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# Outcomes of the ocular surface squamous neoplasia following primary excision with narrow margins and cryotherapy

**Demet YOLCU**
Department of Ophthalmology, University of Health Sciences, Abdurrahman Yurtaslan Oncology Training and Research Hospital, Ankara, Türkiye

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**Abstract**

The aim of the current study was to evaluate the outcomes of the surgical excision of the ocular surface squamous neoplasia (OSSN) with 2 mm margins and adjuvant cryotherapy.The medical records of the subjects who were diagnosed with OSSN and treated with surgical excision with 2 mm margins and adjuvant cryotherapy at the University of Health Sciences, Abdurrahman Yurtaslan Oncology Training and Research Hospital, Department of Ophthalmology, between May 2016 and April 2022 were analyzed. According to the margin status, subjects were divided into two groups (margin positive/indeterminate or negative), and, recurrence rate, treatment methods and complications were analyzed. Out of the 26 OSSN cases, 12 were margin positive/indeterminate and 14 were negative, and, 7 showed recurrence. Of the 14 margin negative cases, 4 showed recurrences, and, of the 12 margin positive/indeterminate cases 3 showed recurrence. Of the 12 margin positive/indeterminate cases, 9 received adjuvant treatment and 1 showed recurrence. Treatments were performed by either INF alfa 2-b or recurrent cryotherapy. There was no significant difference in histopathological diagnosis, follow up period or recurrence rate between the groups ($p > 0.05$). Complications were mild and slightly higher in cases who were treated with recurrent cryotherapy when compared with INF alfa 2-b.Adjuvant therapies reduced the recurrence rate in margin positive/indeterminate cases after excision of the OSSN with narrow margins, and margin negative cases had similar recurrence rate with margin positive/indeterminate cases. After removal of the OSSN with narrow margins, subjects should receive adjuvant therapies if the surgical margins are not negative, and, if the surgical margins were negative, then subject should be monitored closely because of the high recurrence risk of this group.

**Keywords:** ocular surface squamous neoplasia, cryotherapy, INF alfa-2b, margin status

## 1. Introduction

Ocular surface squamous neoplasia (OSSN) describes the spectrum of neoplastic squamous epithelial abnormalities of the conjunctiva or cornea, including squamous dysplasia, squamous cell carcinoma in situ and invasive squamous cell carcinoma (SCC) with extension beyond the basement membrane. It represents the most common non-pigmented tumor of the ocular surface (1, 2). The traditional treatment method of the OSSN is surgical excision with 4 mm (wide margins) tumor free margins and adjuvant cryotherapy. However, in the literature, the recurrence rete of the OSSN has been reported up to 33% despite wide margin (4 mm) excisions were performed with negative margins. Hence, in an effort to reduce the recurrence rate following the OSSN excision surgery, adjuvant topical medical treatments (such as INF alfa 2b, 5-FU, MMC) or cryotherapy were used to clear microscopic disease beyond the edge of the margins (3-5). However, in the literature, despite margin status has been stated as a critical issue for OSSN recurrence and excisions were traditionally made with wide margin excisions; narrow margin excisions have also been reported to reduce the conjunctival defects which require an amniotic membrane (6-8).

Because the surgical margin status, margin size, and adjuvant therapies have been reported as the critical factors for the recurrence rate of the surgical excision of the OSSN, current study was planned to achieve more evidence on the recurrence rate and efficacy of the adjuvant treatments on cases who had undergone OSSN excision with 2 mm margins and adjuvant cryotherapy.

## 2. Materials and Methods

The medical records of subjects who diagnosed with OSSN by slit-lamp evaluation and underwent OSSN excision surgery with 2 mm margins and adjuvant cryotherapy between May 2016 and January 2023 were evaluated. Written informed consent was obtained from all patients before the surgery and the study was adhered to tenets of the Declaration of Helsinki. Ethical board approval was obtained from University of the Health and Sciences, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital (2022-08/144). All surgeries were performed by one surgeon (DY). From the records; age, sex, histopathological diagnosis, tissues involved (conjunctiva/cornea), location on the ocular surface, growth type, margin status, treatments and complications during the follow-up period were evaluated. Cases were excluded if the lesion was primarily treated with topical chemotherapeutics without surgical excision, other chemotherapeutics were used for

*Correspondence: drylcdemet@hotmail.com

treatment instead of the INF alfa-2b or cryotherapy, surgical excision was made with 4 mm margins or initial excision was done elsewhere. The lesion was classified according to the American Joint Committee on Cancer classification system (AJCC) (8th addition) and subjects were divided into two groups according to the margin status to compare the surgical outcomes.

