



ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

DEPARTMENT OF OPHTHALMOLOGY

PREVALENCE AND ASSOCIATED FACTORS OF OCULAR  
SURFACE SQUAMOUS NEOPLASIA AMONG PATIENTS  
PRESENTING WITH OCULAR SURFACE LESIONS AT SPHMMC  
IN ADDIS ABABA, ETHIOPIA

BY: BISRAT AMSALU (OPHTHALMOLOGY RESIDENT)

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# ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

## DEPARTMENT OF OPHTHALMOLOGY

Prevalence and associated factors of ocular surface squamous neoplasia among patients presenting with ocular surface lesions at SPHMMC in Addis Ababa, Ethiopia.

Investigator: Bisrat Amsalu, MD, ophthalmology resident

Advisors: 1. Dr. Tewodros Woretaw (consultant ophthalmologist, cornea specialist)

2. Mr. Dereje Hayilu (MSc, MPH)

3. Dr. Girum W/Gebriel (Consultant ophthalmologist, Glaucoma specialist)

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## Abstract

**Background:** Ocular Surface Squamous Neoplasia (OSSN) is the most common tumor of the ocular surface in adults, with a notably higher reported incidence in African populations. Ultraviolet (UV) radiation exposure and HIV infection are established major risk factors. Despite its clinical significance, data on the prevalence and associated factors of OSSN in Ethiopia remain scarce.

**Objective:** This study aimed to determine the prevalence and associated factors of OSSN among patients presenting with ocular surface lesions at the Ophthalmology Unit of St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

**Methods:** An institution-based cross-sectional study was conducted from February 28, 2024, to December 4, 2025. A total of 70 consecutive patients with excised ocular surface lesions were included. Data on sociodemographic, behavioral factors, and clinical features were collected via interviews and slit-lamp examination. Histopathological analysis of excised tissue confirmed the OSSN diagnosis. Data were analyzed using SPSS version 26, employing descriptive statistics, bivariate, and multivariate logistic regression.

**Results:** The overall prevalence of histologically confirmed OSSN among studied ocular surface lesions was 57.14% (40/70). Among OSSN cases, 77.5% (31/40) were invasive Squamous Cell Carcinoma (SCC) and 22.5% (9/40) were Conjunctival Intraepithelial Neoplasia (CIN). The mean age of participants was 44.17 years ( $\pm 15.42$ ). Multivariate analysis identified prolonged daily outdoor exposure ( $>6$  hours) as the strongest independent risk factor (Adjusted Odds Ratio [aOR]=11.84; 95% CI: 2.12-66.21;  $p=0.005$ ). Age 40-60 years was also a significant risk factor (aOR=4.23; 95% CI: 1.17-15.31;  $p=0.028$ ). Retroviral infection (RVI, predominantly HIV) status showed a strong association with OSSN (aOR=10.14; 95% CI: 1.00-102.50;  $p=0.050$ ). Higher educational attainment (college and above) demonstrated a protective trend (aOR=0.30; 95% CI: 0.08-1.08;  $p=0.065$ ). Clinically, leukoplakic appearance and the presence of feeding vessels were common features.

**Conclusion:** This study reveals a high prevalence of OSSN among patients with ocular surface lesions at a major Ethiopian tertiary center. Prolonged UV light exposure, middle age, and HIV infection are significant risk factors, while higher education may be protective. The findings

underscore the need for public health strategies promoting sun protection, targeted screening of high-risk groups and enhanced clinical suspicion for early diagnosis and management of OSSN in Ethiopia.

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## **Abbreviations and Acronyms**

5 FU: 5 fluorouracil

AIDS: Acquired immunodeficiency syndrome

ART: Anti-retroviral therapy

CD4: Clusters of differentiation 4

CIN: Conjunctival intraepithelial neoplasia

CIS: Carcinoma insitu

CT: Computed tomography

GC: Gregorian Calendar

HIC: High income countries

HIV: Human immune deficiency virus

HPV: Human papilloma virus

HR-OCT: High resolution optical coherence tomography

IVCM: In vivo confocal microscopy

LIC: Low income countries

MIC: Middle income countries

MMC: Mitomycin C

MRI: Magnetic resonance imaging

OSSN: Ocular surface squamous neoplasia

SCC: squamous carcinoma

SPHMMC: Saint Paul's Hospital Millennium medical college

SPSS: Statistical package for the social science

UV: Ultraviolet

# **1. Introduction**

## **1.1. Background**

Ocular surface squamous neoplasia (OSSN) is the most common tumor affecting the ocular surface in adults (1). OSSN was a term suggested by Lee and Hirst to describe all primary dysplastic and carcinomatous lesions that originate from the epithelium of the cornea or conjunctiva.(2) Globally, the estimated incidence of OSSN is less than 0.2 cases per million people annually.(3)

First proposed in 1995 as a distinct clinical entity (2), ocular surface squamous neoplasia (OSSN) is an umbrella term which includes a broad spectrum of conjunctival malignancies, ranging from mild epithelial dysplasia to invasive squamous carcinoma (SCC),(4) with an incidence that ranges from 0.03–1.9 per 100,000/year in the Caucasian population (2,5–7), to 3–3.4 per 100,000/year in African ethnicity populations.(8,9)

The primary risk factor for OSSN is ultraviolet (UV) B radiation, with people chronically exposed to direct solar light and those involved in outdoor occupations being at the most risk.(8) Non-modifiable risk factors include male gender and advancing age. Modifiable risk factors are cigarette smoking(2), vitamin A or retinol deficiency(10) , chronic trauma or inflammation(11), and exposure to petroleum products; more recently, the use of topical voriconazole has been proposed as a predisposing factor.(12,13) Infectious diseases such as human immunodeficiency virus types 1 and 2 (HIV-1 and -2)(14)(15), human papillomavirus (HPV) serotypes 16 and 18(16), and hepatitis B and C virus play a putative role in the pathogenesis of OSSN.(17)

Typically, OSSN presents as a vascularized limbal mass, located unilaterally in the interpalpebral fissure. However, although rare, it can also manifest as a bilateral or multifocal mass.(2) In even rarer cases, the tumor may involve the tarsal conjunctiva or be associated with pterygia or other benign conditions.(18) The most common signs and symptoms of OSSN include a red eye, ocular irritation, and the emergence of a new mass in the eye. In advanced stages, it can lead to necrotizing scleritis, characterized by severe pain and visual loss.(19,20)

The gold standard for the diagnosing OSSN remains histopathologic evaluation, which involves examining tissue samples obtained through incisional or excisional biopsy.(21) However, advancements in technology over the past three decades have introduced alternative methods that are less invasive or non-invasive for diagnosing OSSN. These methods include impression

cytology, in vivo confocal microscopy (IVCM),(15,22,23) and high-resolution or ultra-high-resolution anterior segment optical coherence tomography (HR-OCT).(14) Despite the advancements in non-invasive imaging techniques, histopathology remains crucial for the early identification of potential invasion of OSSN into the cornea, sclera, intraocular structures, or orbit.(24)

## **1.2. Statement of the Problem**

Ocular surface squamous neoplasia (OSSN) is the most common tumor affecting the ocular surface in adults. It encompasses a wide range of conjunctival malignancies, from mild epithelial dysplasia to invasive squamous carcinoma (SCC). The incidence of OSSN varies, with rates ranging from 0.03 to 1.9 per 100,000 per year in the Caucasian population(2,5,7) and 3 to 3.4 per 100,000 per year in African ethnic populations.(8,25)

Compared to other oculo-orbital tumors, ocular surface squamous neoplasia (OSSN) is relatively uncommon. Previous population surveys have indicated that the proportion of OSSN cases among all oculo-orbital tumors ranges from 4% to 29%, with an average of 14%. In the older population, OSSN ranks as the third most common ocular tumor, following melanoma and lymphoma. The average age of onset for OSSN is 56 years, with a wide age range spanning from 4 to 96 years old. Notably, there have been reports of OSSN occurring in young individuals, such as a 4-year-old Caucasian female who developed a conjunctival lesion after strabismus surgery and a 13-year-old African American female who was otherwise healthy. Furthermore, several studies have observed that patients with carcinoma in situ tend to be 5-9 years younger on average compared to those diagnosed with invasive squamous cell carcinoma.(2)

It is important to highlight that there are reports indicating that over 50% of individuals diagnosed with ocular surface squamous neoplasia (OSSN) in Sub-Saharan Africa, including Ethiopia, have underlying Human Immunodeficiency Virus (HIV) infection. Given the high prevalence of HIV in the sub-Saharan region, it becomes imperative to study OSSN in order to better comprehend its prevalence and associated factors within the Ethiopian context. Despite its frequent occurrence in clinical settings, the true prevalence of OSSN in Ethiopia is largely unknown.(26)

This research aims to address the lack of data on the prevalence of Ocular Surface Squamous Neoplasia (OSSN) in Ethiopia.

