factors for malignant melanoma include congenital nevi, solar excessive exposure, ultraviolet sensibility, primary acquired melanosis, family history, age, and Caucasian ethnicity. Malignant melanoma is 12 times more frequent in Caucasians than in Black population and seven times more frequent in Caucasians than in Hispanic ethnicity [11]. A major risk factor in malignant melanoma is a history of severe solar burns, comparing to other tumors (such as basal cell carcinoma or squamous cell carcinoma) in which cumulative exposure is the major risk factor [12].

Oral mucosa self-grafting has been practiced for a long time in ocular conjunctival surgery. Due to the synthetic materials, which have been successfully used in the recent past years, oral mucosa self-grafting has been neglected in the ophthalmic reconstructive surgery.

Patient, Methods and Results

A 68-year-old male patient presented in June 2011 in the Ophthalmology Clinic of the Emergency County Hospital of Craiova, Romania, accusing foreign body sensation and the appearance of a tumor in the lower fornix of his right eye. The patient noticed the tumor about two weeks before presentation. Anamnesis revealed no history of primary acquired melanosis nor of congenital nevus.

Ophthalmologic macroscopic examination and slit

lamp examination were performed, followed by physical examination, with special care regarding the regional lymph nodes. The patient took several paraclinic tests: complete hemoleucogram, blood glucose, liver and kidney specific tests, urinary summary examination, C-reactive protein and ESR, coagulation tests, electrocardiogram, ocular and liver ultrasonography, MRI.

Tumor excision with oncology safety margins was performed, followed by lower fornix reconstruction with oral mucosa self-graft. The graft was taken from the lower lip and used for covering the sclera denudation and the lack of forniceal conjunctiva; the graft size should be double than the defect size. The graft was sutured with 8-0 non-absorbable sutures.

The excised tumor was placed in a container with formaldehyde solution and sent to the Laboratory of Pathological Anatomy, where it was fixed in a paraffin block. Several sections were cut and stained with Hematoxylin–Eosin (HE). Ki-67 antibody was used for immunohistochemical staining.

Macroscopic clinical ophthalmologic examination revealed a dark, protuberant, nodular tumor in the lower fornix of the right eye. The tumor size was 10/5 mm. Slit lamp examination showed a highly vascularized dark-brown tumor. No other melanotic lesions were noticed (melanosis, nevus) on the right eye conjunctiva, nor on the fellow eye conjunctiva. Preauricular, retroauricular, submandibular and latero-cervical lymph nodes were clinically normal. The rest of the physical examination was also normal. All blood tests were normal; imaging investigations showed no invasion of the eyeball, no regional metastasis and no distance metastasis. The only modified paraclinic test was the urinary summary examination, which was positive for melanuria. The clinical diagnosis was RE-*de novo* nodular conjunctival melanoma of the lower fornix (Figure 1).

Microscopically, the tumor consisted of spindly epithelial cells with brown pigmentation and marked atypia, tumor-cells nuclei were irregular with atypical mitoses. The tumor thickness was 2.5 mm and subjacent the conjunctival epithelium presented a large ulcerated area and other several small-ulcerated areas. The histo-pathological diagnosis was *de novo*, invasive, ulcerated melanoma of the conjunctiva (Figures 2 and 3).

Conjunctival malignant melanocytes were positive for Ki-67 immunohistochemical staining (Figure 4).

