Case Report: Conjunctival Intraepithelial Neoplasia

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Abstract
Background:
Conjunctival intraepithelial neoplasia (CIN) is a dysplastic lesion of the conjunctiva and is the most common tumor of the ocular surface. CIN typically presents as a fleshy, gelatinous lesion that is typically located at the limbus. Risk factors include ultraviolet light exposure, immunocompromised state, history of smoking and human papilloma virus (HPV).

Case Report:
We present a case of CIN in a 76 year-old African American patient whose only complaint was of dry eye symptoms. Anterior segment examination revealed a gelatinous, vascularized lesion on the bulbar conjunctiva of the right eye. Excisional biopsy revealed a diagnosis of CIN with positive margins. The patient was treated with topical interferon alpha 2b drops.

Conclusion:
CIN is a neoplastic disorder that has a relatively favorable prognosis with treatment which includes both topical and surgical management. Careful anterior segment examination is crucial in detecting these lesions with prompt referral for treatment.
Key words: CIN, ocular neoplasia

Introduction
Conjunctival intraepithelial neoplasia (CIN) is a dysplastic lesion of the conjunctiva and is the most common tumor of the ocular surface. There are many well established risk factors for CIN including human papillomavirus (HPV), human immunodeficiency virus (HIV), ultraviolet light exposure, and exposure to petroleum products. In this paper we describe a case of a 76-year-old African American male presenting with a gelatinous conjunctival lesion consistent with CIN.

Case Report
A 76-year-old African American male presents for a comprehensive eye exam. He had a chief complaint of dry eyes, worse in the right eye than the left, but otherwise reported good and stable vision. Medical history was remarkable for hypertension, mantle cell lymphoma, and mucosa-associated lymphoid tissue (MALT) of the gastrointestinal tract that was treated with radiation therapy. The remainder of his history was unremarkable, and his medications and allergies were non-contributory. His best corrected vision was 20/20 in both eyes and entrance testing was unremarkable. Posterior segment exam was unremarkable in both eyes. Anterior segment exam revealed a 1mm elevated, gelatinous lesion with vascularization and adjacent calcification on the right temporal bulbar conjunctiva in the right eye [Figures 1a-b]. The patient was referred for excision and biopsy. The pathology report revealed histological findings consistent with conjunctival intraepithelial neoplasia with positive margins. The patient was treated with interferon alpha 2b drops, 1-million units/mL, one drop four times per day for 1 month and was to follow up in 1 month. Unfortunately, the patient never presented for the scheduled follow up due to the COVID pandemic. Seven months later the patient presented for follow up and there was no recurrence of the lesion noted.

Discussion
Ocular surface squamous neoplasia (OSSN) is the most common conjunctival malignancy in the United States and includes conjunctival intraepithelial neoplasia (CIN) and conjunctival squamous cell carcinoma (cSSC). In a study by Aliakbar-Navahi et al, 631 conjunctival lesions were reviewed and categorized based on malignant potential (either benign, premalignant, malignant). Benign lesions were the most prevalent (81.8%), followed by premalignant and malignant lesions constituting 10.8% and 7.4% of lesions respectively. This study also found the most common premalignant lesion to be CIN (94.1%) and the most common malignant lesion to be SCC (93.6%). Categorization of these lesions is based on their location in the conjunctival layers. CIN describes a lesion that is confined to the surface epithelium of the conjunctiva. Conjunctival squamous cell carcinoma (cSSC) are lesions that have broken through the basement membrane of the conjunctiva and have invaded the underlying stroma therefore making them much more invasive.
more invasive2]. Lesions that invade the cornea are defined as conjunctival-corneal intraepithelial neoplasia.

Clinically, CIN appears as a fleshy, sessile or minimally mobile lesion, usually at the limbus, and less commonly in the fornices and tarsal conjunctiva2. Lesions can appear with or without vascularization. CIN is typically described as either gelatinous or leukoplakic, the latter if the lesion undergoes secondary hyperkeratosis2.

Squamous cell carcinoma is characterized by extension of abnormal epithelial cells through the basement membrane to gain access to the conjunctival stroma2. SCC appears clinically similar to CIN but may appear more elevated or larger. In most cases, biopsy is needed to differentiate between the two types of lesions.

