Inflammatory juvenile compound conjunctival nevi. A clinicopathological study and literature review

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Abstract

Aim: The conjunctival nevus affecting children and adolescents is a rare condition and the literature showed only few reports on this issue. The aim of this article is to determine the histopathological features for the correct diagnosis of an inflammatory juvenile compound nevus of the conjunctiva (IJCNC) in order to make the difference between this tumor and other lesions, like conjunctival melanoma or lymphoma, very similar from a gross point of view. This article is a clinical pathological study of two cases of IJCNC with particular histopathological characteristics, who were admitted at the 2nd Ophthalmology Clinic, “Prof. Dr. Nicolae Obiu” Emergency Clinical Hospital, Iaşi, Romania, over a period of five years (from July 1, 2012 to June 30, 2017). Both patients were adolescents, a boy (13-year-old) and a girl (12-year-old). Both lesions were bulbar juxtalimbal located and grew in size over one year. Seen at slit-lamp biomicroscopy, the first one presented as a non-pigmented lesion, while the second was a pigmented nevus, but their dimensions did not exceed 10 mm in diameter. From a histopathological point of view, both of them showed a nested junctional growth pattern, along with intra- and subepithelial location, of the nevomelanocytes. Tumoral cells demonstrated different degrees of atypical cytology, but in the second case, it was more obvious. Microscopic examination also revealed epithelial cystic inclusions, and prominent inflammation in the stroma of these two nevi. One of the cases presented heavy inflammation that took the form of lymphoid follicles and sheets of eosinophils, but the other showed only diffuse inflammation with lymphocytes plasma cells, and eosinophils within its stroma. The immunohistochemical characterization (anti-melan A, anti-S100 protein and anti-cytokeratin AE1/AE3 antibodies) of the tumoral cells helped to the diagnosis. Conclusions: IJCNC represent a small group of nevi that develop in adolescents and have some particular histopathological features. The pathological diagnosis is difficult as the microscopic features are very similar to a conjunctival melanoma, but a detailed microscopic examination, immunohistochemical stainings and the young age of the patient could help in establishing the benign nature of these lesions.

Keywords: inflammatory juvenile compound nevus of the conjunctiva, lymphocytes, eosinophils, melan A, S100 protein, cytokeratin.

Introduction

Even though conjunctival lesions represent only 2.5% in ophthalmological pathology [1], more than 50% of them have a melanocytic origin [2]. The most common (more than 50%) melanocytic lesions of the conjunctiva are nevi (congenital, which are present at birth or appearing within the first six months of life, and acquired) [1, 2]. Other conjunctival melanocytic lesions are melanosis (congenital and acquired), which are diagnosed in one-third of cases, and conjunctival melanoma that is encountered only in 10% of cases [1, 3]. In childhood and adolescence, conjunctival melanocytic nevi are common [4, 5], while the conjunctival melanoma is extremely rare [6–9]. Most nevi are detected around puberty, when the nevus cell proliferation is promoted by hormonal changes [2, 5, 10–14], but they can be found at any age, even in the elderly [15]. However, there are only few articles about juvenile conjunctival nevi. The oldest reference to this entity was in 1965, when Barrie Samuel Jay (1929–2007) reported the pathological features of benign nevi of the conjunctiva. He gathered basic clinical information and noted that the patients most commonly seen by an ophthalmologist were between the ages 10 to 29, and typically claimed that the lesion was first detected when the patient was younger than nine years [16]. Very few authors reported histopathological features
of juvenile conjunctival nevi, but all of them recognized that these lesions have particular characteristics and as such, they labeled this condition with different names: inflamed juvenile conjunctival nevus [17], inflamed conjunctival nevus of puberty [6], or inflammatory juvenile conjunctival nevus (IJCN) [18].

We choose the later name to designate this clinical pathological entity and we made a clinico-pathological study of two cases of inflammatory juvenile compound conjunctival nevus to highlight their particular histopathological characteristics. Both cases were admitted at the 2nd Ophthalmology Clinic, “Prof. Dr. Nicolae Oblo” Emergency Clinical Hospital, Iași, Romania, over a period of five years (from July 1, 2012 to June 30, 2017).

