The past decade has seen a paradigm shift in the understanding of diagnosis and management as well the pathogenesis of ocular surface squamous neoplasia (OSSN). OSSN was a term proposed by Lee and Hirst\(^1\) to incorporate three broad categories:

I. Benign dysplasia: Papilloma, Pseudotheliomatous hyperplasia, Benign hereditary intraepithelial dyskeratosis

II. Preinvasive OSSN: Conjunctival/corneal carcinoma in situ

III. Invasive OSSN: Squamous carcinoma, mucoepidermoid carcinoma

**Epidemiology: Incidence, Racial & Geographic Distribution**

Various authors have cited an incidence ranging from 0.13 to 1.9 /100,000.\(^2\) The tumour accounts for about 14% (4-29%) of all oculo-orbital tumours. It is the third most common ocular tumor of the elderly, after melanoma and lymphoma.

OSSN primarily occurs in older males (78.5%). It is predominantly seen in dark skinned Caucasians, the age of onset being significantly higher in latitudes closer to the equator than 30 degrees. The average age of occurrence has been noted to be 60 years, range 20 to 88 years.\(^3\) Most studies have shown that the average age of incidence of carcinoma in situ lesions is 5-9 years lower than invasive OSSN. This difference may represent the time taken for progression from intraepithelial neoplasm to invasive carcinoma. Patients of xeroderma pigmentosum develop OSSN at a younger age. Young patients of HIV are more prone to develop aggressive OSSN.
Etiopathogenesis

1. Limbal transition zone/stem cell theory

OSSN may represent the abnormal maturation of corneal and conjunctival epithelium as a result of a combination of factors such as UV-B irradiation and HPV. Other risk factors include dust, wind, traumatic lid closure, chemical exposure like trifluridine, beryllium, arsenicals, petroleum products, cigarette smoke, vitamin A deficiency, and viruses like HSV type I.

2. Ultraviolet-B light

UV-B light is known to cause DNA damage and formation of pyrimidine dimers. Failure or delay in repair can lead to neoplasia as in xeroderma pigmentosum. Also major risk factors like pale skin, pale iris, sunburn, sun exposure, and actinic solar keratosis can be attributed to UV-B irradiation. UV-B has also been shown to cause p53 gene mutation, which is associated with OSSN. Histological evidence of solar injury, which is recognised as a major risk factor for conjunctival OSSN, has been reported in 50-100% cases of OSSN.

3. Human Papilloma Virus

HPV genotypes 6 and 11 have been demonstrated in a large number of papillomas as well as dysplastic and malignant lesions of the cornea and conjunctiva. Scott et al demonstrated HPV 16 or 18 DNA and mRNA in CIN cases proving a causal relationship. It has been demonstrated that the protein coded by the E6 region of HPV 16 and 18 forms a complex with the protein coded by the p53 tumor suppressor gene in the host. It is likely thus, that the HPV does not act alone, but in conjunction with cofactors like UV radiation etc.

Clinical Features

OSSN lesions mostly are slightly elevated, and have a pearly grey appearance with tufts of vessels commonly known as sentinel vessels (Fig 1), with or without well defined borders. They usually straddle the limbus in the interpalpebral area but may be limited to the conjunctiva and less commonly, the cornea.

MORPHOLOGICAL CLASSIFICATION

1. Gelatinous
2. Leucoplakic
3. Pappiliform

Circumscribed gelatinous lesions are the most common, the other two variants being nodular (Fig 2a) and diffuse (Fig 2b). The nodular type is fast growing with a propensity to metastasize to adjacent lymph nodes. The diffuse is the least common and in the early stages masquerades as persistent redness of the conjunctiva without associated papillae or follicles. These are slow growing, mimic chronic conjunctivitis and tumefaction occurs in the late stages only. Although it is difficult to differentiate between benign and malignant lesions clinically benign OSSN-pappilomatous type typically are exophytic, strawberry like with a stippled red appearance corresponding to its fibrovascular core.

Corneal OSSN lesions typically are preinvasive, with an opalescent mottled ground glass sheet appearance. They have sharply defined fimbriated borders, are avascular, the convex leading edge spreads in an arc away from the limbus, and often white dots are present over the grey epithelium.