**Epidemiology**

There are several well-established risk factors for CIN which include ultraviolet light exposure, immunosuppression (HIV/AIDS), a history of smoking, and petroleum product exposure. The relationship between risk of CIN and HPV has also been well established. In a study by Ateenyi-Agaba et al 21 eyes with SCC of the conjunctiva and 22 control subjects were tested using PCR based assays for HPV3. Of the 21 eyes with SCC tested, epidermodysplasia verruciformis, a form of HPV, was found in 86% of cases, and 36% of the control eyes, creating an odds ratio of -123. Scott et al further investigated the role of HPV serotypes 16 and 18. In this study of 10 eyes with CIN, HPV 16 was detected in 5 eyes and HPV 18 was detected in the other 5 eyes4. Neither HPV 16 nor 18 were detected in the control specimens4. In a study of 112 patients with primary CIN lesions, 24 (21%) tested positive for HPV with polymerase chain reaction testing5. HPV 16 was the most prevalent genotype6. These studies suggest a need for close monitoring and screening for HPV with a history of SCC. Lighter-skinned individuals and middle-aged males are most commonly affected. Human immunodeficiency virus and human papilloma virus should always be considered and investigated if OSSN presents in a young patient. Geographically, patients living closer to the equator are typically more affected.

The association between the expression of tumor markers and the presence of conjunctival intraepithelial neoplasia has been widely explored. P63 is expressed in basal squamous epithelium and is a homologue of the tumor suppressor gene p536. Some studies have shown that p63 plays a direct role in the development of neoplasms of the squamous epithelium, despite its homology to p536. Auw-Haedrich et al found p63 to be preferentially expressed in specimens of conjunctival intraepithelial neoplasia but did not find a direct correlation between the number of cells staining positive for the tumor marker p63 and differentiation stage of the specimens6.

**Differential Diagnoses**

Characteristic clinical features of CIN can be helpful in differentiating CIN from other common conjunctival conditions. Pinguecula is described as a benign elevation of the conjunctival tissue, typically caused by exposure to ultraviolet light and persons exposed to dry, windy environments. Pterygium is also a condition that should be ruled out when considering CIN. Pterygium shares a similar etiology as pinguecula but unlike pterygium, pinguecula remains on the conjunctiva and does not progress onto the cornea. Other less common differentials include squamous papilloma, solar elastosis, and epithelial hyperplasia. Early identification of CIN is critical in order to implement proper treatment and to reduce the risk of metastasis.

Differentiating conjunctival intraepithelial neoplasia from other conjunctival lesions is important, as confirmation of diagnoses changes the treatment and management of the existing condition. Surgical resection and pathologic evaluation are considered the gold standard, as many conjunctival lesions may appear similar clinically and biopsy provides a definitive diagnosis. Obtaining a biopsy of the suspicious lesion not only aids in confirming diagnoses but removes the lesion, leaving less chance of increase in growth and ability to progress. Details on the surgical procedures are further discussed in the surgical management section.
**Histopathology-Staging**

The American Joint Committee on Cancer (AJCC) provides the most recent classification of conjunctival intraepithelial neoplasia. Category Tis includes mild, moderate, and severe dysplasia, as well as carcinoma in-situ. Mild, moderate, and severe dysplasia are categories used to describe the level at which the epithelium is involved. Mild describes the stage in which one-third of the epithelium is involved, moderate when two thirds of the epithelium is involved, and severe when the entire thickness of the epithelium is involved. Severe stage is also known as carcinoma in-situ. Stages T1 and T2 describe squamous cell carcinoma. Stage T1 is used when less than five millimeters of the tumor invades through the conjunctival basement membrane, without invasion to the adjacent structures. Stage T2 is used when more than five millimeters of tumor has invaded through the conjunctival basement, without invasion to other adjacent ocular structures.

**Treatment and Management**

Treatment for CIN includes both surgical management and medical therapy. Surgical removal of the lesion allows for both removal of the lesion as well as providing a definitive diagnosis. Disadvantages of surgical removal include risk for residual cells left on the conjunctival surface, increasing risk of recurrence, and increased risk of conjunctival scarring and limbal stem cell deficiency. Chemotherapy agents are typically used in conjunction with surgical removal and include interferon alpha 2b (IFNa-2b), mitomycin C (MMC), and 5-fluorouracil (5-FU). Recent studies have also explored the use of anti-VEGF for the treatment of CIN. Advantages of treating topically include treatment of the entire ocular surface which reduces the risk of recurrence and of conjunctival scarring.

