Impression cytology features of conjunctival nevi reported as more noticeable

Características da citologia de impressão de nevos conjuntivais referidos como mais perceptíveis

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ABSTRACT

Purpose: To report the impression cytology features of conjunctival nevi reported as more noticeable. **Methods:** 35 patients who reported that a conjunctival lesion had become more noticeable after color or size change were enrolled. On slit-lamp examination, a clinical diagnosis of nevus was made and lesions underwent impression cytology using acetate cellulose strips and a combined staining with PAS, H&E and Papanicolaou. At patient's or parents' request, excision of the lesion was performed and the tissue was submitted to histopathological study. **Results:** Impression cytology examination revealed nests or cluster of nevus cells within the epithelium layer containing or not mucous-secreting goblet cells in 32 cases (91.4%). Ten patients (28.5%) had the tumor removed and histopathological diagnosis was compound nevus in 8 eyes (1 from caruncle, 1 from plica semilunaris and 6 from bulbar conjunctiva) and subepithelial nevus from bulbar conjunctiva (2 eyes). **Conclusion:** Optical microscopy analysis of the impression cytology specimens confirmed the clinical diagnosis by demonstrating typical histopathological features of the superficial layers from conjunctival nevi in 91.4% of the cases. For amelanotic nevi IC can also allow a differential diagnosis from other nonpigmented lesions. The technique does not replace histopathological examination, but additionally, may assist in evaluating nevus cells in children and adults.

Keywords: Nevus/diagnosis; Conjunctiva/cytology; Conjunctiva/pathology; Cytological techniques; Anterior chamber/ultrasonography; Diagnostic techniques ophthalmological

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Recebido para publicação em 24.08.2008
Última versão recebida em 12.02.2009
Aprovação em 26.02.2009

INTRODUCTION

A variety of tumors and simulating lesions may occur in the conjunctiva. Lesions of melanocytic origin are as common as epithelial tumors[1] and include conjunctival racial melanosis, primary acquired melanosis, secondary melanosis, nevus and melanoma. Although most of the melanocytic lesions are benign, some can carry a potential for malignancy, which makes it important to distinguish between these various conjunctival lesions[1-4].

Conjunctival nevi can account for up to 52% of the diagnoses from all the melanocytic tumors[1] and are the most common ocular surface tumor in children[3]. These benign lesions are more frequently found in the bulbar conjunctiva (72%), caruncle (15%) or plica semilunaris (11%). The diagnosis is typically made by recognition of the spectrum of classical clinical features using slit-lamp biomicroscopy: from heavy pigmentation (65%) to a complete lack of pigments (16%), from a small size lesion to extensive

tumors occupying two quadrants of the ocular surface and from presence of cysts (65%) to a complete lack of cysts. Cysts are most common in compound nevus (70%), decreasingly common in subepithelial nevus (58%), junctional nevus (40%) and absent in blue nevus[2-3].

The recognition of tumor cysts is a key point in differentiating conjunctival nevus from malignant melanoma as many other features overlap and conjunctival melanoma rarely, if ever, displays cysts[1-4]. Pigmented conjunctival nevi may obscure cysts under slit-lamp examination and ultrasound biomicroscopy (UBM) can be a useful adjunct in the clinical diagnosis of cysts in these cases. However, it is not possible for the current UBM to distinguish nevi from melanomas[5]. In vivo confocal microscopy can be a tool in the differential diagnosis of pigmented conjunctival tumors by demonstrating hyperreflective cells[6] but the high cost of the equipment can limits its use.

