Original Article

Impression Cytology in a Series of Clinically Diagnosed Ocular Surface Melanocytic Lesions

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Abstract

Purpose: To report impression cytology (IC) results of clinically diagnosed ocular surface melanocytic lesions.

Methods: Ten patients with a clinical diagnosis of an ocular surface melanocytic lesion underwent IC using cellulose acetate strips and Periodic acid Schiff-Papanicolaou staining. Excisional biopsy of lesions was performed in case of observing atypical cells on IC or at the patient's request, and excised specimens were subjected to histopathological analysis. Agreement between clinical diagnoses and IC results and between IC results and histopathology were evaluated.

Results: Clinical diagnoses were nevi in 6, primary acquired melanosis (PAM) with atypia/melanoma in 2, and atypical nevus versus pigmented conjunctival intraepithelial neoplasia (CIN) in 2 cases. IC results were suggestive of a benign nevus in 7, PAM with atypia/melanoma in 2 and CIN versus an atypical epithelioid type melanocytic lesion in 1 case. IC results were consistent with the clinical diagnoses in 9 cases (Cohen's kappa index of 0.83) and excluded CIN in 1. Histopathology in 6 cases disclosed benign melanonevus in 3, malignant melanoma in the context of PAM with atypia in 2, and CIN in 1 case. Histologic results were well correlated with the IC features (Cohen's kappa index of 0.74).

Conclusion: By demonstrating typical cytomorphological features of ocular superficial layers IC diagnosed the true nature of melanocytic ocular surface lesions in the majority of cases. Although IC does not substitute histopathology, given the high correlation between IC results and histopathology, it can be of great assistance in diagnosis and management of ocular surface melanocytic lesions.

Keywords: Impression Cytology; Ocular Surface Melanocytic Lesion; Primary Acquired Melanosis; Nevus

J Ophthalmic Vis Res 2017; 12(1): 17-22

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Received: 16-04-2016 Accepted: 03-11-2016

Access this article online

Quick Response Code:

Website: www.jovr.org

DOI: 10.4103/jovr.jovr_72_16

INTRODUCTION

Ocular surface melanocytic lesions can be listed as conjunctival racial melanosis, benign acquired melanosis (BAM), primary acquired melanosis (PAM), secondary conjunctival melanosis, conjunctival nevi, and melanomas.[1-4] Most of the ocular surface melanocytic lesions are benign. Conjunctival melanomas are

How to cite this article: Kanavi MR, Hosseini SB, Aliakbar-Navahi R, Aghaei H. Impression cytology in a series of clinically diagnosed ocular surface melanocytic lesions. J Ophthalmic Vis Res 2017;12:17-22.

relatively rare with the overall mortality rate of 26%.[1-3] Conjunctival melanomas mostly arise from the preexisting primary acquired melanosis with atypia which are potentially malignant. Distinguishing benign from malignant or potentially malignant melanocytic lesions of the conjunctiva is of high significance for proper management of such lesions, monitoring the progression of a melanocytic lesion, and following up the effect of a therapeutic intervention.[1,5,6] For instance, avoiding an unnecessary excisional biopsy in the perilimbal areas is pivotal for the preservation of limbal stem cells which are responsible for renewal of corneal epithelium.[7,8]

Cytologic diagnosis of ocular surface melanocytic lesions using invasive biopsies may cause patients' discomfort.[5] The exfoliative or brush cytology may also induce morphologic changes in cellular structure.[6] Impression cytology (IC), however, has been used as a noninvasive, rapid, inexpensive, outpatient-based, and easy to perform method for sampling superficial epithelium in various ocular surface disorders such as dry eyes, limbal stem cell deficiency, microbiological infections, and ocular surface neoplasms.[9-14]

IC features of the conjunctival melanocytic lesions have been previously reported in several studies.[5,13,14] The IC method was reported to have well correlation (73%) with histopathological diagnoses in 24 conjunctival pigmented lesions.[5] It could also discriminate the amelanotic melanocytic lesions from the non-pigmented non-melanocytic ones and confirm clinical diagnosis of 35 conjunctival nevi in 91.4%.[15] However, there is no report regarding the sensitivity and specificity of IC in the diagnosis of ocular surface melanoma or any other melanocytic lesion. Furthermore, given the superficial sampling nature of this technique, it has to be performed repeatedly to recover the melanocytic cells. Additionally, although this method may not replace the gold standard mode of histopathology, it can play an essential role in the diagnosis and management of patients with ocular surface melanocytic lesions.[6,15] In the present study, the application of IC for the diagnosis of clinically diagnosed ocular surface melanocytic disorders was evaluated.