**Pharmacological Management**

Interferons are leukocyte-derived proteins that work by enhancing phagocytic and cytotoxic mechanisms, which inhibit biosynthetic enzymes and decrease blood vessel proliferation, therefore inducing apoptosis and inactivating viral RNA. Interferon α-2b (IFNa-2b) is a cytokine containing 165 amino acid residues with immunomodulatory effects. Intralesional injections of IFNa-2b enhance the production of IL-2 and IFN-y mRNA by the immune system as well as lower the production of IL-10. These well-established mechanisms aide in targeting neoplastic cells.

Interferon alpha 2b can be used topically as an eye drop, or subconjunctivally as an injection. Occasionally both modalities are used. The dosage of topical interferon alpha 2b therapy has been explored in multiple studies. Galor et al compared the efficacy of one million international units (IU)/mL versus three million IU/mL. The study found no statistically significant difference between the two doses, making the standard dosage of treatment for conjunctival intraepithelial neoplasia to be one million IU/mL four times per day when used topically. Treatment is usually continued for one to two months after resolution of the lesion. A case report by Esquenazi et al examined the rate of regression of treatment with interferon alpha 2b. The study reported two cases of biopsy proved CIN that were both treated with interferon alpha 2b four times per day topically. One lesion regressed in 44 days and the other in 84 days. No recurrences were seen at 6 months and 3 months respectively. In another study by Kusumesh et al 24 eyes were treated using topical IFNa-2b (1 million IU/mL) 4 times daily. This study found that 22 patients (92%) had complete resolution with treatment, with a mean time to resolution of 3.25 months. Two patients (8%) did not respond to treatment. In another study by Nanji et al a 3% recurrence rate was found in patients who were treated with IFNa-2b, as compared to a 5% recurrence rate in patients who were treated surgically.

5-fluorouracil (5-FU) is another pharmacological agent that is typically used for the treatment of CIN. 5-FU is a pyridine analog that blocks thymidine synthase which inhibits DNA formation. This leads to a reduction in RNA synthetase, therefore causing poor cell growth and cell death. Similar to interferon alpha 2b and mitomycin C, 5-FU can be used as a primary treatment or adjuvant for the treatment of CIN. 5-FU is typically found in concentrations of 1% and dosed four times per day for one week, followed by three weeks of drug holiday. This regimen describes one cycle which is typically repeated on average of 4 to 6 times or until resolution. Due to the effects 5-FU has on the proliferation of normal rapidly dividing epithelial cells and fibroblasts, the incidence of side effects is typically higher when compared to the other treatment modalities. These side effects include transient lid edema, conjunctival hyperemia, superficial keratitis, filamentary keratitis and on rare occasions, superficial stromal melting.

In one study of 44 eyes, 5-FU was used as therapy to treat CIN and was dosed four times per day for four weeks. Twenty-two patients (53.7%) underwent treatment with topical 5-FU only and 19 patients (46.3%) as adjuvant therapy. This study found a 7.3% recurrence rate in tumors treated with 5-FU only. These tumors were successfully treated with additional cycles of 5-FU.

Mitoxyacin-C (MMC) is an alkylating agent that contains anti-metabolite/antibiotic properties that works by binding to DNA during DNA synthesis therefore inhibiting synthesis and function. At higher concentrations, MMC inhibits nucleotide synthesis which in turn interferes with RNA transcription and protein synthesis. MMC works by inducing apoptosis of fibroblasts, as it is toxic to proliferating and nonproliferating cells. Similar to interferon alpha-2b, MMC may be used as the primary form of treatment for CIN, as well as adjuvant to excisional biopsy or post-operatively for lesions with positive margins. Different concentrations of MMC have been used for the treatment of CIN, with higher concentrations having greater ability to cause epithelial toxicity. One case series reported using a concentration as low as 0.002% of mitomycin C, although the most common concentrations used to treat conjunctival intraepithelial neoplasia are 0.02% and 0.04%. When used as the primary form of treatment, MMC is typically dosed 4 times per day for one week, followed by two to three
through the soft tissue structures. Thus, it is often necessary to excise neoplasms that have the ability to invade through the corneal epithelium and sclera and into the anterior chamber, as well as spread to adjacent tissues along the course of a biopsy needle.