### **1.3. Rationale of the Study**

Studying Ocular Surface Squamous Neoplasia (OSSN) in Ethiopia holds significant scientific and public health importance. While this condition is frequently encountered in clinical settings, its true prevalence remains largely unknown, with only one study conducted at Gonder University Hospital in 2018 focusing on the clinical and histopathological characteristics of OSSN. The scarcity of research on this topic emphasizes the need for further investigation.

The objective of this study is to address the current gap in published literature regarding the prevalence, associated factors and histopathological characteristics of ocular surface squamous neoplasia (OSSN) in Ethiopia, particularly at SPHMMC. Although a report on the pattern of ophthalmic lesions at histopathology centers exists, there is a lack of specific information on OSSN in the country.

OSSN poses a serious threat to vision when not diagnosed early and treated promptly. Therefore, understanding the prevalence and characteristics of this condition in Ethiopia is crucial for effective management and prevention of visual impairment. By conducting a comprehensive study, valuable insights can be gained into the epidemiology, risk factors, and clinical manifestations of OSSN in the Ethiopian population.

Moreover, this study will serve as a vital baseline for future research endeavors. Establishing a foundation of data and knowledge through this research will enable subsequent studies to build upon and expand the understanding of OSSN in Ethiopia

## **2. Literature Review**

### **2.1. Prevalence of OSSN**

In a retrospective records review conducted in Harare, Zimbabwe, the period prevalence of ocular surface squamous neoplasia (OSSN) was studied by analyzing biopsies of ocular surface tumors performed between January 1996 and August 2000. The study found that the overall period prevalence of OSSN was 38.8%, with 615 cases out of a total of 1,587 cases. The annual prevalence of OSSN among ocular surface tumor biopsies showed an increasing trend, ranging from 33% (115 cases) in 1996 to 57.9% (165 cases) in the year 2000. (31)

In a retrospective study conducted in South Western Uganda, the records of patients diagnosed with ocular surface squamous neoplasia (OSSN) at the pathology laboratory were analyzed. The study included patient data from January 2015 to October 2021. Among the 1,133 cases of ocular neoplasia recorded during this period, OSSN accounted for 26.7% (n=303) of the cases. The majority of OSSN cases were observed in females, comprising 59.1% of the total cases. Invasive squamous cell carcinoma was the most common type of OSSN, accounting for 60.4% of the cases. The age group most frequently affected by OSSN was individuals between 30 and 44 years, representing 41.3% of the total OSSN cases.(32)

A clinicopathological evaluation was conducted on a series of clinically suspected ocular surface squamous neoplasia (OSSN) cases at Gondar University Hospital, a tertiary eye care and training center in Ethiopia, from January 2015 to June 2016. The study revealed that the mean age of the participants was 41.26 years, ranging from 18 to 75 years, with 72% of the cases being males. Histopathological examination confirmed ocular surface squamous neoplasia in 96% of the cases. Among these cases, 36% had carcinoma in situ, 36% had invasive squamous cell carcinoma, 18% had conjunctival/corneal intraepithelial neoplasia, and 10% had associated pterygium both clinically and histologically.(26)

### **1.2. Associated factors of OSSN**

A retrospective case-control study was conducted at the Miami Veterans Administration Hospital Eye Clinic involving 55 patients with biopsy-proven ocular surface squamous neoplasia (OSSN) who were treated between 2010 and 2018, and 55 age-matched controls. The results of the study indicated that the strongest risk factor for OSSN was lifetime sun exposure. The odds ratio (OR)

for lifetime sun exposure was 5.4 (95% confidence interval (CI) 2.27-12.847,  $p < 0.005$ ). Additionally, the presence of basal cell carcinoma (OR 3.157, 95% CI 1.286-7.748,  $p = 0.010$ ) and pingueculae (OR 5.267, 95% CI 2.104-13.186,  $p < 0.005$ ) served as surrogate markers for sun exposure and were associated with an increased risk of OSSN. (33)

A retrospective study of ocular surface squamous neoplasia (OSSN) with histopathologic confirmation was conducted at National Taiwan University Hospital between 1994 and 2014. Demographic characteristics, clinical features, tumor staging, pathological findings, lesion location, treatment modalities, and recurrence rates were evaluated. A total of 36 eyes from 36 patients were included. The mean age at diagnosis was  $63.4 \pm 13.0$  years (range, 23–87 years). The condition was more common in males than females, with a male-to-female ratio of 21:15. Nineteen of the 36 affected eyes were right eyes, and none of the patients were infected with human immunodeficiency virus (HIV). Among the 31 cases involving the conjunctiva or corneconjunctival region, the predominant clinical presentations were papilliform (17 eyes), gelatinous (11 eyes), and leukoplakic (3 eyes). The remaining five cases were confined to the cornea and were classified as corneal intraepithelial neoplasia. (36)

A systematic review was conducted to examine the epidemiology and propose an etiological model for ocular surface squamous neoplasia (OSSN) in Africa. PubMed, EMBASE, and Web of Science were searched for systematic reviews, meta-analyses, and case-control studies to identify relevant studies. The initial search was performed on January 2, 2013, and updated on May 31, 2013. The risk of developing OSSN increased with exposure to direct daylight, with odds ratios (ORs) of 1.7 (95% confidence interval [CI]: 1.2–2.4) for 2–4 hours of exposure and 1.8 (95% CI: 1.1–3.1) for 5 or more hours of exposure. Outdoor occupations were also associated with an increased risk of OSSN, with an OR of 1.7 (95% CI: 1.1–2.6). The study's meta-analysis demonstrated a strong association between OSSN and human immunodeficiency virus (HIV) infection, with an OR of 6.17 (95% CI: 4.83–7.89) based on six studies. Additionally, human papillomavirus (HPV) infection was also strongly associated with OSSN, with an OR of 2.64 (95% CI: 1.27–5.49) based on seven studies. The effects of atopy, xeroderma pigmentosa, and vitamin A deficiency on OSSN were found to be unclear in this study and recommended further investigation. (8)

In a prospective interventional study conducted between December 2019 and February 2022 at a tertiary eye hospital in Johannesburg, South Africa, a total of 130 cases and 45 controls were included. The median age of the participants was 44 years, with an equal distribution of gender among the cases. The study found that the prevalence of HIV among the cases was 74%, and there was a strong association between HIV and ocular surface squamous neoplasia (OSSN) ( $p < 0.001$ ). Clinical features such as vascularization, leukoplakia, and pigmentation were observed to be distinguishing factors between the cases and controls.(34)

In a case-control study conducted in Kampala, the odds of various factors were compared between 60 individuals with conjunctival squamous cell carcinoma and 1,214 controls with different cancer sites or types. The study took place between August 1994 and February 1998. Among the cases, 43% were men and 57% were women. The seroprevalence of anti-HIV-1 antibodies was significantly higher among cases compared to controls (OR 10.1, 95% CI 5.2-19.4,  $P < 0.001$ ). Individuals with a higher personal income had a significantly lower risk of conjunctival carcinoma (OR 0.4, 95% CI 0.2-0.7,  $P < 0.001$ ). The risk of conjunctival carcinoma increased significantly with increasing time spent cultivating per week (ORs 1.0, 1.9, and 2.4 for 0-9 h, 10-19 h, and 20+ h respectively,  $P = 0.03$ ). (35)