METHODS

This case series included patients with the clinical appearance of an ocular surface melanocytic lesion. They were referred from ophthalmology centers to the ocular pathology unit of the Central Eye Bank of Iran. The study was approved by the Ethics Committee of the Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Patients' demographic data, clinical features of the lesion including laterality, location, multiplicity, and any previous histopathological report were recorded.

Following biomicroscopic examination and taking slit-lamp photographs by 2 ophthalmologists who were also ocular pathologists (MRK and SBH, Ocular Pathology Department, Central Eye Bank of Iran), patients were subjected to IC sampling. The IC technique has already been reported.[11] Briefly, the eye surface was anesthetized with a drop of 0.5% tetracaine eye drop (Sina Darou Laboratories Company, Tehran, Iran) and a 5 × 5 mm precut cellulose acetate filter paper (47 mm, pore size 0.45 μm, Schleicher and Schuell Microscience GMBH, Dassel, Germany) was placed onto the surface of the lesion. After gentle pressure for a few seconds, the filter paper was carefully peeled off the lesion surface and fixed in a cytology fixative. Each area of the lesion received IC by two consecutive applications of cellulose acetate filter paper. The filter papers were stained with Periodic acid Schiff-Papanicolaou (PAS/PAP) and mounted on glass slides using a mixture of distyrene, a plasticizer, and xylene (DPX) mountant. The slides were examined under light microscopy (BX41, Olympus, Japan) by two ocular pathologists (MRK and SBH, Ocular Pathology Department, Central Eye Bank of Iran) in terms of the presence of intraepithelial nests or clusters of melanocytic cells with any degree of pigmentation and the presence of pleomorphic atypical melanocytes.

Impression Cytology Criteria for Benign Versus Malignant or Potentially Malignant Ocular Surface Melanocytic Lesions

The IC criteria for benign ocular surface melanocytic lesions comprised of the presence of nests or clusters of melanocytes with bland-looking nuclei, containing or lack cytoplamic pigment, no mitosis, adhesion of the melanocytic nests to the surrounding normal-looking epithelial cells containing or lack goblet cells, and with or without squamous metaplasia. Such IC criteria were common between benign melanocytic nevi and PAM without atypia.[4,5,16]

The IC analysis was reported as malignant or potentially malignant ocular surface melanocytic lesions when clusters of pleomorphic atypical cells not resembling epithelial cells were present. The atypical cells expressed different sizes, with or without cytoplasmic pigment, irregular nuclear chromatin pattern, anisokaryosis characterized by large and irregular nuclei with prominent nucleoli, and mitoses.[5,6,17] PAM with atypia, defined by Folberg et al[18] was diagnosed on IC when the relative proportion of atypical melanocytes were low. Malignant ocular surface melanocytic lesions, equivalent to malignant melanoma, were diagnosed cytologically when an abundant number of atypical melanocytes were observed.[5,18]

Histopathological Criteria for Benign Versus Malignant or Potentially Malignant Ocular Surface Melanocytic Lesions

The presence of contiguous nests of round to spindle-shaped melanocytes with bland-looking oval nuclei within the conjunctival epithelium and/or stroma was diagnosed as a benign melanonevus on histopathology.[4,5,16,19]

Histopathological criteria for PAM with atypia, so called melanoma *in situ*, were the presence of atypical melanocytes within the epithelium without breaking through the epithelial basement membrane and immune reactivity of the atypical cells for S-100 and HMB-45. Considering invasive malignant melanoma, the histopathological criteria included a vertical growth phase of the epithelial atypical melanocytes into the substantia properia and violation of epithelial basement membrane, pagetoid involvement of the epithelium, and immune reactivity of the atypical melanocytes for S-100 and HMB-45. In order to distinguish conjunctival nevi from malignant melanomas, immune reactivity for Ki-67 as a proliferative marker in melanoma cells was evaluated.[3,4] The histopathological examinations were performed in the pathology laboratory of Rassoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

Statistical Analysis

Frequency and percentage were used to describe the data. The agreement between the clinical diagnosis and IC results and between the IC results and histopathology were evaluated by calculating the Cohen's Kappa index.