Anti-VEGF agents are monoclonal antibodies that block the interaction of vascular endothelial growth factor and its receptor therefore interfering with the growth of blood vessels. Few studies have reported the efficacy of bevacizumab as therapy for CIN. In one study of 6 eyes, topical bevacizumab (5 mg/ml) was given 4 times per day for 8 weeks. Of those 6 eyes, two patients (34%) had complete CIN resolution while 4 (66%) had partial resolution and underwent excisional biopsy. No recurrences were noted after 6 months of follow up. Studies on the efficacy of anti-VEGF therapy for the treatment of CIN are limited, therefore their role remains uncertain.

Surgical Management

Incisional biopsy is indicated for larger tumors, typically occupying greater than four clock hours on conjunctiva. This technique describes a procedure in which part of the tumor is removed followed by therapy depending on the results of the biopsy.

Excisional biopsy is reserved for smaller tumors that occupy less than four clock hours of the conjunctiva, symptomatic lesions, or lesion suspected of being malignant. This technique is indicated for smaller tumors in order to avoid inadvertent tumor seeding. Tumor seeding occurs when cancer cells are spread to adjacent tissues along the course of a biopsy needle.

Most primary malignant tumors of the conjunctiva arise in the interpalpebral area at the limbus and require a different surgical technique compared to tumors arising in the fornices. Limbal neoplasms have the ability to invade through the corneal epithelium and sclera and into the anterior chamber, as well as through the soft tissue structures. Thus, it is often necessary to remove a thin piece of the sclera to achieve tumor free margins and to decrease the chance of tumor recurrence. A “no touch” technique is also employed in order to reduce the risk of tumor seeding. After excision of the tumor, cryotherapy is typically applied to the remaining margins of the bulbar conjunctiva tissue.

Cryotherapy is typically used as supplemental therapy to excisional biopsy and is performed following removal of the tumor. This is performed by freezing the surrounding bulbar conjunctiva as it is lifted away from the sclera using the cryoprobe. Cryotherapy reduces the risk of recurrence by eliminating microscopic tumor cells. Disadvantages of cryotherapy include chemosis that may persist beyond one week, and rarely cataract, uveitis, corneal thinning and phthisis bulbi.

Prognosis

 Conjunctival intraepithelial neoplasia has been widely known to recur, with choice of initial treatment being a risk factor for recurrence. Traditionally, surgical intervention along with cryotherapy has been the treatment of choice. However, over the years, surgical intervention alone has been proven to be inadequate for the prevention of recurrences as residual tumor cells are frequently left behind after surgical removal leading to proliferation and possibility of tumor formation. The status of the margins in tumors has been shown to influence risk of recurrence, with tumors with positive margins having a much higher risk of recurrence when compared to tumors with negative margins. One study by Tabin et al found a 39% recurrence rate in surgically removed lesions with negative margins, whereas lesions with positive margins had a 56% recurrence rate. Most studies show a low rate of recurrence when lesions are treated with topical therapy alone, however the regimen of choice is typically topical therapy in combination with surgical intervention. Details on the recurrence rates of the various topical agents have been further discussed in previous sections. Although rare, CIN has the ability to invade the orbit, sinuses, and the globe. In a study by Shields et al, size and area of involvement were observed in CIN and compared to SCC. This study revealed SCC with a greater diffuse diameter when compared to CIN (8% vs 1%), and SCC involves a larger median basal diameter (8 mm vs 7 mm) and thickness (2 mm vs 1 mm) when compared to CIN. Annual monitoring of these patients is critical given the tumor’s malignant potential.

Conclusion

 Conjunctival intraepithelial neoplasia can have a variety of presentations mimicking other common benign ocular conditions and should be differentiated from these conditions. Fortunately, CIN is a neoplastic disorder that responds well to topical chemotherapeutic agents and surgical intervention, with more recent studies trending towards topical therapy. Common topical agents include interferon alpha-2b, 5-FU, and mitomycin C. Given the malignant potential of CIN, patients should be monitored closely.

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