### 2.1. Surgical approach

All surgeries were performed by one surgeon. Prior to the surgery, the borders of the thickened conjunctival epithelium and amount of the corneal involvement was identified and the border of the lesion was marked with 2 mm margins by using a fine tipped bipolar electrocautery. Surgical excision for OSSN was performed by using the Shields 'no-touch' technique. Cryotherapy was performed in a double freeze-slow thaw method on conjunctival edges. For tumors with corneal components, corneal epitheliectomy was performed by applying absolute alcohol and lesion was excised in one piece. Special attention was paid to manipulate only at the marked margins and keep the surgical field dry to prevent potentially seeding of tumor cells. Conjunctival closure was performed by primary or with amniotic membrane replacement. The excised tissue was placed on a filter paper and marked carefully and sent to pathological evaluation. After histopathological diagnosis, cases were divided according to the margin status and if the margin was indetermined/positive then adjuvant treatment (either INF alfa-2b or recurrent cryotherapy) was performed in majority of the cases.

During the follow-up period, recurrent cases were treated by either topical INF alfa-2b or recurrent cryotherapy and complications were evaluated. Complete response was defined as 100% reduction of the visible tumor on the biomicroscope.

### Adjuvant INF alfa-2b treatment

Interferon alfa 2b (Intron A, Schering Corporation) was used in a topical formulation, which was performed by 1 million IU/mL (by reconstruction of 1 mL of interferon alfa-2b, 10 million IU/mL, with 9 mL of distilled sterile water and stored in refrigeration) eye drops, was administered 4 times daily and for 2 months in margin positive/indeterminate cases for adjuvant treatment, and, until at least complete clinical resolution was achieved in recurrent cases.

### Recurrent cryotherapy

A second course of freeze and thaw cryotherapy was performed to the conjunctival edges, and, in some cases, to the limbus and cornea. Additional margin or recurrent suspected lesion excision (with no touch technique) was also performed.

### 2.2. Statistical methods

The SPSS program version 22.0 (SPSS Inc, Chicago, IL) was used for the analyses and $P<0.05$ was considered as statistically significant. Chi square and student-t tests were used to compare the results.

## 3. Results

In the current study, 26 cases met the inclusion criteria. The mean age was 57 ± 22.3 (23-81) years and mean follow-up time was 34 ± 13 (range 9-70) months. Out of 26 OSSN cases, 15 had nodular mass, 14 had leukoplakic lesion, 11 had gelatinous lesion,18 had dysplasia (mild or moderate), 4 were CIN and 4 were SCC (Table 1). Out of 26 cases, 24 underwent primary closure of the conjunctiva with some bare sclera, and, 2 required amniotic membrane. Conjunctival margins were positive/indeterminate in 12 cases and negative in 14 cases. Out of 12 margin positive/indeterminate cases, 4 underwent adjuvant recurrent cryotherapy, 5 were treated with adjuvant INF alfa-2b and 3 were observed. Of the 12 margin positive/indeterminate cases, 3 showed recurrence. Out of 9 cases who were margin positive/indeterminate and treated with adjuvant therapies, 1 showed recurrence and underwent recurrent cryotherapy, out of 3 cases who were margin positive/indeterminate but were not received adjuvant therapy, 2 showed recurrence and treated with topical INF alfa 2b therapy. Out of 14 margin negative cases, 4 showed recurrence and 1 treated with recurrent cryotherapy and 3 with INF alfa-2b therapy (Table 1). Out of 26 cases, 5 underwent recurrent cryotherapy and 8 received topical INF alfa-2b treatment (Fig.1). Out of 26 cases, 7 showed recurrence during the follow-up period and 1 was SCC and others were mild/moderate dysplasia. There was no statistically significant difference in age, gender, tumor location, histopathological diagnosis, AJCC classification, margin status, corneal involvement, growth type and recurrence rate between the margin negative and positive/indeterminate groups ($p > 0.05$) (Table 1).