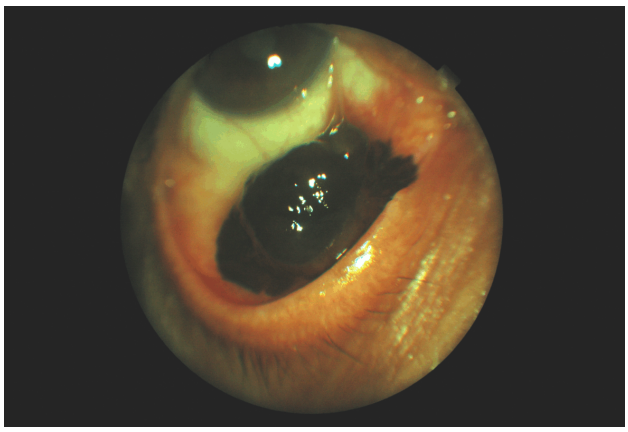

Figure 1 – ***Conjunctival malignant melanoma in the lower fornix.***

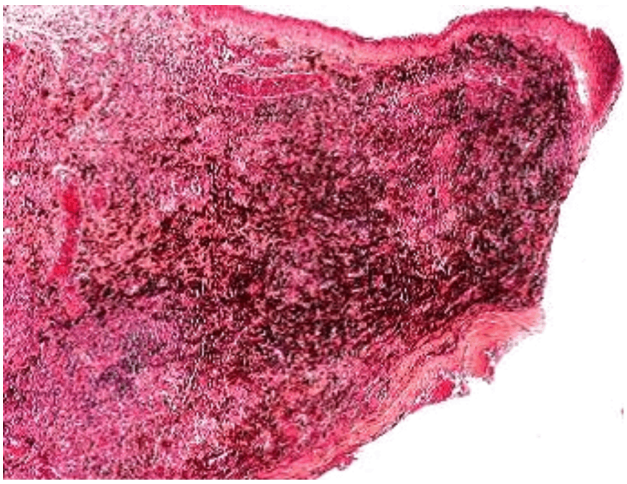

Figure 2 – ***Conjunctival melanoma. Brown pigmented cells and large ulcerated area subjacent the conjunctival epithelium (HE staining, ×100).***

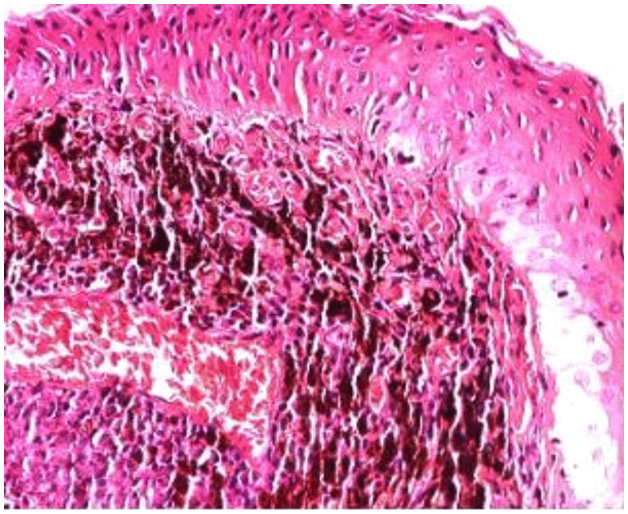

Figure 3 – ***Conjunctival melanoma. Spindly epithelial cells with brown pigmentation and marked atypia, nuclei with atypical mitoses (HE staining, ×200).***

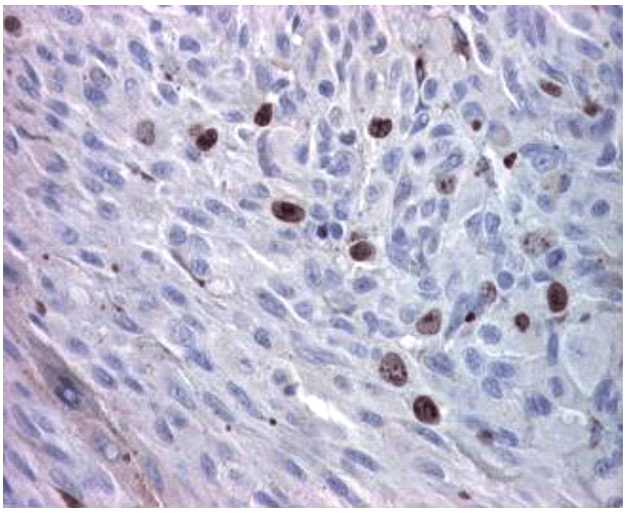

Figure 4 – ***Conjunctival melanoma. Melanocytes nuclei are positive for Ki-67 (Ki-67 immunostaining, ×400).***

Post-operatory outcome was very good and the oral mucosa self graft integrated quickly (Figures 5 and 6) due to histological structure similarities with the conjunctiva (Figures 6 and 7). Ten days after surgical excision, melanuria was absent.