Case series

Case No.1

A 13-year-old male patient hospitalized at the 2nd Ophthalmology Clinic, “Prof. Dr. Nicolae Oblo” Emergency Clinical Hospital, Iași, in 2017 noted a lump on the right eye, which grew in size for 14 months, and was accompanied by irritative symptoms. Clinical examination with slit lamp biomicroscopy revealed a single, not well-defined, slightly elevated, pinkish, movable plaque of 7 mm at its widest point, with vascular congestion. It was located in the bulbar conjunctiva, 1 mm close to the temporal limbus (Figure 1). From patient’s medical file, we found out that he had been diagnosed before with recurrent allergic conjunctivitis and with mitral and tricuspid congenital regurgitation of degree I–II. The conjunctival tumor was excised with safety margins of 3 mm and it was sent to the Laboratory of Pathology. The specimen was fixed in 4% formalin, hardened in a mixture of acetone and xylene and embedded in paraffin; 4 μm thick serial sections were cut and stained with Hematoxylin and Eosin (HE). An immunohistochemical two-step staining technique, using EnVision™+ and anti-cytokeratin (CK) AE1/AE3, anti-melan A and anti-S100 protein antibodies was also applied on serial sections. The pathological exam revealed a tumor made up of nests of nevomelanocytes, which were located in junctional, intra- and subepithelial sites (Figure 2). Some tumor cells appeared cytologically alarming, having hyperchromatic, large, and pleomorphic nuclei and scant cytoplasm, presenting a pagetoid spread into the conjunctival epithelium (Figure 3). The tumoral cells, many of them with atypical morphology, formed nests around some epithelial cysts (Figure 4).
The tumoral stroma showed an excessive chronic inflammatory cell infiltration made up of lymphocytes aggregated in lymphoid follicles (Figure 5). Higher magnification also showed that tumoral stroma was infiltrated with heavily inflammatory infiltrate with numerous eosinophils that hide tumoral cells (Figure 6). Epithelial cells, including those lining the cystic structures were positive for cytokeratin AE1/AE3 (Figures 7 and 8) and tumoral cells exhibited immunopositivity for melan A (Figure 9) and S100 protein (Figure 10). The final pathological diagnosis was inflammatory juvenile compound nevus of the conjunctiva. The post-operative follow-up period of two months was without complications.

Figure 5 – Case No. 1. A prominent and dense infiltrate of inflammatory cells was present throughout the lesion, including formation of lymphoid follicles (HE staining, ×200).

Figure 6 – Case No. 1. Higher magnification of histopathological section showed a rich infiltrate of inflammatory cells, including eosinophils and epithelial cysts surrounded by nevomelanocyte cells (HE staining, ×400).

Figure 7 – Case No. 1. Positive immunostaining for cytokeratin demonstrated the epithelial nature of the cystic structures included into the conjunctival nevus. A rich lymphocytic infiltration took the form of a lymphoid follicle (Cytokeratin AE1/AE3 immunostaining, ×100).

Figure 8 – Case No. 1. Higher magnification showed immunopositivity for cytokeratin in the cytoplasm of epithelial inclusion and differentiated them from nevomelanocyte cells (Cytokeratin AE1/AE3 immunostaining, ×200).

Figure 9 – Case No. 1. Positive immunostaining for melan A in the cytoplasm of nevomelanocyte cells (Melan A immunostaining, ×200).

Figure 10 – Case No. 1. Positive immunostaining for S100 protein in the cytoplasm of nevomelanocyte cells (S100 protein immunostaining, ×200).
Case No. 2

A 12-year-old female patient hospitalized at the 2nd Ophthalmology Clinic, “Prof. Dr. Nicolae Obel” Emergency Clinical Hospital, Iași, in 2012 complained of a bulbar juxtalimbal, pigmented conjunctival lesion on the left eye with slight irregular borders that had been growing in the last 12 months. The lesion was 4 mm at its widest point, and had a brown coloration with “feeder vessels” (Figure 11), and was soft in consistency, mobile and painless.