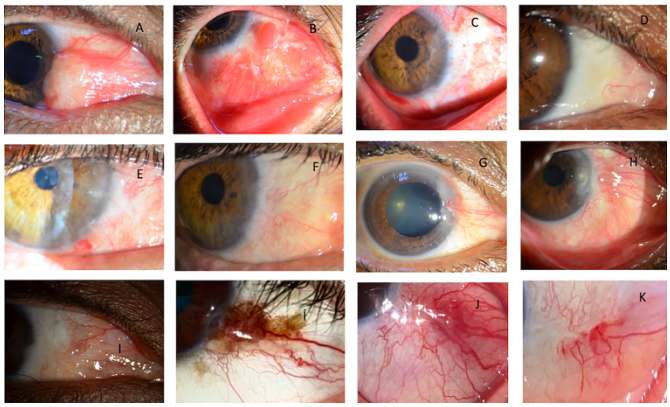


**Fig. 1. (**A) Anterior segment photography of the nasal nodular SCC with corneal involvement. (B) The appearance of the lesion after removal with 2 mm margins and subject was given adjuvant treatment because of the indeterminate margin status. (C) Complete healing of the operation field with topical adjuvant INF alfa 2b treatment (D) No recurrence was detected during the 70 months follow up period. (E) Anterior segment photography of an OSSN with diffuse corneal recurrence following excision with 2 mm at 8th month visit. (F) Appearance of the complete resolution of the OSSN following topical INF alfa 2b treatment for 3 moths. (G) Anterior segment photography of a nasally located leukoplakic OSSN. (H) The appearance of the case after 2 mm margin excision and direct closure of the conjunctiva. Because the positive margin status, a second course of cryotherapy and margin excision was performed. (I) The appearance of the case at the 1th month after second course of the cryotherapy. (İ) Anterior

segment photography of the temporally located hyperpigmented OSSN involving the cornea. (J) Appearance of the lesion after 2 mm margin excision and because indeterminate margin status, a second course of the cryotherapy was performed (K) The appearance of the case at the 2th month visit

**Table 1.** Demographic and clinical properties of the OSSN lesion according to the margin status

| | Margin negative | Margin positive/ indeterminate | p |
|---|---|---|---|
| **Number of patients** | 14 | 12 | 0.34 |
| **Age (years), mean (SD)** | 53 ± 42.3 (23-54) | 49 ± 42.3 (31-64) | 0.65 |
| **Gender, male n (%)** | 7 | 5 | 0.31 |
| **Properties of the lesion** | | | |
| **Nasal Location*** | 9 | 8 | 0.81 |
| **Corneal involvement** | 8 | 9 | 0.85 |
| **Leukoplakia, n** | 6 | 4 | 0.43 |
| **Nodular mass** | 7 | 5 | 0.32 |
| **Gelatinous mass** | 7 | 7 | 1 |
| **Recurrence rate** | 4 | 3 | 0.39 |
| **Adjuvant cryotherapy** | 1 | 4 | 0.09 |
| **Adjuvant INF alfa-2b** | 3 | 5 | 0.12 |
| **Follow-up time** | 22 ± 13 (9-56) m | 32 ± 33 (23-70) m | 0.42 |
| **Histopathological and AJCC classification of the OSSN** | | | |
| **Dysplasia (mild-moderate)** | 10 | 8 | 0.32 |
| **CIN** | 2 | 2 | 1 |
| **SCC** | 2 | 2 | 1 |
| **T1 (AJCC)**** | 8 | 6 | 0.34 |
| **T2 (AJCC)**** | 6 | 3 | 0.08 |
| **T3 (AJCC)**** | 1 | 2 | 0.23 |

n: number of individuals in group; INF alfa-2b: Interferon alfa-2b, OSSN: ocular surface squamous neoplasia; CIS: conjunctival intraepithelial neoplasia, SCC: squamous cell carcinoma, AJCC: American Joint Committee on Cancer clinical stage; T: tumor *Tumors could involve more than one quadrant and only horizontal orientation is recorded **The classification according to the AJCC.

Complication rates of the adjuvant treatments were presented on table 2 and there was no significant difference among the two adjuvant treatment modalities. Pain, irritation, redness, pyogenic granuloma or symblepharon incidence was higher following the adjuvant cryotherapy when compared with INF alfa-2b. Flue like symptoms were observed in 2 cases following INF alfa 2-b treatment and were well tolerated. Severe complications such as limbal stem cell deficiency or scleral melting were not observed during the follow-up period.

**Table 2.** Treatment complications in patients with OSSN treated by adjuvant cryotherapy or INF alfa-2b

| | Margin excision + cryotherapy n:5 | Topical INF alfa-2b n:8 | P |
|---|---|---|---|
| **Pain, n, %** | 2 | - | 0.09 |
| **Irritation, n, %** | 3 | 1 | 0.07 |
| **Redness** | 2 | 1 | 0.14 |
| **Pyogenic granuloma** | 2 | - | 0.09 |
| **Flue like symptoms** | - | 2 | 0.07 |
| **Limbal stem cell deficiency** | - | - | - |
| **Symblepharon** | 1 | - | 0.21 |

## 4. Discussion

Current paper directly compares the recurrence rate of the OSSN removal surgery between margin negative and positive/indeterminate cases who had undergone OSSN excision surgery with 2 mm margins and intraoperative cryotherapy in order to conserve normal conjunctival tissues and, analyze the treatment modalities, in those cases. According to the current study, recurrence rate was similar in both margin positive/indeterminate and negative cases after excision of the OSSN with 2 mm margins and cryotherapy, because adjuvant topical INF alfa 2b or recurrent cryotherapy was performed to the margin positive/indeterminate cases to reduce the recurrence rate in those cases.