After surgery, the patient was guided to the Department of Oncology, where he followed specialized treatment with interferon – 6 million units for two months.

Every six months, he came at follow-up and until June 2013 (the last follow-up when the patient presented) he did not present any clinical sign of local or general recurrent disease.

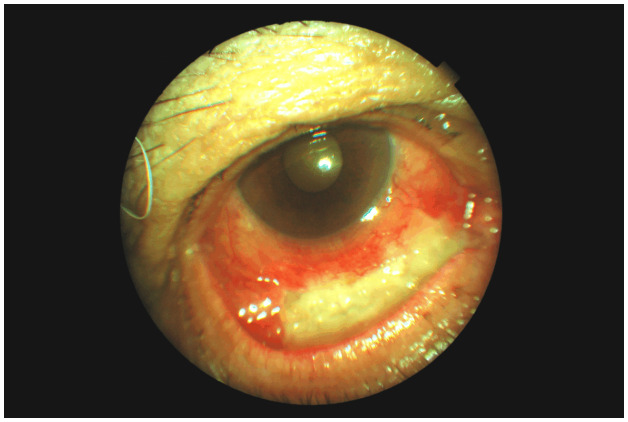

Figure 5 – ***Conjunctival melanoma. First day after surgery: oral mucosa self-graft covering the denudated sclera and the lack of forniceal conjunctiva.***

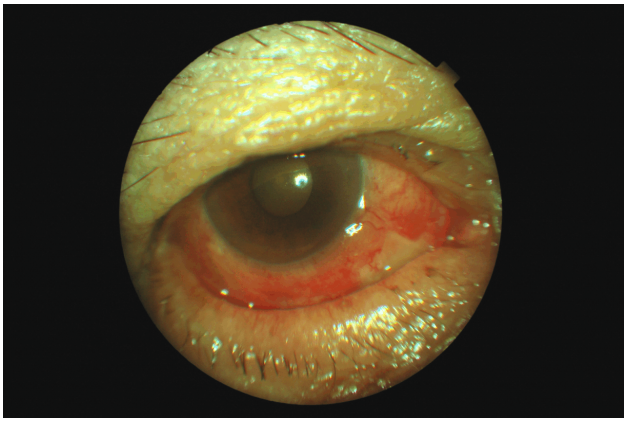

Figure 6 – ***Conjunctival melanoma. One week after surgery: oral mucosa self-graft integrated; lower fornix reconstructed.***