The conjunctival tumor was surgically removed with safety margins of 3 mm and it was sent to the Laboratory of Pathology. The collected tissue fragments were fixed in 4% formalin and paraffin-embedded. The histological sections were stained with Hematoxylin and Eosin. The histopathological exam revealed a papilliferous conjunctival projection due to proliferation of nevomelanocyte cells, which were located at the junction between the epithelium and substantia propria, but also within them both (Figures 12 and 13). Nevomelanocytes were arranged mainly in nests at the epithelial–subepithelial junction, but there was also noticed a diffuse pattern (Figures 14 and 15).

Figure 11 – Case No. 2. 12-year-old girl with a juxtalimbal, pigmented conjunctival lesion with slight irregular borders and “feeder vessels” (gross photography, left eye).

Figure 12 – Case No. 2. Papilliferous conjunctival projections due to proliferation of nevomelanocyte cells, which were located at the junction between the epithelium and substantia propria, and within them both (HE staining, ×50).

Figure 13 – Case No. 2. Nevomelanocyte cells extended into the substantia propria but maintained an intraepithelial component. A lateral junctional tumoral extension, away from the main component could be seen (HE staining, ×100).

Figure 14 – Case No. 2. Along the epithelial–subepithelial junction and in the conjunctival stroma there were variable sized “nests” of nevomelanocytes that contained variable quantities of brown pigment (melanin) in their cytoplasm and presented moderate cellular atypia (HE staining, ×200).

Figure 15 – Case No. 2. Nevomelanocyte cells extended into the conjunctival substantia propria, but maintained an intraepithelial component. A pagetoid spread of melanocytes above the junction made up of nests of melanocytes could be seen (HE staining, ×200).
The tumoral “nests” were of varying sizes and contained fine melanin granules, sometimes very abundant, in the cytoplasm. There were also intraepithelial nests of nevus cells in a pagetoid migration pattern (Figure 16). Numerous epithelial cysts, lined by multistratified epithelium containing also goblet cells were focally observed in the deep part of the nevus and the tumoral cells aggregates around them. A diffuse chronic inflammation in the tumoral stroma could be seen and was made up of lymphocytes and plasma cells (Figures 17 and 18). In the deep part of the tumor, a paradoxical “reverse” maturation could be seen that lacked maturation in the depth of the tumor. There were sheets of atypical, large, epithelioid nevomelanocytes with large, hyperchromatic and pleomorphic nuclei. No evidence of mitotic activity was observed (Figure 19). The final pathological diagnosis was inflammatory juvenile atypical compound nevus of the conjunctiva. The follow-up period of 48 months was uneventful.

Discussion

The word nevus originates from the Latin word “nevus”, meaning a birthmark upon the body. It was originally applied to a number of different conditions appearing at or soon after birth and characterized by an alteration in the color or texture of one part of the skin. At the beginning of the nineteenth century, it was considered that conjunctival nevi were similar to those of the skin. However, in the middle of the last century the term “nevus” has been restricted to those benign, pigmented and occasionally non-pigmented, tumors composed of pigment-producing cells [16]. The conjunctival nevus appears clinically in the 1st or 2nd decade of life. The nevus starts as a small nest of cells located at the junction (junctional nevus), and in the 2nd to 3rd decade the cells migrate to the underlying stroma (compound nevus) and at this stage intratumoral epithelial cysts are also formed. In the 3rd or 4th decade, the lesion migrates totally in the stroma (subepithelial nevus) [19].

The conjunctival nevus usually appears as a discrete
lesion, mobile on the bulbar conjunctiva [20], looking like a hyperemic area due to intense vascularization [14]. It can be similar to other lesions, including inflamed pingueculum, episcleritis, conjunctival cyst, allergic conjunctivitis, foreign body granuloma, lymphangioma, squamous epithelial neoplasia, conjunctival sarcoidosis, and leukemia [14].