The proposed etiology of these lesions varies from a denovo dysplasia to centripetal sliding of limbal dysplastic cells. These lesions are typically indolent, slow growing and remarkably prone to recurrence. OSSN typically presents as a growth on the ocular surface and gives rise to symptoms like foreign body sensation, redness or irritation and rarely, diminution of vision.

Differential Diagnosis

The differential diagnosis of OSSN includes
pannus, actinic disease, vitamin A deficiency, benign intraepithelial dyskeratosis, pinguecula, pyogenic granuloma, keratoacanthoma, pseudoepitheliomatous hyperplasia, malignant melanoma and nevi.

**Preoperative Diagnostic Tests**

**Exfoliative and Impression Cytology**

Exfoliative cytology using a cytobrush is particularly suited as malignant cells have poor cell to cell adherence and tend to desquamate when located on the mucosal surface. Impression cytology using cellulose acetate paper (CAP) is as simple and inexpensive as exfoliative cytology with the added advantage of maintained cell-to-cell relationship. However, CAP specimens require immediate processing. Biopore membrane has better cell adherence and can be stored for subsequent analysis making it the procedure of choice. Within the intraepithelial group, keratinized dysplastic cells, often accompanied by hyperkeratosis, syncytial-like groupings, and nonkeratinized dysplastic cells are seen. Within the invasive group, cases with significant keratinization and an additional group of cases with little keratinization and sometimes also prominent macronucleoli are described. Keratinized cases are the most numerous in both the intraepithelial and invasive groups. Cytological features that reliably differentiate carcinoma in situ (CIS) from invasive carcinoma are yet to be identified. It may also be used to monitor regression of lesion and response of the lesion to chemotherapeutic modulators.

Several patients may have histological CIN or partial thickness epithelial atypia adjacent to the invasive disease, which would not necessarily yield sheets of atypical cells if sampled by impression cytology. Endophytic lesions and orbital invasion cannot be identified with impression cytology, limiting its use as a diagnostic aid.

**Histopathology**

Papillomas demonstrate papillary fibrovascular fronds covered by acanthotic epithelium. This epithelium may show varying degrees of dysplasia, however, the cells have normal polarity and the basal layers are often unremarkable.

Preinvasive OSSN are classified as mild, moderate or severe depending on the degree of involvement of the dysplastic epithelium (Fig 3).

(i) Mild- CIN grade I: dysplasia confined to lower third of the epithelium (Fig 3a).

(ii) Moderate- CIN grade II: dysplasia extends into the middle third.

(iii) Severe- CIN grade III: full thickness dysplasia, also called carcinoma-in-situ (Fig 3b).

Invasive OSSN show nests of infiltrating cells that have penetrated the epithelial basement membrane and spread into the conjunctival stroma. These cells can either be well differentiated (Fig 3d) and easily recognized as squamous, or poorly differentiated and difficult to distinguish (Fig 3c). The latter are more uncommon and more aggressive. Two types of cells may be seen interspersed with squamous cells in these tumours: spindle cells and mucoepidermoid cells.

**Electron Microscopy**: reveals excessive mitochondria, tonofilaments and endoplasmic reticulum; decreased desmosomes, alteration/absence of basement membrane and deposition of fibrillogranular material between the basement membrane and bowmans layer.

**Therapeutic modalities**

**Surgery**

Surgical treatment has traditionally been the treatment of choice as a tissue diagnosis is essential before initiation of adjunctive therapy. Superficial excision remains the important initial
step in management as it is impossible to exclude invasive disease on clinical grounds or with impression cytology. Excision allows an immediate histopathological diagnosis, surgical debulking, and excludes life threatening invasive carcinoma. The disadvantage of primary excision alone is the high recurrence rate which ranges from 15% to 52%. Dissection of all abnormal tissue with a wide surgical margin of 4-5mm, with or without delineation with rose Bengal staining, is usually sufficient. Conjunctival defect so created can be closed primarily (if less than three clock hours in diameter). Larger defects require tissue replacement transpositional conjunctival flaps, free conj flaps from the other eye, or amniotic membrane grafts. Lamellar techniques may be indicated in lesions with deep invasion. Frozen section can be used to assess the adequacy of excision, and is accurate in delineating horizontal tumor spread. Bunn’s modification of Moh’s technique of tumor margin surveillance may also be used. In this the free conjunctival edges are excised by 2mm if residual tumor is evident even after excision of a 2mm surgical margin. Enucleation, and rarely exenteration may be required in cases of intraocular or intraorbital spread. In all cases a no touch technique is used, and direct manipulation of the tumour is avoided to prevent tumour seeding.