RESULTS

Between 2005 and 2015, 10 patients with the clinical appearance of an ocular surface melanocytic lesion were referred to the Ocular Pathology Department of the Central Eye Bank of Iran for IC evaluation. The patients' demographic data as well as their clinical, IC and histopathological diagnoses were listed in Table 1. All patients were Caucasians with the mean age of 36 (range, 9–72) years and 60% of the subjects were male. All lesions were moderately pigmented and unilateral. Excluding 2 patients who presented with multiple ocular surface pigmented lesions, the remaining cases had single conjunctival lesions. The anatomical location of the pigmented lesions was the bulbar conjunctiva in all cases except in one subject who had an additional forniceal lesion. In overall, when taking the above 2 cases with multiple lesions into account, the involved quadrant was temporal (8 eyes, 80%), nasal (4 eyes, 40%), superior (2 eyes, 20%), and inferior (2 eyes, 20%). Clinical diagnoses were nevi in 6, recurrent conjunctival melanoma in the context of PAM with atypia in 1, PAM with atypia in 1, and atypical nevus versus pigmented conjunctival intraepithelial neoplasia (CIN) in 2 patients.

IC disclosed the presence of moderately pigmented benign-looking melanocytes amongst the epithelial cells and was suggestive of a benign nevus in 7 (70%), presence of moderately pigmented atypical-looking melanocytes suggestive of PAM with atypia/melanoma in 2 (20%), and the presence of atypical epithelioid type cells together with melanin pigment suggestive of a pigmented CIN versus an atypical epithelioid type melanocytic lesion in 1 (10%) eye. The IC results were consistent with the primary clinical diagnoses in 9 (90%) cases (Cohen's kappa index, 0.83) and excluded the presence of CIN in the remaining one (case #9). However, the IC results in case #3, could not differentiate between a pigmented CIN and an atypical epithelioid type melanocytic lesion although the findings revealed the presence of atypical cells, and necessitated histopathology for the definite diagnosis.

Six (60%) patients underwent excisional biopsy of the lesion and the histopathological diagnoses were benign melanonevus in 3, malignant melanoma in the context of PAM with atypia in 2 and CIN in 1 case. The benign nevi had less than 10% immune reactivity for Ki-67. Two cases who were diagnosed as malignant melanoma in the context of PAM with atypia demonstrated immune reactivity for S100 and HMB45. The histopathological features were all well correlated with the IC results in our series (Cohen's kappa index, 0.74). In the only case (case #3) that the IC was suggestive of either pigmented CIN or an atypical epithelioid type melanocytic lesion, histopathology demonstrated the former diagnosis. Examples were illustrated in Figures 1 to 3.

DISCUSSION

Our study demonstrated that IC results had a critical role in the diagnosis and further management of patients with clinically diagnosed ocular surface melanocytic lesions. The IC results in our series were highly consistent with the primary clinical diagnoses, and were subsequently approved by histopathology, particularly in cases that had atypical cells on cytology. IC, as a minimally invasive method, assisted to diagnose both the primary and recurrent lesions in our series.

In malignant melanoma, incisional biopsy should be avoided because of the risk of local tumor spreading.[20] The IC features, in our series, not only demonstrated the cytopathological diagnosis of the lesions, but also obviated the need for performing subsequent incisional biopsy of the malignant lesions, which may lead to local tumor dissemination. However, in cases that had atypical cells on cytology, excisional biopsy was performed as a critical step in the management of such lesions.

Table 1. Demographic data, impression cytology, and histopathological features of 10 clinically diagnosed ocular surface melanocytic lesions