During a case-control study conducted in Kenya from July 2012 to July 2014, 131 cases and 131 controls were recruited, with a similar distribution of males and females. HIV infection without antiretroviral therapy (ART) use was strongly associated with OSSN, with an odds ratio (OR) of 48.42 and a 95% confidence interval (CI) ranging from 7.73 to 303.31. Additionally, HIV infection with ART use was also significantly associated with OSSN, with an OR of 19.16 and a 95% CI of 6.60 to 55.57. For each additional hour of sun exposure per day, the odds of developing OSSN increased, with an OR of 1.24 and a 95% CI of 1.10 to 1.40. A history of allergic conjunctivitis was strongly associated with OSSN, with an OR of 74.61 and a 95% CI of 8.08 to 688.91. On the other hand, wearing hats was found to be a protective factor against OSSN, with an OR of 0.22 and a 95% CI of 0.07 to 0.63.(10)

A clinicopathological evaluation was conducted on a series of clinically suspected ocular surface squamous neoplasia (OSSN) cases at Gondar University Hospital, a tertiary eye care and training center in Ethiopia, from January 2015 to June 2016. The study revealed that the mean age of the participants was 41.26 years, ranging from 18 to 75 years, with 72% of the cases being males. The

majority of the participants (40%) were farmers. Human immunodeficiency virus (HIV) was found to be positive in 22% of the participants. The most common clinical appearance of the lesions was leukoplakic (68%), and the nasal side of the conjunctiva was the most frequent location (72%). Feeding vessels were observed in 84% of the participants. (26)

### **3. Objectives**

#### **3.1 General Objectives:**

- To assess the prevalence and associated factors of OSSN among patients presenting with ocular surface lesions at SPHMMC Ophthalmology unit from February 28, 2024 – December 04, 2025 GC.

#### **3.2 Specific objectives:**

- To assess the prevalence OSSN among patients presenting with ocular surface lesions at SPHMMC ophthalmology unit
- To describe the clinical presentation and demographics of patients with OSSN
- To identify associated factors with OSSN
- To examine clinico- pathological correlation between lesion characteristics (size, appearance, pigmentation, vascularity) and OSSN subtypes

## **4. Methods and materials**

### **4.1. Study design and period**

An institution based cross-sectional study was done in a period between February 28, 2024 – December 04, 2025 GC.

### **4.2. Study setting**

The study was conducted in ophthalmology clinic of St. Paul's Hospital Millennium Medical college; Addis Ababa, Ethiopia. The Medical College was a prestigious institution with a rich history and a strong commitment to medical education, patient care, and research. It was founded in 1968 by Emperor Haile Selassie. Since its establishment, the college had evolved and grown significantly, culminating in its current form in 2010. The college is governed by a board under the Federal Ministry of Health. This ensures that it operates within the regulatory framework set by the government and aligns with national healthcare policies.

St. Paul's Hospital Millennium Medical College actively engages in basic and applied research. By conducting research, the college aims to advance medical knowledge, develop innovative healthcare practices, and improve patient outcomes. This research engagement enhances the overall quality of healthcare delivery and contributes to the scientific community's understanding of various medical conditions and treatments.

The Ophthalmology department at St. Paul's Hospital Millennium Medical College plays a crucial role in providing comprehensive eye care training to students from different regions of Ethiopia. This department focuses on training future ophthalmologists, ensuring that they acquire the necessary knowledge and skills to address the eye health needs of the population effectively.

By offering specialized training in ophthalmology, the college contributes to the development of a skilled workforce that can provide quality eye care services across the country. This is especially important considering the high prevalence of eye conditions and the need for accessible and affordable eye care services in Ethiopia.

### **4.3. Source population**

All patients with ocular surface lesions who visit the ophthalmology clinic of SPHMMC throughout the study period.

### **4.4. Study population**

All patients who undergone excisional surgery for the ocular surface lesions at SPHMMC during the study period

### **4.5. Inclusion and exclusion criteria**

#### **4.5.1. Inclusion criteria**

- The study involved patients who had undergone histopathological examination of excised ocular surface lesions at SPHMMC between February 28, 2024, and December 04, 2025 GC.
- Complete histopathological reports available in the hospital registry

#### **4.5.2. Exclusion criteria**

- Patients with neurological conditions that hindered study investigations (Cognitive impairment, Communication disorders)
- Patients with history of excisional biopsy performed at an outside facility, providing only the biopsy results

### **4.6. Sample size determination**

Single population proportion formula was used to calculate the estimated sample size with p (prevalence of OSSN extracted from other studies)

Single population proportion formula,

$$n = \frac{(Z_{\alpha/2})^2 p(1-p)}{d^2}$$

where n = sample size,

$Z_{\alpha/2}$  = prevalence at 95% confidence interval (1.96)

d = 5% margin of error (0.05)

Due to the absence of prior research with a similar study design that could accommodate a large sample size, we have considered a prevalence of 50% (0.5) as there is no available data to inform a more precise estimate.

$$n = \frac{(1.96)^2(0.5)(1-0.5)}{(0.05)^2}$$

Then putting all together, calculated sample (n) size was 384 and Considering 10% non-response rate the total sample size was 422.

Due to the limited number of previous cases of Ocular Surface Squamous Neoplasia (OSSN) observed at the ophthalmology clinic in the hospital, the study included all consecutive patients suspected with OSSN during the study period.

#### **4.8. Study variables**

##### **4.8.1. Dependent variables**

- Prevalence of OSSN among ocular surface lesions

##### **4.8.2. Independent variables**

##### **Sociodemographic variables**

- Age
- Sex
- Geographic location
- Family history of OSSN
- Race and ethnicity
- Occupation
- Income
- Educational level

##### **Behavioral, Environmental and OSSN related factors**

- Average time spent outdoors every day
- Use of tobacco products
- HIV and the use of ARVs
- Immunosuppression: such as organ transplant recipients or those with autoimmune diseases
- Previous ocular injury
- Previous or current chronic inflammatory ocular surface disease
- History of regular exposure to petroleum product

#### **4.9. Operational definition**

**OSSN:** A histopathologically confirmed spectrum of epithelial neoplastic lesions originating from the ocular surface, including the conjunctiva and cornea. This includes various subtypes such as conjunctival intraepithelial neoplasia (CIN), carcinoma in situ, and invasive squamous cell carcinoma (SCC) of the conjunctiva and cornea.

**Prevalence of OSSN:** The proportion of patients with histologically confirmed OSSN among all patients who underwent excisional biopsy of ocular surface lesions at SPHMMC during the study period.

**Conjunctival intraepithelial neoplasia (CIN):** Partial involvement of the corneal/conjunctival epithelial layer by neoplastic cells.

**Carcinoma insitu (CIS):** Full thickness involvement of corneal/ conjunctival epithelium by neoplastic cells.

**Invasive squamous cell carcinoma:** Corneal/conjunctival basement membrane invasion by neoplastic cells.

**Papillomatous OSSN:** refers to a benign form of Ocular Surface Squamous Neoplasia that manifests as a pink to cherry red, exophytic growth resembling a strawberry in appearance.

**Leukoplakic OSSN:** Preinvasive lesions, characterized by surface hyperkeratinization, which leads to thickening and a whitish appearance of the tumor. These lesions are in the early stages and have not yet invaded surrounding tissues.

**Gelatinous OSSN:** lesions that exhibit a well-defined and circumscribed nature, displaying a distinctive hairpin configuration of conjunctival vessels.

**Chronic inflammatory ocular surface disease:** Defined as a persistent inflammatory condition affecting the ocular surface, including the conjunctiva, cornea, and surrounding tissues, lasting for a duration of three months or longer. It is characterized by clinical signs such as redness, swelling, irritation, discomfort, and may be associated with biomarkers of inflammation.

**Ocular surgery:** Defined as any prior surgical procedure performed on the ocular surface or adjacent structures, excluding surgeries specifically targeting OSSN lesions. This includes surgeries such as pterygium excision, limbal stem cell transplantation, corneal transplantation, or excision of conjunctival lesions, which may potentially increase the risk of developing OSSN in the operated area or surrounding tissues.