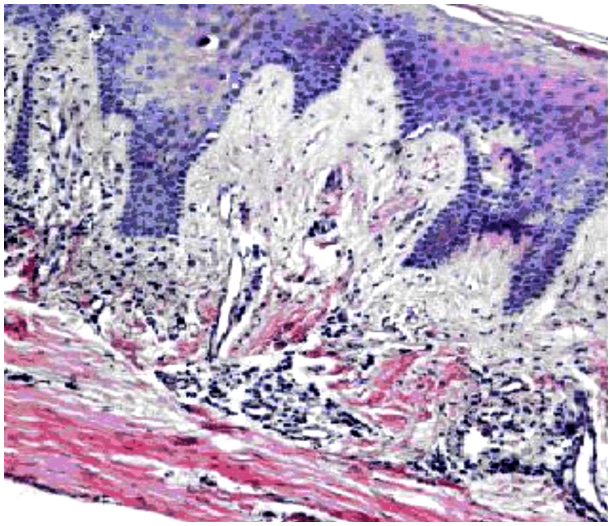

Figure 7 – ***Oral mucosa histological structure (HE staining, ×200).***

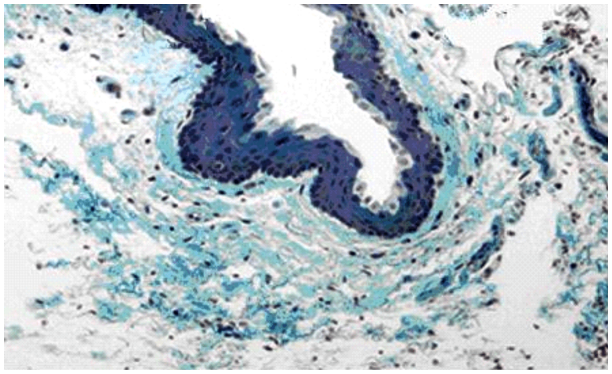

Figure 8 – ***Bulbar conjunctiva histological structure (Trichromic Goldner–Szekely staining, ×200).***

Discussion

Conjunctival melanoma is a potentially lethal neoplasm. It is identified frequently in the perilimbal conjunctiva, in the palpebral or forniceal conjunctiva, or caruncle, plica semilunaris, or eyelid margins. It appears like a solitary black or grey nodule containing feeder vessels and may become fixed to episclera.

Based on histopathological findings, conjunctival melanoma arises in an area of PAM with atypia in 75% of cases, *de novo* in 12% of cases, and from a pre-existing nevus in 13% of cases [1, 13].

PAM (primary acquired melanosis) is an uncommon unilateral condition [1], which affects middle-aged white people, with two histological types: PAM without atypia, a benignant proliferation of normal melanocytes; PAM with atypia, a pre-malignant condition characterized by an increased number of large melanocytes with prominent nucleoli, involving all conjunctival layers.

PAM with and without atypia presents the same clinical features, only biopsies assisted by immunohisto-chemistry can make the difference [14].

The overall mortality of conjunctival melanoma is 30% at 10 years, the main sites for metastasis are regional lymph nodes, lung, brain, and liver [15, 16].

The thickness of the tumor can be measured objectively using a calibrated microscope; tumors thicker than 0.8 mm carry a higher risk for dissemination [13]. However, lesions less than 0.8 mm thick have resulted in patient mortality. Various thicknesses have been assigned with a poor prognosis, ranging from 0.8 mm to over 2 mm [1].

Poor prognostic indicators are other histological characteristics as well: invasion of the cornea, episclera, or sclera; over five mitotic figures for every 10 high-power fields; involvement of the fornix, caruncle or palpebral conjunctiva; the presence of high or moderate atypia; the existence of melanoma cells permeating the entire thickness of the epithelium when PAM is present; lymphatic or orbital spread [16, 17].

Tumoral ulceration represents a sign of increased malignancy of the cutaneous melanoma. Some authors suggest that the tumoral ulceration is also a sign of increased malignancy of the conjunctival melanoma. They

have noticed that four out of 10 cases of ulcerated conjunctival melanoma presented regional lymphatic nodules metastasis and distance sites metastasis, in comparison to the non-ulcerated conjunctival melanomas, which presented normal regional lymphatic nodules [18]. Next to the histopathological examination, the immunohistochemistry examination confirmed the diagnosis and guided further treatment.

Ki-67 is a nuclear protein present in all the active phases of the cell cycle and absent in resting cells. It is used as a cellular marker of proliferation, being frequently correlated with the clinical evolution of patients with different type of tumors. It is one of the most common markers, used because the proportion of cells engaged in the cell cycle can be easily evaluated using Ki-67. This marker identifies an antigen expressed in G1, S and G2 phases of the cell cycle [19].

Ki-67 is frequently used for the differentiation between benignant and malignant conjunctival melanocytes proliferation. In this kind of study, the average of the proliferation index Ki-67 was 1.89% for nevus and 17.3% for conjunctival melanoma [20, 21].