Case	Sex	Age	Eye	Race	Multiplicity	Anatomical location	Quadrant	Clinical diagnosis	Atypical melanocytes on cytology	Benign-looking melanocytes on cytology	IC diagnosis	Histopathology
1	F	51	R	Caucasian	Multiple	Corneoscleral limbus Upper fornix	All	Recurrent conjunctival melanoma with PAM with atypia	+	-	A. PAM with Atypia B. Recurrent melanoma	Melanoma in the context of PAM with atypia
2	M	25	R	Caucasian	Single	Corneoscleral limbus	TB	Nevus	-	+	Benign-looking melanocytic lesion	Compound Nevus
3	M	30	L	Caucasian	Single	Corneoscleral limbus	NB	Atypical nevus vs Pigmented CIN	+	-	Pigmented CIN vs Atypical Nevus	CIN
4	F	9	L	Caucasian	Single	Corneoscleral limbus	TB	Nevus	-	+	Benign-looking melanocytic lesion	NP
5	F	14	R	Caucasian	Single	Corneoscleral limbus		Nevus	-	+	Benign-looking melanocytic lesion	NP
6	M	72	L	Caucasian	Single	Corneoscleral limbus	TB	Nevus	-	+	Benign-looking melanocytic lesion	Compound Nevus
7	M	20	L	Caucasian	Single	Corneoscleral limbus	NB	Nevus	-	+	Benign-looking melanocytic lesion	NP
8	F	70	R	Caucasian	Multiple	Corneoscleral limbus	SB, TB, IB	PAM with atypia	+	-	PAM with atypia	Malignant Melanoma in the context of "PAM with atypia"
9	M	57	R	Caucasian	Single	Corneoscleral limbus	TB	Atypical nevus vs Pigmented CIN	-	+	Benign-looking melanocytic lesion	NP
10	M	13	R	Caucasian	Single	Corneoscleral limbus	TB	Nevus	-	+	Benign-looking melanocytic lesion	Compound Nevus

IC, impression cytology; M, male; F, female; R, right; L, left; IB, inferior bulbar; NB, nasal bulbar; SB, superior bulbar; TB, temporal bulbar; CIN, conjunctival intraepithelial neoplasia; PAM, primary acquired melanosis; NP, not performed

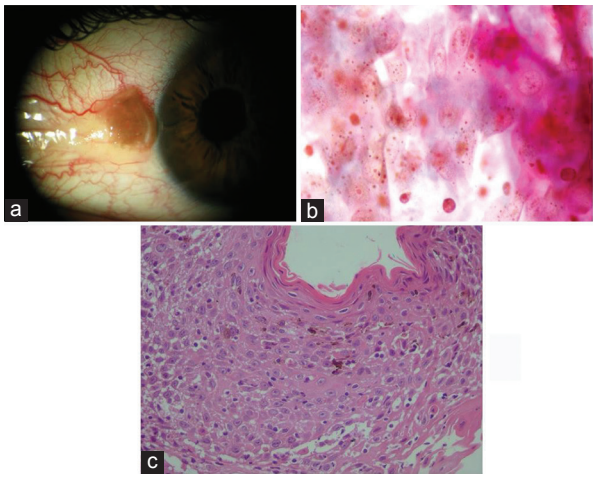

Figure 1. Representative images of case #3: (a) clinical picture of a pigmented conjunctival mass suspicious to an atypical nevus versus a pigmented CIN in the right eye; (b) impression cytology demonstrating atypical epithelioid type cells with nuclear pleomorphism, distinct nucleoli and together with melanin pigment (original magnification, ×1000); (c) photomicrograph of the excised lesion disclosing acanthosis with mild surface keratinization, altered cellular polarity, and significant nuclear pleomorphism involving full thickness of the epithelium (original magnification, ×400).

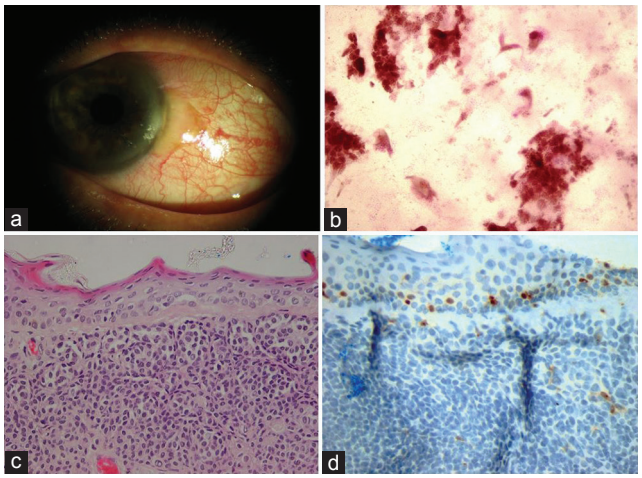

Figure 2. Representative images of case #2: (a) clinical picture of a slightly pigmented conjunctival nevus that had become noticeable in the left eye; (b) impression cytology demonstrating clusters of nevus cells with bland-looking nuclei, containing cytoplasmic pigment, and no mitosis (original magnification, ×400); (c) histopathological examination of the excised lesion exhibiting contiguous nests of round to spindle-shaped melanocytes with bland-looking oval nuclei within the conjunctival epithelium and stroma and with mild immune reactivity (d) for Ki-67 (original magnification, ×400).