**Average time spent outdoors every day:** was defined as self-reported hours per day spent on direct sunlight

#### **4.10. Data collection tool and techniques**

For the study, face-to-face interview was conducted with patients using a structured questionnaire that had been adapted with minor modifications from similar research studies. The questionnaire gathered demographic data, presenting history, and information on associated risk factors. The interviews took place in private rooms to ensure confidentiality. The interview primarily aimed to gather information on various risk factors associated with Ocular Surface Squamous Neoplasia (OSSN). These factors involved evaluating the patient's own historical information regarding their HIV status and the use of antiretroviral medications, investigating the presence of other systemic conditions or medications linked to immunosuppression, understanding the average daily time spent outdoors, examining the use of tobacco products, exploring any history of previous ocular injury, evaluating previous or current chronic inflammatory ocular surface diseases.

The data collection process took place one day prior to the surgery as part of the preoperative evaluation. The principal investigator or, in his absence, a senior resident at the cornea and oculoplasty clinic were responsible for conducting the data collection. To ensure continuity of communication and prevent loss of patients, their phone numbers were obtained when they were

sent to the pathology department. Additionally, communication was established with the pathology department to obtain biopsy results in case any patients were lost during the study.

A slit-lamp examination was conducted to assess the clinical features, dimensions, and morphology of the conjunctival mass. The dimensions of the mass were measured as the largest two perpendicular dimensions, and the surface area was calculated based on these measurements. The extent of involvement in terms of limbal clock hours was recorded. To determine the thickness of the mass, a comparison was made with the assumed thickness of the lid margin, which was considered to be 1.5 mm.

The histological examination of the biopsy samples determined the presence of CIN, squamous cell carcinoma in situ (CIS), or SCC. All cases were unilateral, and only one eye per patient was included in the analysis. This approach ensured that each participant contributed a single data point, thereby maintaining statistical independence and minimizing potential bias in the results.

#### **4.11. Quality control measures**

The questionnaire was prepared in English. A pretest was conducted on 5 % of the total sample size in a separate population to check for completeness. Additionally, data collectors were given training prior to actual data collection. Each clinical parameter was recorded using an interviewer-administered questionnaire conducted by the principal investigator and data collector residents in the ophthalmology clinic.

#### **4.12. Data analysis**

Data analysis was conducted using SPSS version 26. Descriptive statistics including frequencies, percentages, means, and standard deviations were calculated to summarize participant characteristics. The prevalence of OSSN was calculated as the proportion of histologically confirmed cases among all excised ocular surface lesions.

Bivariate analyses were performed using chi-square tests for categorical variables and independent t-tests for continuous variables to identify factors associated with OSSN. Crude odds ratios with

h 95% confidence intervals were calculated using univariate logistic regression. Variables with p-value < 0.2 in bivariate analysis were considered candidates for multivariate analysis.

Multivariate logistic regression using backward stepwise elimination ( $p < 0.10$  for retention) was employed to identify independent predictors of OSSN while controlling for potential confounders. Adjusted odds ratios with 95% confidence intervals were reported, with statistical significance set at  $p < 0.05$ . Model fit was assessed using likelihood ratio tests and pseudo  $R^2$ .

Clinical-pathological correlations were analyzed using cross-tabulation and chi-square tests to examine associations between clinical features (lesion appearance, pigmentation, feeding vessels) and histological subtypes (CIN vs. SCC). All statistical tests were two-tailed with  $\alpha = 0.05$ .

#### **4.13. Dissemination plan**

The final output of this study will be presented to the college of SPHMMC and a copy will be submitted. Finding from this study will be published to reputable journal for it to be assessable throughout the world.

#### **4.14. Ethical consideration**

An ethical clearance letter was granted from the ethical committee of SPHMMC. Prior to enrollment, each study participant received detailed information about the study, including its purpose and procedures. Informed consent was obtained orally from each participant. It was emphasized that their participation was voluntary and they had the right to decline participation without any negative consequences. To ensure confidentiality, data were collected anonymously without any patient identifiers, such as names or personal details.

## 5. Results

### 5.1 Descriptive Characteristics of Study Participants

A total of 70 patients presenting with ocular surface lesions and who had undergone excisional surgery at SPHMMC during the study period were included in this study. The overall prevalence of Ocular Surface Squamous Neoplasia (OSSN) was 57.14% (40/70), indicating that more than half of the ocular surface lesions evaluated were malignant or pre-malignant squamous lesions.

The non-OSSN group (n=30) comprised patients with various benign ocular surface lesions confirmed by histopathology. These included: pterygium (n=10, 33.3%), compound nevus (n=5, 16.7%), pingueculae (n=4, 13.3%), conjunctival granuloma (n=2, 6.7%), benign squamous growth (n=2, 6.7%), and others including calcific conjunctivitis, conjunctival nevus, and granulomatous inflammation (n=7, 23.3%).

#### 5.1.1. Demographic Profile Socioeconomic and Behavioral Characteristics

The mean age of participants was **44.17 years** (SD = 15.42), with a range from 14 to 80 years. Age distribution revealed that the majority of participants were relatively young, with 45.71% (32/70) being under 40 years of age. Middle-aged individuals (40-60 years) constituted 38.57% (27/70), while those above 60 years represented 15.71% (11/70) of the study population.

Regarding gender distribution, males were slightly more predominant, comprising **58.57%** (41/70) of participants compared to 41.43% (29/70) females. The majority of participants resided in urban areas (75.71%, 53/70), while rural residents constituted 24.29% (17/70).

Educational attainment showed considerable variation among participants. Approximately one-quarter were illiterate (23.19%, 16/69), another quarter had primary education (23.19%, 16/69), 21.74% (15/69) had secondary education, and the highest proportion had college or higher education (31.88%, 22/69). One participant had missing education data.

Smoking behavior was uncommon in this population, with only **4.29%** (3/70) reporting history of smoking. The vast majority (95.71%, 67/70) were never-smokers.

### **Demographic, Socioeconomic and Behavioral Characteristics of OSSN Patients**

Among the 40 patients diagnosed with OSSN, the mean age was 47.93 years (SD = 13.86), with ages ranging from 24 to 80 years. Age distribution revealed that 35.00% (14/40) were under 40 years of age, 50.00% (20/40) were in the 40-60 years' age group, and 15.00% (6/40) were above 60 years of age.

Regarding gender distribution among OSSN cases, males were more predominant, comprising 62.50% (25/40) of cases compared to 37.50% (15/40) females. The majority of OSSN patients resided in urban areas (72.50%, 29/40), while rural residents constituted 27.50% (11/40) of cases.

Educational attainment among OSSN patients showed that 32.50% (13/40) were illiterate, 27.50% (11/40) had primary education, 17.50% (7/40) had secondary education, and 22.50% (9/40) had college or higher education.

Smoking behavior was uncommon among OSSN patients, with 92.50% (37/40) being never-smokers and only 7.50% (3/40) reporting a history of smoking.

#### **5.1.2. Sun Exposure Patterns**

Daily outdoor time, as a proxy for ultraviolet light exposure, showed a distinct distribution. Half of the participants (50.00%, 35/70) reported spending 4-6 hours outdoors daily, while 30.00% (21/70) spent more than 6 hours' outdoors, and 20.00% (14/70) spent less than 4 hours' outdoors. The mean outdoor time was **5.57 hours per day** (SD = 2.29), indicating substantial cumulative sun exposure in this population.

#### **Sun Exposure Patterns in OSSN and Non-OSSN Patients**

Among the 40 patients diagnosed with OSSN, the mean daily outdoor time was 6.63 hours (SD = 2.12), with a range from 2 to 10 hours. In comparison, the 30 patients without OSSN had a mean daily outdoor time of 4.17 hours (SD = 1.70), with a range from 2 to 8 hours.

The difference in mean outdoor time between the two groups was statistically significant ( $t = 5.21$ ,  $p < 0.0001$ ), with OSSN patients spending an average of 2.46 hours more outdoors daily compared to non-OSSN patients (95% CI: 1.52 to 3.40 hours).

Categorically, among OSSN patients, only 7.50% (3/40) spent less than 4 hours outdoors daily, while 47.50% (19/40) spent 4-6 hours, and 45.00% (18/40) spent more than 6 hours' outdoors. In contrast, among non-OSSN patients, 36.67% (11/30) spent less than 4 hours' outdoors, 53.33% (16/30) spent 4-6 hours, and only 10.00% (3/30) spent more than 6 hours' outdoors.