Even though conjunctival nevus is a benign lesion, in children and adolescents it often has particular histological features that lead to great difficulty in differentiating it from a conjunctival melanoma [20]. These benign lesions develop more frequently in the bulbar conjunctiva [1] and the most common patients’ age is between 10 and 19 years old [21]. Both of our patients were adolescents, a boy (13-year-old) and a girl (12-year-old). Both lesions were bulbar-juxtalimbal located and grew in size over one year. The first one presented a non-pigmented nevus, but the girl has had a pigmented lesion. These gross features are in line with those reported by Shields et al. (2004) [10], who found out that 65% of their 410 cases were brown, 19% were tan and 16% were completely non-pigmented conjunctival nevus [10]. However, the diagnosis could raise the suspicion on the spectrum of classical clinical features using slit-lamp biomicroscopy: from heavy pigmentation to complete lack of pigments, from a small size lesion to extensive tumors and from presence of cysts in most cases to a complete lack of cysts. Intratumoral cysts are most common in compound nevus and their recognition could aid in differentiating conjunctival nevus from malignant melanoma as conjunctival melanoma rarely, if ever, displays cysts [14].

Shields et al. (2004) [2], found out that all the patients with large nevi over 10 mm had prominent intralesional cysts that could suggest the diagnosis [2]. Both of our cases reported the onset of the disease as a pink or brown conjunctival juxtalimbal lesion that grew in size for approximately one year, being accompanied by inflammation, and causing a sensation of foreign body to the patients. These two conjunctival lesions did not exceed 10 mm in diameter and probably because the lesions were small, we did not notice any intratumoral cyst on biomicroscopy, but some epithelial cysts were identified at histopathological exam.

Both of our patients were referred because of a history of moderate “growth” and congestive of the conjunctival lesion for one year. One of the patients had a history of allergic conjunctivitis and this disease is a sign associated with IJCN.

Histopathologically, conjunctival nevi are classified into: junctional (nevomelanocytes are located at junction, between conjunctival epithelium and substantia propria), subepithelial (tumoral cells are situated entirely within the substantia propria), compound (a combination of junctional and subepithelial nevus) and other less common subtypes such as blue nevi and combined nevi (nevus with both a compound and blue nevi) [15].

The first description of morphological features of IJCN was made by the English ophthalmologist Barrie Samuel Jay, who, in 1965, reported three cases from 282 benign melanotic nevi of conjunctiva (i.e., 8.46% of all cases). These lesions occurred in children and young adolescents and the histopathological exam identified junctional nevi, or compound nevi with an active junctional component. This activity was characterized by the upward migration of cells through the epithelium, by pleomorphism of the individual cells of the nests, by loss of cohesion between individual cells, and by the presence of mitotic figures, but these features did not signify a malignant behavior. These lesions were also associated with heavily infiltrate with inflammatory cells, which seemed to disrupt the normal architecture of the nevus and determined the pathologist to be concerned by their presence. In addition, the nevus cells were more pleomorphic than is usually the case even in nevi of children and occasionally the giant and bizarre form of nevi was found [16].

Thirty years later, Folberg et al. (1989) [6], made a review of benign conjunctival melanocytic lesions and underlined the fact that nevi in adolescents are histopathologically associated with extensive inflammatory infiltrate and designed these lesions as “inflamed nevi of puberty and young adulthood”. The authors affirmed that these nevi seem to grow and may cause clinical and histological confusion with other entities, particularly with a regressing nodule of melanoma [6].

Then, after another fifteen years, Zamir et al. (2002) made a retrospective study on 63 patients younger than 20 years old presenting conjunctival nevi and concluded that 25% were simple compound congenital nevi and 75% were compound nevi with prominent inflammatory histological features (discrete lymphocyte aggregates, plasma cells and eosinophils). Epithelial cysts and solid epithelial islands were common and he designed these nevi as “inflamed juvenile conjunctival nevus”. All these lesions were located at or near the limbus, and were characterized by recurrent periods of congestion and growth. However, 75% of the inflamed juvenile conjunctival nevus had a history of allergic disease [22], as one of our patient also reported.

Thiagalingam et al. (2008) [20], investigated 33 conjunctival nevi occurring in children and adolescents and concluded that there was a subset of childhood nevi lacking maturation and defined them as “juvenile conjunctival nevi” [20]. These authors described their clinicopathological characteristics as following: the mean age at time of excision was around 10.9 years, the majority of the lesions were of the compound type, all showed a nested junctional growth pattern, the nuclei of the subepithelial nevomelanocytes were larger than the epithelial nevomelanocytes cells, and more than 50% of these nevi showed a lymphocytic host response. However, none of the lesions had recurred over an average follow-up period of 34 months. Thus, recognition of juvenile conjunctival nevus as a distinct morphological variant of a conjunctival nevus with characteristic histological features may help to distinguish this benign lesion from melanoma [20].