Comparable with the previous studies,[5,12] our IC results were highly correlated with the histopathological features. Similar to our results, a 73% correlation between IC and histopathology was reported in a series of epibulbar pigmented tumors.[5] Out of 10 cases in the current series, 6 underwent excisional biopsy and histopathological investigations, of which the IC results of 5 subjects were approved by histopathology. In the one remaining case with the histopathologic diagnosis of a pigmented CIN, although IC identified the presence of atypical cells, it could not differentiate between an atypical epithelioid type melanocytic lesion and a pigmented CIN. However, the IC results were suggestive of an atypical lesion necessitating therapeutic interventions. The high agreement between IC and histopathology in our series might be due to the presence of epithelial components in the melanocytic-looking lesions as well as high experience of our ocular pathologists in ocular surface sampling, processing, and microscopic investigations. However, further investigation using a large number of patients with ocular surface melanocytic lesions is needed to elucidate the sensitivity and specificity of IC when it is performed in experienced hands.

Although IC has been capable to differentiate amelanotic melanoma from other non-pigmented lesions,[17] it could not differentiate pigmented CIN from an atypical epithelioid-type melanocytic lesion in one case of our series. In such cases, the novel combination of IC and immunocytochemistry for S100 and/or cytokeratins may be of diagnostic value. This combination method, described by Krenzer and Freddo,[21] assists simultaneous assessment of cytomorphology as well as immunocytochemical analysis of IC specimens. However, the sensitivity and reliability of this combination method in the diagnosis of ocular surface melanocytic lesions needs further investigation.

In our series, all of the lesions were located at the bulbar conjunctiva adjacent to the corneoscleral limbus excepting one case in which an additional lesion in the upper fornix was present and by using cellulose acetate strips, IC sampling was possible in these anatomical locations. The simplicity of sampling of conjunctival areas other than corneoscleral limbus by using cellulose acetate strips has been previously reported[15] in comparison to sampling difficulties when Biopore membranes are used.[16]

In conclusion, IC is a minimally invasive method that can be of great assistance in the diagnosis, management, and follow-up of clinically diagnosed ocular surface melanocytic lesions and has a high correlation with corresponding tissue histology when performed in experienced hands. Although the numbers of cases in our series were limited, for the first time in Iran and in the Middle East region, we tried to highlight the importance of IC in the proper diagnosis and management of patients with clinical suspicion of ocular surface melanocytic lesions. Given the results of this study, we would strongly suggest performing IC as

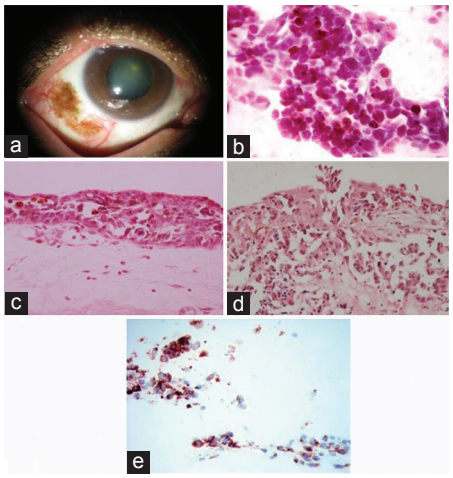

Figure 3. Representative images of case #1: (a) slit-lamp photograph of epibulbar pigmented patches suspicious to malignant melanoma in the context of PAM with atypia in the right eye; (b) abundant numbers of atypical melanocytes with large and irregular nuclei with prominent nucleoli are demonstrated in impression cytology (original magnification, ×400); (c, d) photomicrographs of the excised lesion exhibiting the presence of atypical melanocytes within the epithelium (c) together with violation of epithelial basement membrane (d). The atypical melanocytes are immune reactive (e) for HMB45 (original magnification, ×400).

the first and non-invasive diagnostic method in cases with clinical diagnosis of either benign or malignant melanocytic lesion. The IC results would be beneficial for further management of the patient, avoiding unnecessary surgical biopsies. However, in cases with negative or uncertain IC results, histopathologic evaluation of the excisional biopsy specimens is required.