Other authors have reached the conclusion that conjunctival melanoma develops *de novo* only in 18% of the cases; most of these tumors (45%) develop from PAM lesions. In the same study, these authors pointed that only 9% of the cases presented history of conjunctival nevi. They also noted that the average age was less than 63 years and the incidence was higher in men [22].

The melanoma coming from our study was a melanic, brown tumor, but it is well known the fact that the conjunctival melanoma can be amelanotic [23, 24]. This kind of tumor is very rare though. Unfortunately, in amelanotic conjunctival melanomas early diagnosis and adequate treatment are delayed [24].

Despite the histological poor prognosis factors present in our patient (involvement of the fornix, presence of atypia, tumor ulceration, tumor thickness over 0.8 mm), the clinical outcome was very good, two years after surgery and chemotherapy the patient had no clinical sign of local or distance recurrent disease.

The oral mucosa is a choriopapillary mucosal type. It is formed of epithelium and of chorion, divided and at the same time united by a basal membrane. The epithelium is stratified squamous Malpighian non-keratinized type, but orthokeratinized or parakeratinized areas can be found too. The chorion has two areas: the superficial chorion and the profound chorion. The superficial chorion is formed of connective tissue rich in cells and the profound chorion is formed of dense connective tissue, rich in collagen fibers. The bulbar conjunctiva is formed of non-keratinized stratified squamous epithelium and chorion. The chorion is rich in glands, blood vessels, lymphatic vessels and nerves. Histological similarities between these tissue types are obvious, fact also proved by the graft clinical evolution, which was quickly integrated.

Nowadays, oral mucosa grafts are still used by surgeons for eyeball annexes reconstruction. Kumar *et al.*, in 2006 [25], after a comparative study reached the conclusion that using the amniotic membrane in the treatment of contracted anophthalmic socket could represent a good alternative to oral mucosa grafts [25].

We consider that oral mucosa self graft is the graft that integrates the fastest (we have not recorded any graft rejection), being accessible for the surgeon and relatively easy to sampling.

Conclusions

Though rare, conjunctival melanoma is a life-threatening tumor, especially when associated to clinical and histological poor prognosis factors. Unfortunately, our patient presented some of these factors and despite very good clinical outcome on short term, continuous ophthalmological and oncological surveillance is mandatory. The epithelial similarities between oral mucosa and conjunctiva explain the fact that oral mucosa self-grafts are very well integrated at bulbar and forniceal conjunctival level.

Author contribution

All authors equally contributed to this paper.

References

[1] Kanski JJ, *Clinical ophthalmology*, 5th edition, Butterworth–Heinemann International Edition, 2003, 88–92.

[2] Zimmermann P, Dietrich T, Bock F, Horn FK, Hofmann-Rummelt C, Kruse FE, Cursiefen C, *Tumour-associated lymphangiogenesis in conjunctival malignant melanoma*, Br J Ophthalmol, 2009, 93(11):1529–1534.

[3] Hu DN, Yu G, McCormick SA, Finger PT, *Population-based incidence of conjunctival melanoma in various races and ethnic groups and comparison with other melanomas*, Am J Ophthalmol, 2008, 145(3):418–423.

[4] Tuomaala S, Eskelin S, Tarkkanen A, Kivelä T, *Population-based assessment of clinical characteristics predicting outcome of conjunctival melanoma in whites*, Invest Ophthalmol Vis Sci, 2002, 43(11):3399–3408.

[5] Kurli M, Finger PT, *Melanocytic conjunctival tumors*, Ophthalmol Clin North Am, 2005, 18(1):15–24, *vii*.

[6] Brownstein S, *Malignant melanoma of the conjunctiva*, Cancer Control, 2004, 11(5):310–316.

[7] Shields JD, Borsetti M, Rigby H, Harper SJ, Mortimer PS, Levick JR, Orlando A, Bates DO, *Lymphatic density and metastatic spread in human malignant melanoma*, Br J Cancer, 2004, 90(3):693–700.