In the literature, the margin status and adjuvant therapy have been evaluated as the important factors for predicting the recurrence rate of the OSSN (3). In many studies, in an effort to obtain negative surgical margins, OSSN removal surgery was performed with 4 mm margins and often resulted with large conjunctival defect (9). However, despite the excision of the OSSN was performed with wide margins, the recurrence rate was reported up to 56% in pathologically margin positive cases who were not treated with adjuvant treatment following the surgery. In order to conserve normal conjunctival tissue, excision of the OSSN with 2 mm margins were also has been reported with similar risk of recurrence (4-19%) compared to wide margin excision (3)(10). In the literature, the recurrence rate of the OSSN according to the pathological margin status has also been evaluated, and, in a study, while much lower (1.5%) recurrence rate has been reported in margin negative cases when compared with margin positive cases (17%); in another study, the recurrence rate has been reported at the 18% of the cases, however, all recurrences were observed in margin negative cases (1). There are also some studies where the margin status had not been discussed (11). In the current study, the rate of the margin positive/indeterminate cases was 46% (12/26) and recurrence rate of margin negative cases was 28% (4/14) and positive/indeterminate was %25. In the light of the

information above, it can be stated that, the inconsistencies between the studies could be due to the extent of the thickened epithelium could not be identified carefully prior to surgery or conjunctival margins could not be evaluated properly in the pathological evaluation process and resulted with false margin positive results.

In recent studies, adjuvant treatments have been reported as one of the important factors reducing the recurrence rate following surgical excision of the OSSN. (12). In a study, evaluating excision with cryotherapy, as performed in the current study, has found recurrence rate 12% in cases who had negative margins. In another study, intraoperative cryotherapy and adjuvant topical INF alfa-2b has been reported with significantly reduced risk of recurrence in cases with positive margins (13). In the current study all excisions were made with cryotherapy, and, while the recurrence rate was 28% in margin negative cases; it was 11% in margin positive or indeterminate cases who received adjuvant therapy. In the end, current study supported the recent literature in terms of the usefulness of the adjuvant therapy following surgical removal of the OSSN especially in cases with positive margins.

In the literature, follow-up duration has also been stated as one of the important factors for the recurrence rate, and, a 1-year recurrence rate of 10% and a 5-year recurrence rate of 21% has been reported after surgical excision. In the current study, during the 34 ± 13 (range 9-70) months follow up period, 28% recurrence rate has been reported (1).

In the literature, the side effects of INF alfa-2b have been reported with significant improved patient acceptance over the other medical therapies with proven efficacy and was in line with the current study. However, cryotherapy has been reported with significant side effects such as; ptosis, cataract, symblepharon, uveitis, hypotony and scleral thinning, and, Shields stated that lifting off the conjunctiva from the globe could help decrease the chances of ocular complications (12). In the current study, despite cryotherapy was performed twice or more in some cases (involving the limbus or cornea), none of the significant complications were observed during the follow up period probably due to the proper handling and careful application of the instrument.

The limitations of the current study were; the retrospective nature of the study and the side effect data was obtained by examiner during the clinical visit, the pathological evaluations could not be performed by one individualized pathologist or borders of the lesion could not be analyzed by advanced devices, such as HR-OCT or UBM prior to the surgery to achieve more accurate margin status, and, the study sample was small to make more accurate comparisons.

In conclusion, according to the current study, recurrence use of the cryotherapy or topical INF alfa 2b treatment reduced the recurrence rate in margin positive/indeterminate cases after excision of the OSSN with narrow margins, and margin negative cases had similar recurrence rate with margin positive/indeterminate cases. After removal of the OSSN with narrow margins, subjects should receive adjuvant therapies if the surgical margins are not negative, and, if the surgical margins were negative, then subject should be monitored closely because of the high recurrence risk of this group.

**Conflict of interest**
The authors declared no conflict of interest.

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**Authors' contributions**
Concept: D.Y., Design: D.Y., Data Collection or Processing: D.Y., Analysis or Interpretation: D.Y., Literature Search: D.Y., Writing: D.Y.

**Ethical Statement**
Approval was obtained from Health Science University Dr. Abdurrahman Yurtaslan Noninvasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 25/08/2022 and the number of ethical committee decisions is 2022-08/144.

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