Acknowledgements

The authors sincerely thank Mrs. Faranak Mohammadi Roshanagh for her assistance in performing IC stainings.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Shields CL, Demirci H, Karatza E, Shields JA. Clinical survey of 1643 melanocytic and nonmelanocytic conjunctival tumors. *Ophthalmology* 2004;111:1747-1754.
2. Shields CL, Schields JA. Tumors of the conjunctiva and cornea. *Surv Ophthalmol* 2004;49:3-24.
3. Eagle Jr. RC. Conjunctiva. In: Eye Pathology: An atlas and textbook. Chapter 5 (Conjunctiva). 2nd edition. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2011. pp. 70.
4. American Academy of Ophthalmology, The Eye MD Association. Conjunctiva. In: Ophthalmic Pathology and Intraocular Tumors. Section 4. San Francisco; 2014-2015. pp. 69.
5. Paridaens AD, McCartney AC, Curling OM, Lyons CJ, Hungerford JL. Impression cytology of conjunctival melanosis and melanoma. *Br J Ophthalmol* 1992;76:198-201.
6. Barros JN, Almeida SRA, Lowen MS, Cunha MC, Gomes JAP. Impression cytology in the evaluation of ocular surface tumors: Review article. *Arq Bras Oftalmol* 2015;78:126-132.
7. Nolan GR, Hirst LW, Bancroft BJ. The citomorphology of ocular surface squamous neoplasia by using impression cytology. *Cancer* 2001;93:60-67.
8. Tole DM, McKelvie PA, Daniell M. Reliability of impression cytology for the diagnosis of ocular surface squamous neoplasia employing the biopore membrane. *Br J Ophthalmol* 2001;85:154-158.
9. Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface: Dry eye states. *Arch Ophthalmol* 1983;101:1869-1872.
10. Puangsricharern V, Tseng SC. Cytologic evidence of corneal diseases with limbal stem cell deficiency. *Ophthalmology* 1995;102:1476-1485.
11. Kanavi MR, Hosseini B, Javadi F, Rakhshani N, Javadi MA. Impression cytology in eyes with clinical and confocal scan features of acanthamoeba keratitis. *J Ophthalmic Vis Res* 2013;8:207-212.
12. Tananuvat, T, Lertprasertsuk N, Mahanupap P, Noppanakeepong P. Role of impression cytology in diagnosis of ocular surface neoplasia. *Cornea* 2008;27:269-274.
13. Barros JN, Lowen MS, Ballalai PL, Mascaro VL, Gomes JA, Martins MC. Predictive index to differentiate invasive squamous cell carcinoma from preinvasive ocular surface lesions by impression cytology. *Br J Ophthalmol* 2009;93:209-214.
14. Nolan GR, Hirst LW, Wright RG, et al. Application of impression cytology to the diagnosis of conjunctival neoplasms. *Diagn Cytopathol* 1994;11:246-249.
15. Barros JN, Lowen MS, Mascaro VL, Andrade TP, Martins MC. Impression cytology features of conjunctival nevi reported as more noticeable. *Arq Bras Oftalmol* 2009;72:205-210.
16. Keijser S, Missotten GS, De Wolff-Rouendaal D, Verbeke SL, Van Luijk CM, Veselic-Charvat M, et al. Impression cytology of melanocytic conjunctival tumours using the Biopore membrane. *Eur J Ophthalmol* 2007;17:501-506.
17. Barros J de N, Motono M, Costa FD, Cunha MC, Chojniak MM. Amelanotic corneally displaced malignant conjunctival melanoma: A case report evaluated with impression cytology. *Arq Bras Oftalmol* 2014;77:57-59.
18. Folberg R, McLean IW, Zimmerman LE. Primary acquired melanosis of the conjunctiva. *Hum Pathol* 1985;16:129-135.
19. Font RL, Craxatto JO, Rao NA. AFIP Atlas of tumor pathology, Series IV: Tumors of the eye and ocular adnexa. Washington D.C.: American Registry of Pathology; 2007.
20. Lim L, Madigan MC, Conway RM. Conjunctival melanoma: A review of conceptual and treatment advances. *Clin Ophthalmol* 2013;6:521-531.
21. Krenzer KL, Freddo TF. Cytokeratin expression in normal human bulbar conjunctiva obtained by impression cytology. *Invest Ophthalmol Vis Sci* 1997;38:142-152.