Table 1: Demographic and Clinical Characteristics of Study Participants (N=70)

| Characteristic  | Category    | Frequency (n) | Percentage (%) |
|-----------------|-------------|---------------|----------------|
| OSSN Diagnosis  | Yes         | 40            | 57.14          |
|                 | No          | 30            | 42.86          |
| Sex             | Male        | 41            | 58.57          |
|                 | Female      | 29            | 41.43          |
| Age Group       | 8-39 years  | 32            | 45.71          |
|                 | 40-60 years | 27            | 38.57          |
|                 | >60 years   | 11            | 15.71          |
| Residence       | Urban       | 53            | 75.71          |
|                 | Rural       | 17            | 24.29          |
| Education Level | Illiterate  | 16            | 23.19          |
|                 | Primary     | 16            | 23.19          |
|                 | Secondary   | 15            | 21.74          |
|                 | College+    | 22            | 31.88          |

| Characteristic     | Category     | Frequency (n) | Percentage (%) |
|--------------------|--------------|---------------|----------------|
| Smoking Status     | Never smoked | 67            | 95.71          |
|                    | Ever smoked  | 3             | 4.29           |
| Daily Outdoor Time | <4 hours     | 14            | 20.00          |
|                    | 4-6 hours    | 35            | 50.00          |
|                    | >6 hours     | 21            | 30.00          |

Table 2: Demographic and Clinical Characteristics of OSSN Patients (n=40)

| Characteristic  | Category    | Frequency (n) | Percentage (%) |
|-----------------|-------------|---------------|----------------|
| Age Group       | <8-39 years | 14            | 35.00          |
|                 | 40-60 years | 20            | 50.00          |
|                 | >60 years   | 6             | 15.00          |
| Sex             | Male        | 25            | 62.50          |
|                 | Female      | 15            | 37.50          |
| Residence       | Urban       | 29            | 72.50          |
|                 | Rural       | 11            | 27.50          |
| Education Level | Illiterate  | 13            | 32.50          |
|                 | Primary     | 11            | 27.50          |
|                 | Secondary   | 7             | 17.50          |
|                 | College+    | 9             | 22.50          |

| Characteristic             | Category     | Frequency (n) | Percentage (%) |
|----------------------------|--------------|---------------|----------------|
| Smoking Status             | Never smoked | 37            | 92.50          |
|                            | Ever smoked  | 3             | 7.50           |
| Daily Outdoor Time         | <4 hours     | 3             | 7.50           |
|                            | 4-6 hours    | 19            | 47.50          |
|                            | >6 hours     | 18            | 45.00          |
| RVI Status                 | Positive     | 8             | 20.00          |
|                            | Negative     | 32            | 80.00          |
| Immunosuppression*         | Yes          | 8             | 20.00          |
|                            | No           | 32            | 80.00          |
| Organ Transplant Recipient | Yes          | 0             | 0.00           |
|                            | No           | 40            | 100.00         |

\*Immunosuppression defined as RVI positive status or presence of autoimmune disease requiring immunosuppressive therapy

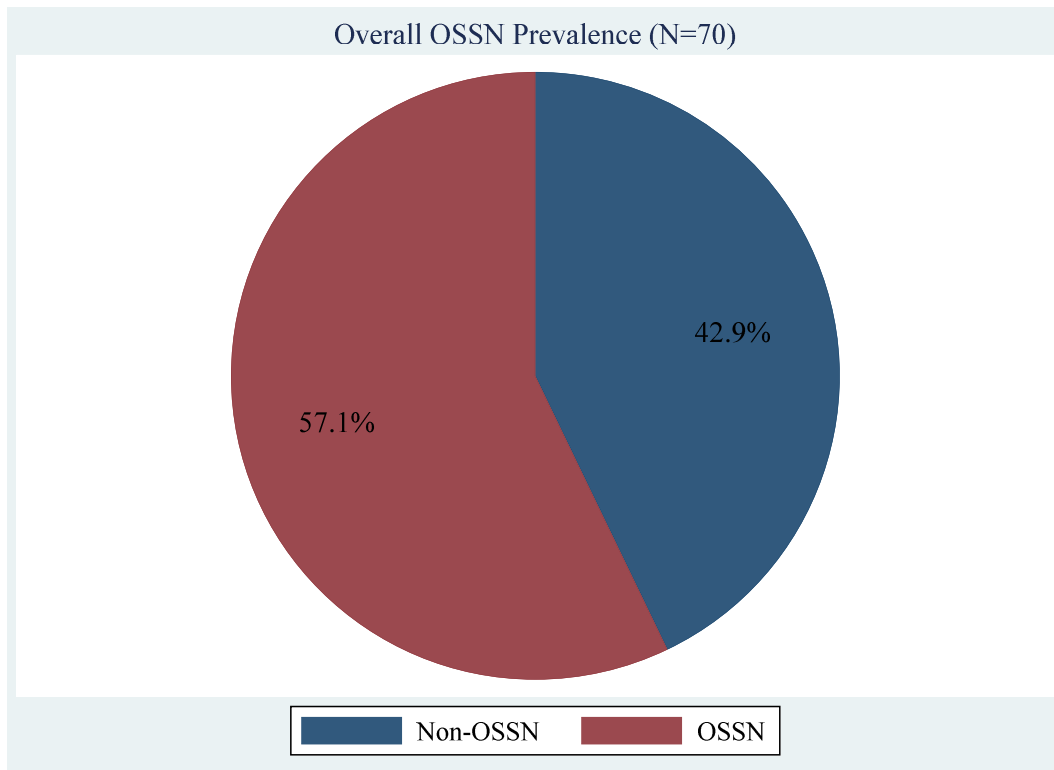


Figure 1: Overall Prevalence of OSSN

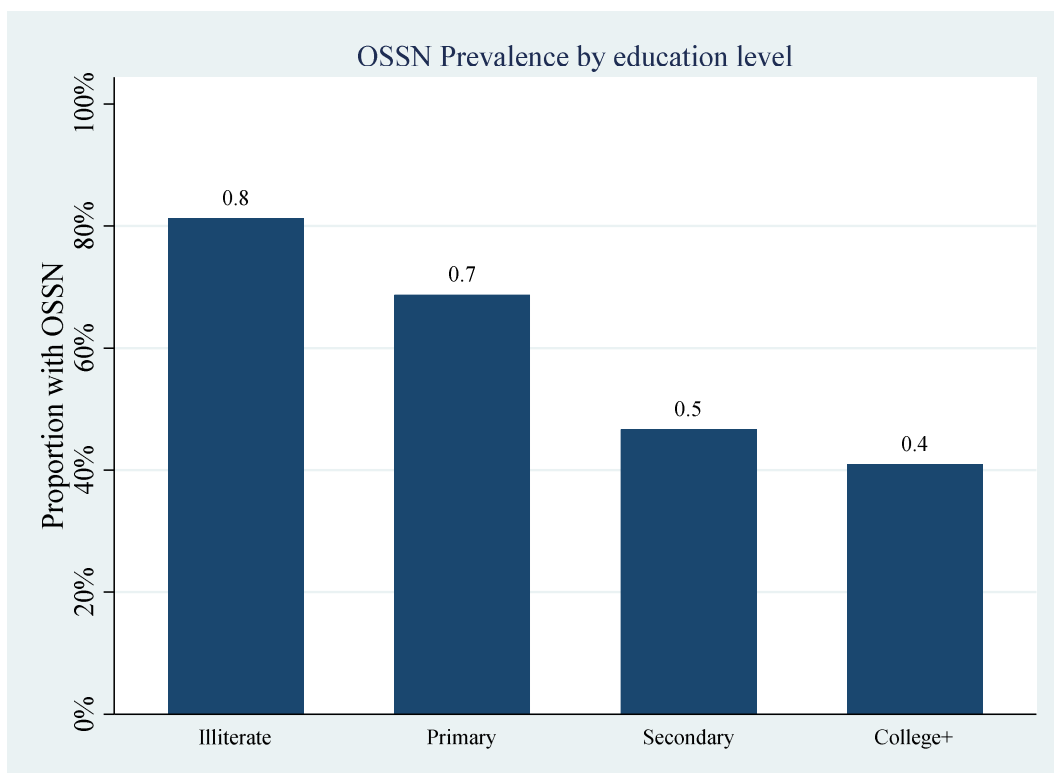


Figure 2: OSSN Prevalence by education level

### 5.1.3. Clinical-Pathological profiles among OSSN Cases

Among the 40 confirmed OSSN cases, histological analysis revealed **31 cases of Squamous Cell Carcinoma (SCC)** (77.50%) and **9 cases of Conjunctival Intraepithelial Neoplasia (CIN)** (22.50%).