Cryotherapy

This modality is often used in conjunction with surgery. It acts by directly destroying the tumor cells by thermal effect as well as by obliterating the microcirculation causing ischemic necrosis. Intraoperative cryotherapy is commonly used as adjunctive therapy as it is known to decrease the recurrence rate by destruction of any residual tumour tissue beyond the horizontal or deep surgical margin of the wound. It has the advantage of reaching both tumor cell islands and deeply infiltrated cells, thus obviating the need for radical surgery. A nitrous oxide cryoprobe tip (2.5 or 5mm) is used to form an iceball extending 2mm for conjunctiva, 1mm for episcleral tissue and 0.5mm for the cornea. A slow duration freeze with a slow thaw, repeated two or three times (freeze-thaw-refreeze) is recommended. It is important to include the limbal region during cryotherapy, and not apply the cryoprobe for more than three seconds. Both extensive surgical excision and cryotherapy can cause limbal stem cell insufficiency, requiring limbal autotransplantation.

Radiotherapy

Various sources such as strontium-90 (beta irradiation) and radium (gamma radiation) were used earlier. But given the high incidence of side effects and prolonged duration of treatment required means it is rarely used.

Chemotherapy

Topical chemotherapy is inexpensive, simple and reduces the risk of limbal stem cell deficiency, and obviates the need for clear tumor margins by treating the entire ocular surface, including the potentially dysplastic cells. However, the obvious limitation is the limited drug penetration in larger tumors, and a possibly deleterious effect on the nasopharyngeal epithelium on prolonged use.

1. Mitomycin C: It is an antitumour antibiotic that preferentially inhibits DNA synthesis in the G1 and S phases. It leads to generation of alkylating species or redox cycling that produces active oxygen species leading to DNA damage. As the hypoxia required for the intracellular reduction of MMC is greater in tumour tissue, it exhibits a certain degree of selectivity. MMC appears to produce cell death in OSSN by apoptosis and necrosis. Cellular changes related to MMC mimic those caused by radiation-cytomegaly, nucleomegaly and vacuolation. MMC related changes may persist in ocular surface epithelium for at least 8 months following MMC therapy. It is used in the concentration of 0.02-
0.04% four times a day with one week on and one week off in alternating cycles for a maximum of 8 weeks. The one week on, one week off regimen prevents damage to more slowly dividing epithelial cells and limbal stem cells, allowing them to repair their DNA. Allowing time for complete epithelial healing before application of MMC is important in avoiding the more serious complications such as corneal epitheliopathy, scleral ulceration, uveitis, cataract, and glaucoma.

2. 5 Fluorouracil: It is an antimetabolite that acts specifically during the S phase of the cell cycle. It is converted to 5-F DUMP, which inhibits thymydilate kinase thus preventing DNA and RNA synthesis. Both MMC and 5FU are currently being used four times daily for 1-2 weeks in a pulsed fashion, the treatment being repeated after every 1-2 weeks. This one week –on and one week off drug regimen has the added advantage of good efficacy and better tolerance.