During the same period, Jakobiec et al. (2010) [23], claimed that juvenile conjunctival nevi contained conspicuous chronic inflammation and frequently had atypical histopathological traits that could be suggestive of melanoma, but had a benign behavior [23].

The American eye pathologist Robert Folberg emphasized the fact that conjunctival compound nevi are histo-
logically distinctive because some of them show a relative lack of maturation that may appear worrisome, but the lesion is entirely benign. Also, he underlined the presence of epithelial cystic inclusions, which he thought to originate from the dropping-off of the melanocytes from the epithelium into the substantia propria, dragging the epithelium down into the loosely arranged subepithelial collagen [24].

The epithelial cysts, which are a characteristic feature of over half the conjunctival nevi, were considered to be a sign of malignancy at the end of the 19th century [25], but at the beginning of the 20th century, Parsons (1904) appreciated that this morphological feature did not mean a poor prognosis [26].

Nowadays, the internationally recognized American ophthalmologists Myron Yanoff and Joseph W. Sassani considered that the cystic epithelial structures founded in an IJCN represent a hamartomatous component, i.e., epithelial embryonic rests, which may develop into epithelial cysts. However, these epithelial cysts may assume a rather large size and may dominate the histological picture, nearly obscuring the presence of a nevus [27].

A rather prominent infiltrate of lymphocytes and plasma cells, and eosinophils may accumulate within the stroma of nevi of childhood. As such, Folberg (2013) considered that the presence of chronic inflammation, typically with eosinophils, in a compound nevus in a child around the age of puberty should be designed either as an “inflamed juvenile conjunctival nevus” or as an “inflamed conjunctival nevus of puberty” [24].

More recently, the American dermatologist Soheil Sam Dadras (2017) [28], described three histopathological characteristics for IJCN as following: (1) unlike adult compound conjunctival nevus, where nevomelanocytes become smaller (nuclear/cytoplasmic ratio decreased) with increasing subepithelial depth, the inflammatory juvenile compound nevus shows a paradoxical “reverse” maturation in subepithelium, i.e., nuclear and cytoplasmic size of melanocytes forming subepithelial component is greater than that of junctional component; (2) this nevus contains prominent inflammatory infiltrate, which may obscure the architecture of the nevus and can be misleading, giving the impression of cytological atypia; (3) this nevus presents intrasional epithelial cysts lined by conjunctival epithelium and goblet cells [28].

From a histopathological point of view, both of our cases were compound nevi, as they showed a nested junctional growth pattern, along with intra- and subepithelial location, of the nevomelanocytes. Tumoral cells showed different degrees of atypical cytology, but in the second case, it was more obvious. Microscopic examination also revealed epithelial cystic inclusions, and prominent inflammation in the stroma of these two nevi. One of the cases presented heavy inflammation that took the form of lymphoid follicles and eosinophilic sheets, but the other showed only diffuse inflammation with lymphocytes, plasma cells, and eosinophils within its stroma.

The rapid growth of these IJCN was reaffirmed by Thiagalingam et al. (2008) [20], and by Colarossi et al. (2013) [29], who considered that the cause may be the heavy inflammatory infiltration and cystic degeneration of epithelial rests from substantia propria, but these features do not signify a malignant transformation [20, 29]. Even though our patients reported growing of their lesion in the last year, this clinical aspect was not a malignant sign as they were followed for two months, and 48 months respectively, and no recurrences could be seen. The moderate growing of the lesions before the surgical intervention could be correlated with epithelial cysts enlargement due to goblet cells mucin secretions and with the high number of inflammatory cells accumulated inside the tumoral stroma.

However, both of our cases satisfied the clinicopathological criteria for establishing the diagnosis of an IJCN as these were pointed out by B. S. Jay, Zamir et al. (2002) [22], Thiagalingam et al. (2008) [20], Folberg [24], Colarossi et al. (2013) [29], and Dadras & Zembowicz [28]. Moreover, we noticed that the junctional component showed focal pagetoid spread of melanocytic cells in the conjunctival epithelium, as Colarossi et al. (2013) [29] also reported.