Several clinical trends were observed between squamous cell carcinoma (SCC) and conjunctival intraepithelial neoplasia (CIN) cases. A leukoplakic lesion appearance was more frequently noted among SCC cases (80.65%) compared with CIN cases (55.56%). Feeding vessels were nearly universal across both groups, present in 93.5% of SCC cases and 100% of CIN cases. Pigmentation was observed in approximately half of SCC cases (48.4%) and one-third of CIN cases (33.3%).

Additionally, SCC cases comprised a higher proportion of patients older than 50 years (45.16%) compared to CIN cases (22.22%).

**Table 3: Anatomical Site and Extent of Involvement by OSSN Subtype**

| Anatomical Feature  | Category                                | SCC (n=31)<br>n(%) | CIN (n=9)<br>n(%) | Total (N=40)<br>n(%) |
|---------------------|-----------------------------------------|--------------------|-------------------|----------------------|
| Site of Involvement | Bulbar conjunctiva alone                | 6 (19.4%)          | 3 (33.3%)         | 9 (22.5%)            |
|                     | Limbus alone                            | 4 (12.9%)          | 1 (11.1%)         | 5 (12.5%)            |
|                     | Cornea alone                            | 2 (6.5%)           | 1 (11.1%)         | 3 (7.5%)             |
|                     | Bulbar conjunctiva +<br>Limbus          | 8 (25.8%)          | 2 (22.2%)         | 10 (25.0%)           |
|                     | Bulbar conjunctiva +<br>Limbus + Cornea | 11 (35.5%)         | 2 (22.2%)         | 13 (32.5%)           |
|                     | Temporal only                           | 15 (48.4%)         | 4 (44.4%)         | 19 (47.5%)           |

|                            |                    |            |           |            |
|----------------------------|--------------------|------------|-----------|------------|
| Location on Bulbar Surface | Nasal only         | 10 (32.3%) | 4 (44.4%) | 14 (35.0%) |
|                            | Inferior only      | 1 (3.2%)   | 0 (0%)    | 1 (2.5%)   |
|                            | Superior only      | 0 (0%)     | 0 (0%)    | 0 (0%)     |
|                            | Multiple quadrants | 5 (16.1%)  | 1 (11.1%) | 6 (15.0%)  |
| Corneal Involvement        | Present            | 16 (51.6%) | 3 (33.3%) | 19 (47.5%) |
|                            | Absent             | 15 (48.4%) | 6 (66.7%) | 21 (52.5%) |
| Limbus Involvement         | Present            | 27 (87.1%) | 6 (66.7%) | 33 (82.5%) |
|                            | Absent             | 4 (12.9%)  | 3 (33.3%) | 7 (17.5%)  |

Table 4: Lesion Size Distribution by OSSN Subtype

| Size Range (Clock Hours) | SCC (n=31) n(%) | CIN (n=9) n(%) | Total (N=40) n(%) |
|--------------------------|-----------------|----------------|-------------------|
| ≤ 2 clock hours          | 4 (12.9%)       | 2 (22.2%)      | 6 (15.0%)         |
| >2 – 3 clock hours       | 9 (29.0%)       | 4 (44.4%)      | 13 (32.5%)        |
| >3 – 4 clock hours       | 10 (32.3%)      | 2 (22.2%)      | 12 (30.0%)        |
| >4 – 5 clock hours       | 8 (25.8%)       | 1 (11.1%)      | 9 (22.5%)         |

SCC cases demonstrated a trend toward larger lesions, with **58.1% (18/31)** measuring greater than 3 clock hours, compared to **33.3% (3/9)** of CIN cases. In contrast, CIN lesions were predominantly smaller, with **66.6% (6/9)** involving 3 clock hours or less.

Table 5: Clinical Appearance by OSSN Subtype

| Lesion Appearance | SCC (n=31) n(%) | CIN (n=9) n(%) | Total (N=40) n(%) |
|-------------------|-----------------|----------------|-------------------|
| Leukoplakic       | 25 (80.6%)      | 5 (55.6%)      | 30 (75.0%)        |
| Papillomatous     | 6 (19.4%)       | 4 (44.4%)      | 10 (25.0%)        |
| Gelatinous        | 0 (0%)          | 0 (0%)         | 0 (0%)            |
| Nodular           | 0 (0%)          | 0 (0%)         | 0 (0%)            |

Leukoplakic appearance was the predominant morphological type overall (75.0%, 30/40), particularly among SCC cases (80.6%, 25/31). Papillomatous appearance was more common in CIN cases (44.4%, 4/9) than in SCC cases (19.4%, 6/31).

**Table 6: Vascularity and Pigmentation by OSSN Subtype**

| Feature                | Category | SCC (n=31) n(%) | CIN (n=9) n(%) | Total (N=40) n(%) |
|------------------------|----------|-----------------|----------------|-------------------|
| <b>Feeding Vessels</b> | Present  | 29 (93.5%)      | 9 (100%)       | 38 (95.0%)        |
|                        | Absent   | 2 (6.5%)        | 0 (0%)         | 2 (5.0%)          |
| <b>Pigmentation</b>    | Present  | 15 (48.4%)      | 3 (33.3%)      | 18 (45.0%)        |
|                        | Absent   | 16 (51.6%)      | 6 (66.7%)      | 22 (55.0%)        |

**Table 7: Age Distribution by OSSN Subtype**

| Age Category | SCC (n=31) n(%) | CIN (n=9) n(%) | Total (N=40) n(%) |
|--------------|-----------------|----------------|-------------------|
| ≤ 8-39 years | 10 (32.3%)      | 5 (55.6%)      | 15 (37.5%)        |
| 41-50 years  | 7 (22.6%)       | 2 (22.2%)      | 9 (22.5%)         |
| 51-60 years  | 8 (25.8%)       | 1 (11.1%)      | 9 (22.5%)         |
| >60 years    | 6 (19.4%)       | 1 (11.1%)      | 7 (17.5%)         |

## 5.2. Bivariate Analysis of Factors Associated with OSSN

**Age differences** between groups were statistically significant. Patients with OSSN were on average **8.76 years older** than those without OSSN (mean age: 47.93 vs. 39.17 years,  $t = 2.434$ ,  $p = 0.0176$ ).

While males constituted 62.50% of OSSN cases compared to 53.33% of non-OSSN cases, this difference was not statistically significant ( $\chi^2 = 0.594$ ,  $p = 0.441$ ).

Residential location showed minimal difference between groups, with urban residents comprising 72.50% of OSSN cases and 80.00% of non-OSSN cases ( $\chi^2 = 0.524$ ,  $p = 0.469$ ).

Educational attainment revealed a striking pattern. Illiterate individuals constituted 32.50% of OSSN cases but only 10.34% of non-OSSN cases. Conversely, those with college or higher education represented only 22.50% of OSSN cases but 44.83% of non-OSSN cases. This educational gradient was statistically significant ( $\chi^2 = 7.737$ ,  $p = 0.052$ ).

Regarding smoking, while all non OSSN cases were never-smokers, 7.50% of OSSN cases (3/40) had a history of smoking. However, this difference was not statistically significant ( $\chi^2 = 2.351$ ,  $p = 0.125$ ), likely due to the small number of smokers in the sample.

**Outdoor exposure** differences were even more pronounced. OSSN cases reported spending an average of **2.46 more hours** outdoors daily compared to non-OSSN cases (6.63 vs]. 4.17 hours,  $t = 5.210$ ,  $p < 0.0001$ ).