[8] Shields CL, *Conjunctival melanoma: risk factors for recurrence, exenteration, metastasis, and death in 150 consecutive patients*, Trans Am Ophthalmol Soc, 2000, 98:471–492.

[9] Tuomaala S, Toivonen P, Al-Jamal R, Kivelä T, *Prognostic significance of histopathology of primary conjunctival melanoma in Caucasians*, Curr Eye Res, 2007, 32(11):939–952.

[10] Rodríguez-Martín M, Rodríguez-Martín J, de Paz NM, Ferrer PC, Cabrera PR, Rodríguez Martín B, Gordillo Santana G, Martín-Herrera A, Noda-Cabrera A, *Conjunctival melanoma: a new clinical and therapeutical approach*, Case Rep Dermatol, 2010, 2(2):149–155.

[11] Rhodes AR, Weinstock MA, Fitzpatrick TB, Mihm MC Jr, Sober AJ, *Risk factors for cutaneous melanoma. A practical method of recognizing predisposed individuals*, JAMA, 1987, 258(21):3146–3154.

[12] McCormick SA, DeLuca RL, Tumors of melanocytic origin. In: Mannis MJ, Macsai MS, Huntley AC (eds), *Eye and skin disease*, Lippincott–Raven, Philadelphia, 1996, 381–393.

[13] Shields CL, Shields JA, *Ocular melanoma: relatively rare but requiring respect*, Clin Dermatol, 2009, 27(1):122–133.

[14] Finger PT, Yu GP, *Conjunctival melanoma: is it increasing in the United States? Author reply*, Am J Ophthalmol, 2003, 136(6):1190–1191.

[15] Richtig E, Langmann G, Müllner K, Smolle J, *Ocular melanoma: epidemiology, clinical presentation, and relationship with dysplastic nevi*, Ophthalmologica, 2004, 218(2):111–114.

[16] Esmaeli B, Reifler D, Prieto VG, Amir Ahmadi M, Hidaji L, Delpassand E, Ross MI, *Conjunctival melanoma with a positive sentinel lymph node*, Arch Ophtalmol, 2003, 121(12):1779–1783.

[17] Werschnik C, Lommatzsch PK, *Long-term follow-up of patients with conjunctival melanoma*, Am J Clin Oncol, 2002, 25(3):248–255.
[18] Savar A, Esmaeli B, Ho H, Liu S, Prieto VG, *Conjunctival melanoma: local-regional control rates, and impact of high-risk histopathologic features*, J Cutan Pathol, 2011, 38(1):18–24.
[19] Cattoretti G, Becker MH, Key G, Duchrow M, Schlüter C, Galle J, Gerdes J, *Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections*, J Pathol, 1992, 168(4):357–363.
[20] Jakobiec FA, Bhat P, Colby KA, *Immunohistochemical studies of conjunctival nevi and melanomas*, Arch Ophthalmol, 2010, 128(2):174–183.
[21] Eagle RC Jr, *Immunohistochemistry in diagnostic ophthalmic pathology: a review*, Clin Experiment Ophthalmol, 2008, 36(7): 675–688.
[22] Kimura K, Usui Y, Goto H, *Clinical findings and prognosis of 11 cases of conjunctival malignant melanoma*, Nihon Ganka Gakkai Zasshi, 2012, 116(5):503–509.
[23] 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.
[24] Kovacević D, Lukanović-Primc K, Markusić V, Babić MB, Ledić D, *Conjunctival amelanotic melanoma – a case report*, Coll Antropol, 2011, 35(Suppl 2):295–297.
[25] Kumar S, Sugandhi P, Arora R, Pandey PK, *Amniotic membrane transplantation versus mucous membrane grafting in anophthalmic contracted socket*, Orbit, 2006, 25(3):195–203.

Corresponding author
Maria Bălăşoiu, Associate Professor, MD, PhD, Department of Physiological Sciences, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40723–319 879, e-mail: balasoiu.maria@yahoo.com

Received: May 10, 2014

Accepted: December 23, 2014