An amelanotic conjunctival nevus can resemble other nonpigmented conditions including inflamed pingueculum, episcleritis, conjunctival cyst, allergic conjunctivitis, foreign body granuloma, lymphangioma and squamous epithelial neoplasia. The differentiation of these various conditions is important as it implies diverse ocular and systemic prognoses[1-3]. Whenever necessary, histopathological findings confirm diagnosis. Nevertheless, the treatment for noninflamed conjunctival nevus is observational and they rarely require surgical removal[4]. Thus, as conjunctiva is an easily accessible tissue source for cytological examination, the analysis of the features from melanocytic cells by the noninvasive and affordable impression cytology (IC) can help in the differentiation of benign from malignant pigmented tumors of the bulbar conjunctiva[7-8]. IC, first described in 1977 for the collection of mucin and cells from the conjunctiva[9], had other diagnostic possibilities, such as: ocular surface squamous neoplasia[10-13], dry eyes[14], vitamin A deficiency[15], allergic conjunctivitis[16], limbal stem cell failure[17] and microbiological infections[18]. The aim of this study was to report the IC features of conjunctival nevi.

METHODS

A transversal study was conducted between March 2005 and April 2008 at the Vision Institute, Department of Ophthalmology of the Federal University of São Paulo, Brazil. The study was approved by the Medical Ethics Committee of the institution.

Thirty-five patients who reported that a conjunctival lesion had become more noticeable after color or size change were examined by the same experienced professional. If the patient was a child, parents' report was also taken into account. Clinical data were registered in the medical records and included: patient demographic features (age, race and gender) and tumor features such as eye involved, lesion color, anatomic location (bulbar conjunctiva, palpebral conjunctiva, fornix, plica semilunaris, caruncle), quadrant location (superior, temporal, inferior, nasal, diffuse) and intralesional cysts presence or absence. None of the patients had prior topical chemotherapy or cryotherapy on the ocular surface before cytological examination.

Following slit-lamp examination, a diagnosis of a conjunctival nevus was obtained and patients were submitted to IC sampling. In brief, after administration of topical anesthesia with 0.5% proximetacaine hydrochloride (Anestalcon® 0,5%, Alcon, São Paulo, Brazil), a strip of acetate cellulose filter paper with a 0.45 micron pore size (Millipore HAWP304, Bedford, EUA) was placed onto the lesion surface, gently pressed for 5 seconds, and then peeled off. Sampling was performed twice over the same region since repeated examinations may increase the sensitivity of this technique. Filters were immediately fixed for approximately ten minutes in a solution containing glacial acetic acid, 37% formaldehyde, and ethyl alcohol in a 1:1:20 volume ratio. All strips were processed for the periodic acid-Schiff (PAS), Gill's hematoxylin and Papanicolaou stain[19]. Glass slides were mounted with *Entellan* (Merck, Darmstadt, Germany) and cells were analyzed under light microscopy by two experienced professionals. From the slide sets, only IC specimens with at least one third of the filter surface filled with visible cells were included.

Criteria for conjunctival nevi diagnosis with impression cytology

In conjunctival nevi normal rounded melanocytes containing or not intracytoplasmic melanin pigment granules typically aggregate in nevus cell nests. Nevus cells tend to adhere to one another and to the surrounding epithelial cells. The nevus cells usually reside in nests aggregated at the epithelial and subepithelial junction[20]. Utilizing criteria derived from histopathological examination, the IC analysis was reported for conjunctival nevus when nests or cluster of nevus cells were seen within the epithelium layer containing or not mucous-secreting goblet cells. Epithelial cell layers could demonstrate normal morphology or signs of squamous metaplasia of the conjunctiva[7-8].

Criteria for histopathological diagnosis

In cases of the patient's or parents' request, excision of the lesion was performed and tissue was submitted to histopathological study. All specimens were evaluated by two experienced ocular pathologists in a masked fashion and consensus existed regarding final diagnosis. Compound nevus was histopathologically diagnosed when nevus cells were observed in the epithelium and subepithelial connective tissue. Junctional nevus was defined for lesions with contiguous nests of round or spindle melanocytes near the basal cell region with oval nuclei and small nucleoli. Subepithelial nevus was identified by the presence of nevus cells only in the subepithelial connective tissue[21].