Table 8: Factors Associated with OSSN (Univariate Logistic Regression)

| Variable        | Odds Ratio (OR) | 95% CI     | p-value |
|-----------------|-----------------|------------|---------|
| Male sex        | 1.46            | 0.56-3.81  | 0.442   |
| Age group       | 1.58            | 0.80-3.12  | 0.186   |
| Urban residence | 0.66            | 0.21-2.04  | 0.471   |
| Education       | 0.54            | 0.34-0.85  | 0.008*  |
| Outdoor time    | 4.70            | 1.95-11.37 | 0.001*  |

Table 2 presents the crude odds ratios from univariate logistic regression analyses. Each unit increase in education category (from illiterate to college+) was associated with a **46% reduction** in odds of OSSN (OR = 0.54, 95% CI: 0.34-0.85, p = 0.008).

Each categorical increase in outdoor exposure (<4 hours to 4-6 hours to >6 hours) was associated with a **4.70-fold increase** in odds of OSSN (OR = 4.70, 95% CI: 1.95-11.37, p = 0.001).

When outdoor exposure was analyzed as a continuous variable each additional hour spent outdoors per day was associated with a 1.94-fold increase in the odds of OSSN (OR = 1.94, 95% CI: 1.39-2.71, p < 0.001).

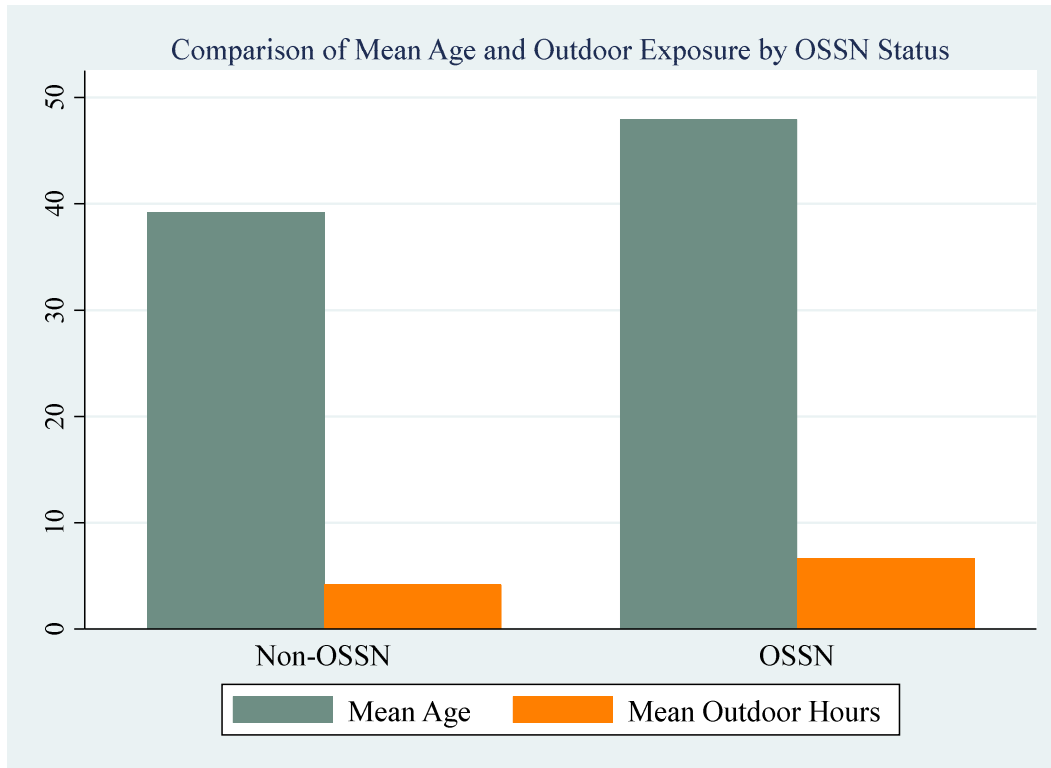


Figure 3: Mean age and outdoor exposure by OSSN

### 5.3. Multivariate Analysis of factors associated with OSSN

A stepwise logistic regression approach was employed to identify independent predictors of OSSN. The final multivariate model retained four significant predictors: outdoor time >6 hours, age 40-60 years, RVI positive status, and college education.

The strongest independent predictor was **outdoor exposure >6 hours daily**, which was associated with an **11.84-fold increase** in odds of OSSN (aOR = 11.84, 95% CI: 2.12-66.21,  $p = 0.005$ ) after adjusting for other factors.

**Age 40-60 years** was associated with a **4.23-fold increase** in odds compared to those under 40 years (aOR = 4.23, 95% CI: 1.17-15.31,  $p = 0.028$ ).

**RVI positive status** showed a borderline significant association with increased odds (aOR = 10.14, 95% CI: 1.00-102.50,  $p = 0.050$ ).

**College or higher education** showed a protective trend, associated with 70% reduced odds of OSSN (aOR = 0.30, 95% CI: 0.08-1.08, p = 0.065), though this approached but did not reach conventional statistical significance.

The final multivariate model demonstrated good explanatory power, with a pseudo R<sup>2</sup> of 0.29, indicating that approximately 29% of the variance in OSSN diagnosis was explained by the included variables. The likelihood ratio chi-square test was highly significant ( $\chi^2 = 27.29$ , p < 0.0001), confirming that the model as a whole was statistically significant.

Table 9: Multivariate Logistic Regression Model for OSSN

| Variable              | Adjusted OR | 95% CI      | p-value |
|-----------------------|-------------|-------------|---------|
| Outdoor time >6 hours | 11.84       | 2.12-66.21  | 0.005*  |
| Age 40-60 years       | 4.23        | 1.17-15.31  | 0.028*  |
| RVI positive status   | 10.14       | 1.00-102.50 | 0.050*  |
| College+ education    | 0.30        | 0.08-1.08   | 0.065   |
| Constant              | 0.58        | 0.25-1.36   | 0.212   |

## 6. Discussion

This study investigated the prevalence and associated factors of Ocular Surface Squamous Neoplasia (OSSN) among patients presenting with ocular surface lesions at Saint Paul's Hospital Millennium Medical College (SPHMMC). The research revealed a high prevalence of OSSN (57.14%) among patients undergoing excision of ocular surface lesions, indicating that squamous neoplasia constitutes a substantial proportion of ocular surface pathologies in this clinical setting. The multivariate analysis identified prolonged outdoor exposure and middle age (40-60 years) as independent predictors of OSSN, while higher education demonstrated a protective trend. Clinical-pathological correlation revealed that leukoplakic appearance, feeding vessels, and temporal location were common findings, with differences observed between SCC and CIN cases. These findings contribute to the growing body of literature on OSSN epidemiology in Ethiopia and sub-Saharan Africa and have important implications for clinical practice and public health interventions.

In this study the 57.14% prevalence of OSSN among patients undergoing excision of ocular surface lesions represents a selected clinical population presenting with lesions requiring surgical intervention, indicating a substantial burden of disease within the referral pathway. Our study differs notably from the Gondar University Hospital study, which reported histopathological confirmation of OSSN in 96% of clinically suspected cases. This substantial difference in diagnostic accuracy (57% vs. 96%) may reflect variations in clinical assessment expertise, patient selection criteria, or differences in the spectrum of benign mimickers presenting at each institution. Both studies, however, underscore the critical importance of histopathological examination as the gold standard, as clinical appearance alone may not reliably differentiate OSSN from its mimics, particularly in early or atypical presentations.

Our finding of 57.14% falls within the range reported by other African studies that examined OSSN among patients with ocular surface lesions undergoing biopsy. In Zimbabwe, a retrospective review of ocular surface tumor biopsies between 1996 and 2000 found that OSSN constituted 38.8% of cases (615 out of 1,587 biopsies), with the annual prevalence increasing from 33% in 1996 to 57.9% in 2000 (31). Our finding of 57.14% is remarkably similar to the 57.9% prevalence reported in Zimbabwe during the year 2000, suggesting a consistent burden of

OSSN among biopsied ocular surface lesions in the region. In Uganda, a study of ocular neoplasia cases from 2015 to 2021 reported that OSSN accounted for 26.7% (303 out of 1,133 cases) of ocular neoplasia (32), which is lower than our finding, possibly reflecting differences in case definitions, referral patterns, or the inclusion of all ocular neoplasms rather than only surface lesions.