Immunotherapy

Interferon alpha2b is a naturally occurring glycoprotein which binds to cell surface receptors affecting intracellular events resulting in anti tumor and anti viral properties. Its efficacy may be explained by this due to the oncogenic link between HPV and OSSN. It has been used for extensive, residual, recalcitrant, multifocal or diffuse lesions ;and for those that involve the visual axis where surgery is not the treatment of choice. Interferon alfa 2-b is an important treatment modality for recalcitrant OSSN, effective in both, primary tumors unresponsive to treatment, as well as recurrences. It is more toxic than MMC and usually takes a longer duration for complete resolution, so is not started as a first line of therapy and preserved for lesions non responsive to topical MMC. IFN-albha2b drops(1millionIU/ml) is used four times a day until resolution, and a month thereafter. Subconjunctival injections have also been used. Median time for resolution has been reported as 54 days (range 28-188 days), with a mean follow up ranging from 2.9 to 18 months.13-15

Recurrence

Recurrence rates of OSSN ranges from 15-52%, average reported being 30%. Recurrences are higher in case of inadequate excision margins, and occur usually within two years of surgery. These typically exhibit a more aggressive behavior because of the tissue disruption associated with the primary excision theoretically enhancing the ability of the tumor cells to enter the eye. The main predictors for recurrence include age, histological grade of the lesion, adequacy of margins at initial excision , corneal location , larger size (> 2 mm), and a high proliferation index ( Immunostaining with antibody to Ki-67, which is a nuclear antigen expressed in proliferating cells, allows evaluation of the growth fraction of normal and neoplastic cells yielding the proliferation index).16

Fig 1: Sentinel Vessels

Fig 2 a
Recommended therapeutic strategy and current therapeutic practice

The recent advances and the current status of the diagnostic modalities and management of squamous neoplasms have been reviewed by Basti et al and have made the following recommendations.

Although the clinical diagnosis of in situ disease is high (86%), invasive carcinoma is much less often recognised (35%). Larger lesions and those with hyperkeratosis are more likely to be correctly diagnosed preoperatively. Impression cytology does not reliably distinguish in situ from minimally invasive disease, and therefore has limitations in the accurate diagnosis of OSSN.

1. Suspected OSSN 1-3 clock hours

Complete excision biopsy

a. If margins show residual tumor, adjunctive chemotherapy with MMC Monthly, with CIC repeated quarterly to evaluate tumor resolution.

Thereafter, follow up every six months

b. If tumor margins free of tumor, quarterly follow up for a year to confirm absence of recurrences; thereafter follow up every six months.

2. Suspected OSSN 3-6 clock hours

Biopsy to evaluate whether invasive or preinvasive

a. Preinvasive: start chemotherapy

Monthly follow up, with CIC repeated quarterly to evaluate tumor resolution. If complete resolution, follow up every six months

b. Invasive: start topical chemotherapy to achieve chemoreduction

Surgical excision of any residual tumor, cryotherapy to bed. Cover bed with amniotic membrane graft. Monthly follow up, with CIC repeated quarterly to confirm absence of tumor recurrence. Thereafter, follow up every three months.

3. Suspected OSSN >6 clock hours

Biopsy to decide evaluate whether invasive or preinvasive

a. Preinvasive: start chemotherapy

Monthly follow up, with CIC repeated quarterly to evaluate tumor resolution. If complete resolution, follow up every six months

b. Invasive: high dose chemotherapy with MMC

i) If complete resolution, monthly follow up for a year, quarterly thereafter.

ii) Partial resolution, chemoreduction achieved, surgical excision of any residual tumor, cryotherapy to bed. Cover bed with amniotic membrane graft.

Monthly follow up, with CIC repeated quarterly to confirm absence of tumor recurrence. Thereafter, follow up every three months

iii) If >6 clock hours inspite of chemotherapy, palliative treatment with radiotherapy.

A majority of ophthalmic surgeons (54%) believe that sufficient evidence exists to justify the use of mitomycin C in the treatment of OSSN, and fewer feel that the published literature justified the use of 5-fluorouracil or interferon (11% and 21%, respectively). About one-half of ophthalmic surgeons always perform a biopsy before institution of topical therapy. The reported use of topical chemotherapy as an adjunct to surgical excision increases with the size of the lesion; 45% of the respondents utilize topical therapy along with surgery for lesions greater than 8 mm in diameter.18

Squamous lesions of the cornea and conjunctiva are uncommon but demand appropriate attention due to the potential for visual loss and systemic morbidity and mortality. Further refinements of modern therapeutic options will allow cell
specific anti-cancer treatment of these lesions with preservation of the limbal stem cells and ocular surface.

References