RESULTS

The general information about patient demographics is listed in Table 1. Of the 35 patients, 20 (about 57%) were white, 12 (about 34%) were Afro-descendant, 2 (about 6%) were Asian and 1 (about 3%) was Brazilian Indian. There were 18 (51%) males and 17 (49%) females with a mean age of 22 years (range, 4-66 years). The nevus was located on the right eye in 18 patients and in the left eye in 17. Lesion was heavily pigmented in 51%, lightly pigmented in 23%, and completely non-pigmented in 26%. The anatomical location of the nevus was the bulbar conjunctiva near the corneoscleral limbus (31 eyes, 89%), caruncle (2 eyes, 6%) and plica semilunaris (2 eyes, 6%). The nevus quadrant was temporal (22 eyes, 63%), nasal (9 eyes, 23%), superior (3 eyes, 9%), and inferior (2 eyes, 6%). Additional features included intralesional cysts (24 eyes, 68.6%).

IC examination of the 35 lesions revealed nests or cluster of nevus cells within the epithelium layer in 91.4% (32 cases). Examples are available in Figures 1 and 2.

Ten patients (28.5%) had the lesion removed and histopathological diagnosis was compound nevus in 8 eyes (1 from caruncle, 1 from plica semilunaris and 6 from bulbar conjunctiva) and subepithelial nevus from bulbar conjunctiva (2 eyes). No specimen presented inflammatory infiltrate.

DISCUSSION

In the present study, 35 conjunctival nevi were diagnosed clinically and evaluated cytologically by IC. Cytological examination is indicated to confirm a clinical diagnosis of a tumor or to promote a differential diagnosis between the various

Table 1. Features of 35 conjuntival nevi

Case	Gender	Age	Eye	Race	Lesion color	Anatomical location	Quadrant	Nevus cells on cytology	Cyst status	Histopathology
1	F	4	R	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
2	F	13	R	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
3	F	6	L	Afro	Lightly pigm	Bulbar conjunctiva	T	+	+	NP
4	F	54	R	Afro	Heavily pigm	Plica semilunar	N	+	+	NP
5	M	8	R	Afro	Lightly pigm	Bulbar conjunctiva	T	+	+	NP
6	F	40	L	White	Heavily pigm	Bulbar conjunctiva	I	+	+	NP
7	F	26	L	White	Lightly pigm	Bulbar conjunctiva	I	+	+	Compound nevus
8	M	10	R	White	Amelanotic	Bulbar conjunctiva	N	+	-	NP
9	M	6	L	White	Amelanotic	Bulbar conjunctiva	T	+	-	NP
10	M	41	R	Afro	Heavily pigm	Bulbar conjunctiva	T	-	-	Compound nevus
11	M	28	R	Indian	Heavily pigm	Bulbar conjunctiva	T	-	+	Compound nevus
12	F	51	R	White	Heavily pigm	Bulbar conjunctiva	N	-	-	Compound nevus
13	F	12	L	Asian	Amelanotic	Bulbar conjunctiva	T	+	-	NP
14	M	38	L	White	Lightly pigm	Bulbar conjunctiva	T	+	+	NP
15	F	9	L	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
16	M	9	R	White	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
17	F	66	L	White	Lightly pigm	Bulbar conjunctiva	T	+	+	Subepithelial nevus
18	M	11	R	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
19	F	11	R	White	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
20	M	17	L	White	Amelanotic	Bulbar conjunctiva	S	+	+	NP
21	M	40	R	White	Heavily pigm	Caruncle	N	+	+	NP
22	M	9	L	White	Lightly pigm	Bulbar conjunctiva	N	+	+	NP
23	F	23	R	White	Amelanotic	Bulbar conjunctiva	N	+	-	Compound nevus
24	F	59	R	White	Lightly pigm	Bulbar conjunctiva	T	+	+	Subepithelial nevus
25	M	27	R	White	Heavily pigm	Caruncle	N	+	+	Compound nevus
26	F	28	L	White	Heavily pigm	Plica semilunar	N	+	+	Compound nevus
27	M	10	L	Afro	Heavily pigm	Bulbar conjunctiva	S	+	-	NP
28	M	45	R	White	Lightly pigm	Bulbar conjunctiva	S	+	-	NP
29	F	8	L	White	Amelanotic	Bulbar conjunctiva	T	+	-	NP
30	F	4	R	Asian	Amelanotic	Bulbar conjunctiva	T	+	+	NP
31	M	4	L	Afro	Heavily pigm	Bulbar conjunctiva	T	+	-	NP
32	M	12	R	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	NP
33	M	12	L	Afro	Heavily pigm	Bulbar conjunctiva	T	+	+	Compound nevus
34	F	19	L	White	Amelanotic	Bulbar conjunctiva	T	+	-	NP
35	M	10	L	White	Amelanotic	Bulbar conjunctiva	T	+	-	NP