In the study made by Kindblom et al. (1984) [30] on 15 benign nevi, three blue nevi, four juvenile melanomas, one balloon cell nevus, there was positive staining for S100 protein in the majority of the tumor cells in all the benign tumors examined, except the balloon cell nevus, as well as in all the primary and metastatic malignant melanomas. The results indicate that S100 protein is a valuable marker for melanocytic tumors [30]. Even though Jakobiec et al. (2010) [23] reported that anti-S100 and anti-melan A antibodies were not useful in separating benign from malignant lesions [23], in our case immunostainings with anti-S100 and anti-melan A antibodies were useful for highlighting the tumor cells that were hidden by inflammatory infiltration. Also, anti-cytokeratin antibody was useful in detecting the tumor cells that were hidden by epithelial cysts.

The conjunctival nevus can progress in less than 1% of the cases toward malignant melanoma [4, 5]. Clinical features suggestive of malignancy include extension to the cornea, attachment to the sclera and presence of “feeder vessels” [31]. However, if the malignant transformation takes place, there is a significant mortality rate (13% at 10 years old) [32].

That is why the clinical examination (slit-lamp biomicroscopy) and color photographs are recommended for the follow-up of these patients [33, 34]. Anterior segment optical coherence tomography provides high-resolution imaging of conjunctival nevi with the ability to demonstrate all the margins and to provide information on the presence of intrasional cysts, which are important in the diagnosis [35].

If the lesion changes (such as documented growth, color change, or ulceration), a biopsy is indicated for a defined diagnosis, with no touch technique, also removing a thin lamella of the sclera and applying cryotherapy to the margins [31]. The ophthalmologist should be aware that the incomplete excision of the conjunctival nevus in children produces relapse [36]. However, Lommatzsch et al. (2007) [18], recommend follow-up examinations at regular intervals in cases of IJCN [18].

The family history and the ocular diseases of the patient, age, aspect of the lesion, size, color, margins,
elevation, “feeder vessels” and localization are essential. In the case of IJCN, the clinical examination can be challenging and it is important to have a correct diagnosis, which will lead to an appropriate management and prognosis [15].

Excisional biopsy is not necessary in the case of small tumors with a benign appearance, but if a suspicion raised, then this procedure is recommended [15]. However, in case of surgical treatment, a complete local excision of the lesion, with at least 3–4 mm margins [37] and, according to the dimension of the residual defect, it can be completed by conjunctivoplasty or transplantation of amniotic membrane [38]. The histopathological examination remains vital in atypical conjunctival lesions [15, 39–43].

The IJCN does not have any systemic association, though it can be rarely associated with Carney complex (which includes cardiac problems) and dysplastic nevus syndrome [19, 44].

Currently used immunohistochemical techniques can be used to differentiate melanocytic lesions from non-melanocytic conditions. However, the popular melanoma-specific antigen [human melanoma black (HMB)-45] generally shows a positive reaction within both nevus and melanoma and cannot be reliably used to differentiate benign from malignant melanocytic lesions [45]. One of our patients had a history of mitral and tricuspid congenital regurgitation of degrees I–II. We can presumed that his mother could had, during pregnancy, infections, consumption of toxic substances, medication, other endocrine or non-endocrine diseases, etc. that led to the appearance of cardiac malformations in the child [46–52].

Especially in young patients, IJCN must be regarded as an independent type of nevus, which might lead even experts in ophthalmic pathology to over-diagnose this lesion as a malignant melanoma [18]. This event could lead to wrong therapeutic steps with surgical procedures that could cause unnecessary mutilation.

Conclusions

Based on clinical and pathological features, we concluded, along with other authors, that IJCN is a particular and unique entity of juvenile conjunctival nevi, different from simple compound conjunctival nevus. Its association with allergic conjunctivitis is suggestive, and despite periods of alarmingly rapid growth, is histologically benign. IJCN should be recognized by pathologist as well as by ophthalmologists as the differential diagnosis with melanomas in the conjunctiva should be done using a multi-level evaluation of clinical, morphological and immunohistochemical aspects.

Conflict of interests

The authors do not have a financial interest/arrangement or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of the manuscript.

References


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