R= right; L= left; F= female; M= male; T= temporal; N= nasal; I= inferior; S= superior; += present; -= absent; NP= not performed; Afro= afro-descendant; pigm= pigmented

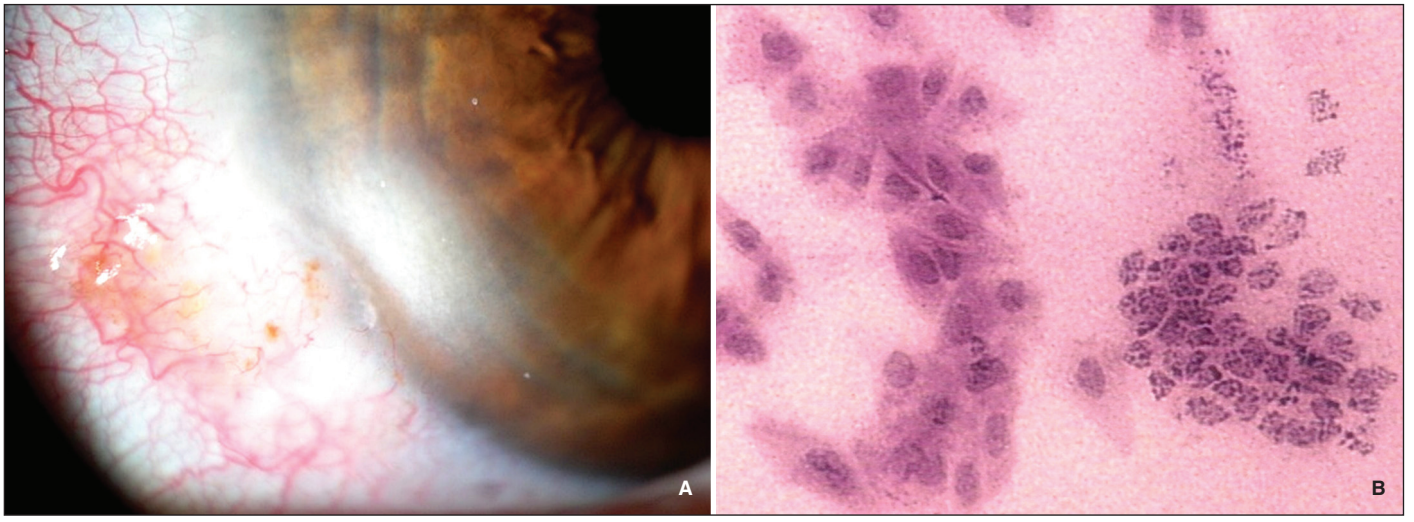

Figure 1 - A) Clinical picture of a slightly pigmented conjunctival nevus that had become noticeable in the right eye and B) Original magnification, × 200, impression cytology demonstrating a cluster of nevus cells without intracytoplasmic melanin pigment granules among epithelial cells

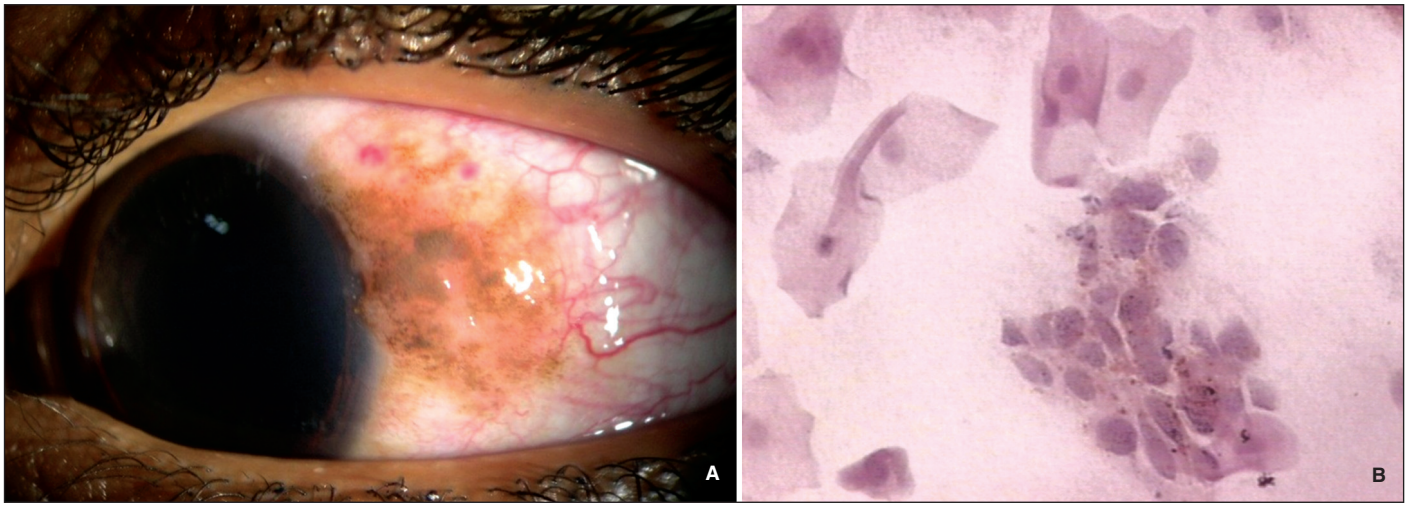

Figure 2 - A) Clinical picture of a pigmented conjunctival nevus that had become noticeable in the left eye and B) Original magnification, × 200, impression cytology demonstrating a cluster of nevus cells with intracytoplasmic melanin pigment granules among epithelial cells.

conjunctival lesions[7-13]. IC examination revealed nests or cluster of nevus cells within the epithelium layer containing or not mucous-secreting goblet cells in 32 cases (91.4%), confirming the clinical diagnosis of a conjunctival nevus. The first application of IC using acetate cellulose paper for the diagnosis of conjunctival lesions occurred in 1992 and reported a correlation of 73% between IC and histopathology in the diagnosis of 23 pigmented tumors of the bulbar conjunctiva, of which 4 were nevi[7]. A few melanocytic lesions, including 4 nevi and 1 malignant melanoma were also examined in another study, and for such cases, results of both IC and histopathological features correlated[10].

Although a great number of lesions evaluated in this paper (31 cases, 89%) were located at bulbar conjunctiva near the corneoscleral limbus, caruncle nevus and plica semilunaris nevus were also included, demonstrating that IC sampling of these anatomic locations is possible. Other authors who performed IC with the Biopore membrane instead of cellulose acetate strips described that sampling of the fornix, caruncula and ocular material in children was difficult and preferred to use exfoliative cytology for those situations[8].

IC has advantages over spatula scrapings that are more traumatic to the patient and give little information about cell to cell relations. It has other advantages such as the preservation of limbal stem cells over incisional biopsy that samples only a very small surface area to minimize scarring[7-8;11-13]. Nevertheless, cytologic sampling can be misleading because melanocytes lodged in the deeper part of the epithelium may not be included and false-negative reports can be presented, as noted on our 3 cases. Although the natural history of conjunctival nevi is benign and

less than 1% evolves to melanoma over time[3], IC does not replace histopathology examination that is the gold-standard method and may be performed to rule out melanoma or other neoplastic tumor when clinically suspected.

In our series, ten eyes (28.5%) were treated surgically and had histopathological confirmation of the disease. Regarding the histopathological study from conjunctival nevi, circumscription of the lesions, lack of mitoses in the substantia propria and lack of pagetoid spread of atypical cells in the adjacent conjunctival epithelium support benign diagnoses[21]. Histopathological evaluation demonstrated these benign features in all cases with no inflammatory infiltrate. Twenty-five patients (71.5%) were followed up conservatively without surgery. Comparatively, in the largest series already published of conjunctival nevi, 62% had follow-up without surgery[2].

Of the entire group, 31.4% of the patients did not demonstrate the classic clinical feature of intralesional cysts at slit-lamp biomicroscopy, a finding that is in agreement with literature[2].

All patients presented with the main complaint of the lesion that became more noticeable after color or size change but claimed that the lesion was first detected when they were younger. Although change in the lesion over the years prior to our examination was reported, it was not supported by photographic follow-up. Regarding lack of this documentation, possible reasons for patients' complaints can be: intralesional change in color/size or earlier and resolved inflammation[22]. According to previous reports from other authors, photographically documented change in conjunctival nevus color was observed in 13% and in size in 8% of all cases (n=149). Both were gradual and visible only on careful comparison of photographs over years[2].

About 26% of the included nevi were amelanotic but could be reported to us as a localized area of hyperemia due to their prominent vascularity. Possibly all conjunctival melanocytic nevi are congenital and may become noticeable after color change within the lesion, size change of the tumor or become elevated at any time during the first and second decades. The mean patient age observed (22 yr) is in agreement with other authors who reported the clinical diagnosis between the ages of 10 to 29 yr[23] or initial manifestation at the mean age of 32 yr[2]. Most nevi will be first detected around the age of puberty when the lesion acquires pigment in previously amelanotic or slightly pigmented regions and nevus cell proliferation is promoted by hormonal changes[1-3,7-8,20-21]. Furthermore, growth of a nevus may be result of factors other than melanocytic proliferation, such as inflammatory cell infiltration[21], allergic inflammation[22], enlargement of the intrinsic cysts, increased pigmentation in previously amelanotic regions[2] or immune response induced by the nevus itself[22].

CONCLUSIONS

Optical microscopy analysis of the IC specimens confirmed the clinical diagnosis by demonstrating typical histopathological features of the superficial layers from conjunctival nevi in 91.4% of the cases. For amelanotic nevi IC can also allow a differential diagnosis from other nonpigmented lesions. The technique does not replace histopathological examination, but additionally, may assist in evaluating nevus cells in children and adults.

RESUMO

Objetivo: Relatar as características da citologia de impressão de nevos conjuntivais referidos como mais perceptíveis. **Métodos:** Trinta e cinco pacientes que notaram uma lesão conjuntival que se tornou mais perceptível, por mudança de cor ou de tamanho, foram avaliados. Ao exame biomicroscópico foi feito o diagnóstico clínico de nevo, sendo obtidas amostras de citologia de impressão das lesões com auxílio do papel filtro de acetato de celulose, coradas com PAS, H&E e Papanicolaou. Para os indivíduos que optaram também pela remoção da lesão, o tecido foi enviado para análise histopatológica. **Resultados:** O exame de citologia de impressão revelou ninhos ou blocos de células névicas na camada epitelial, acompanhados ou não de células caliciformes em 32 casos (91,4%). Dez pacientes (28,5%) tiveram a lesão removida, sendo o diagnóstico histopatológico de nevo composto (um na carúncula, um na prega semilunar e seis na conjuntiva bulbar) e nevo subepitelial (dois na conjuntiva bulbar). **Conclusão:** A análise pela microscopia óptica das amostras de citologia de impressão confirmou o diagnóstico clínico ao demonstrar características típicas das camadas superficiais do nevo conjuntival em 91,4% dos pacientes. Nos nevos amelanóticos a citologia pode auxiliar no diagnóstico diferencial de outras lesões não pigmentadas. A técnica não substitui o exame histopatológico, mas pode ser útil na avaliação de células névicas em crianças e adultos.

Descritores: Nevo/diagnóstico; Túnica conjuntiva/citologia; Túnica conjuntiva/patologia; Técnicas citológicas; Câmara anterior/ultrassonografia; Técnicas de diagnóstico oftalmológico

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