A clinicopathological review from Malawi reported that OSSN was the most common conjunctival malignancy, though specific prevalence figures varied by time period and HIV status (28). In contrast, a study from North Taiwan examining OSSN cases from 1994 to 2014 reported markedly different epidemiological patterns, with older age at presentation and lower association with HIV, reflecting regional differences in risk factor profiles (35). These geographic variations underscore the importance of region-specific data to guide local clinical practice and public health planning.

The mean age of OSSN patients in our study was 47.93 years (SD = 13.86), with the 40-60-year age group being a significant independent risk factor (aOR = 4.23, 95% CI: 1.17-15.31,  $p = 0.028$ ). This is slightly higher than the mean age of 41.26 years reported from Gondar, Ethiopia (26), but lower than the mean age reported in the Taiwan study where patients presented at older ages, typically in the sixth to seventh decades (35). The younger age profile in Ethiopian studies likely reflects the high prevalence of HIV infection in the region, as immunosuppression accelerates OSSN development. In the Ugandan study, 41.3% of OSSN cases were aged 30-44 years (32), further supporting this observation. A substantial proportion (35.0%) of our OSSN cases were under 40 years, indicating that OSSN in Ethiopia affects economically productive adults, with significant public health and socioeconomic implications. This contrasts with high-income countries where OSSN typically presents in older individuals with cumulative sun exposure but lower HIV prevalence.

Males comprised 62.5% of OSSN cases in our study, a male predominance also observed in the Gondar study where 72% of cases were male (26). This male predominance may reflect occupational patterns, with men more likely engaged in outdoor occupations such as farming and daily labor that entail prolonged sun exposure. In our study, farmers, merchants, and daily laborers constituted a substantial proportion of the study population. However, sex was not a

statistically significant independent predictor in multivariate analysis, suggesting that the observed male predominance may be mediated through occupational sun exposure rather than biological sex differences.

Leukoplakic appearance was the predominant morphological type, observed in 75.0% of OSSN cases overall, and more frequently in SCC (80.6%) than in CIN (55.6%). This prevalence is consistent with the Gondar study, which reported leukoplakic appearance in 68% of cases (26). Leukoplakia represents surface keratinization overlying dysplastic or malignant epithelium and is a classic clinical sign of keratinizing squamous neoplasia. The higher frequency in SCC compared to CIN suggests that leukoplakia may indicate more advanced disease with greater keratin production. Papillomatous appearance was observed in 25.0% of cases, more commonly in CIN (44.4%) than SCC (19.4%), suggesting that exophytic growth patterns may be more characteristic of pre-invasive disease. The South African study similarly identified leukoplakia as a distinguishing clinical feature of OSSN compared to benign lesions (34). These findings emphasize the importance of recognizing leukoplakic lesions during routine ocular examination to facilitate early biopsy and intervention.

Temporal involvement was most common in our study (47.5%), followed by nasal (35.0%) and multiple quadrants (15.0%). This contrasts with the Gondar study, which reported nasal predominance in 72% of cases (26). The temporal limbus is typically the site of maximum actinic exposure due to the focusing effect of the tear meniscus and the lateral canthus, making it a plausible predominant site for UV-induced neoplasia. The difference between studies may reflect variations in sun exposure patterns, ocular anatomy, or recording practices. Both studies, however, confirm that the interpalpebral region (exposed to maximum UV) is the predominant site for OSSN development. Corneal involvement was present in 47.5% of cases, and limbal involvement in 82.5%, underscoring the propensity of OSSN to arise from the limbal stem cell niche and extend onto the cornea.

The near-universal presence of feeding vessels (95.0% of OSSN cases) is a striking finding that corroborates existing literature. Among OSSN cases, feeding vessels were present in 93.5% of SCC cases and 100% of CIN cases. This high prevalence of vascularization closely aligns with the Gondar study, which reported feeding vessels in 84% of their OSSN cases (26). The presence

of pronounced, corkscrew, or isolated feeder vessels supplying a conjunctival lesion is a strong indicator of neoplasia and reflects the angiogenic requirements of rapidly proliferating tissue. This feature is integral to clinical algorithms for suspecting OSSN and appears to be a consistent finding across Ethiopian populations. The South African study similarly identified vascularization as a distinguishing feature between OSSN cases and controls (34).

Pigmentation was observed in 45.0% of OSSN cases overall, present in 48.4% of SCC cases and 33.3% of CIN cases. While not as prevalent as leukoplakia or vascularization, pigmentation can be a confounding feature, sometimes leading to misdiagnosis as a melanocytic lesion such as conjunctival nevus or melanoma. Its presence in OSSN, particularly in African populations with higher baseline conjunctival pigmentation, is an important diagnostic consideration and has been noted as a distinguishing feature in other African studies (34). The variation in pigmentation between SCC and CIN may reflect differences in underlying tumor biology, host melanocytic response, or chronicity of the lesion.

Lesion size analysis revealed that SCC cases tended toward larger lesions, with 58.1% measuring 3 or more clock hours compared to 33.3% of CIN cases. The most frequently observed size ranges were 2-3 clock hours (32.5%) and 3-4 clock hours (30.0%). Larger lesion size in SCC likely reflects longer duration of growth, more aggressive biological behavior, or delayed presentation. The association between larger size and invasive disease has clinical implications, suggesting that lesions exceeding 5 clock hours should raise higher suspicion for SCC and may warrant more extensive excision with wider margins and closer postoperative surveillance.

Our study provides robust evidence of a dose-response relationship between daily outdoor exposure and OSSN risk. Each additional hour spent outdoors per day was associated with a 1.94-fold increase in the odds of OSSN (OR = 1.94, 95% CI: 1.39–2.71,  $p < 0.001$ ).

These findings align closely with a Kenyan case-control study which reported that for each additional hour of daily sun exposure, the odds of OSSN increased by 24% (OR = 1.24, 95% CI: 1.10–1.40) (10). The substantially higher per-hour effect size observed in our Ethiopian cohort (1.94 vs. 1.24) may be attributable to several factors. Ethiopia's location near the equator and its high-altitude result in more intense ultraviolet radiation compared to Kenya, where the study was conducted at lower altitudes. UV intensity increases by approximately 4% for every 300-meter

increase in altitude and is more direct near the equator (8). This environmental factor potentially amplifies the carcinogenic impact of each exposure hour. Additionally, differences in occupational patterns, sun-protective behaviors, and baseline skin pigmentation may modify individual susceptibility.

The categorical analysis further revealed a threshold effect: compared to those with <4 hours daily exposure, individuals spending 4–6 hours showed a non-significant increase (OR = 2.83,  $p = 0.299$ ), while those with >6 hours experienced a dramatic 11.84-fold increase (aOR = 11.84, 95% CI: 2.12–66.21,  $p = 0.005$ ). This pattern suggests that risk escalates sharply beyond six hours, consistent with a systematic review from Africa which reported odds ratios of 1.7 for 2–4 hours and 1.8 for  $\geq 5$  hours of exposure (8). The finding that 45.0% of OSSN cases reported >6 hours daily outdoor exposure, compared to only 10.0% of non-OSSN cases, underscores the clinical significance of this threshold.

A retrospective case-control study from the USA identified lifetime sun exposure as the strongest risk factor for OSSN (OR = 5.4, 95% CI: 2.27–12.85,  $p < 0.005$ ), with pingueculae serving as surrogate markers for cumulative sun exposure (33). The consistency of findings across diverse geographic settings from North America to East Africa reinforces the universal importance of UV radiation in OSSN pathogenesis. The biological mechanism involves UV-induced DNA damage in limbal stem cells, leading to mutations in tumor suppressor genes such as p53 and subsequent dysregulated epithelial proliferation (8).

The association between HIV and OSSN in sub-Saharan Africa is well-documented, with odds ratios ranging from 6.17 in meta-analyses to 48.42 in specific studies (8, 10). In our study, RVI positivity showed a strong, borderline significant association with OSSN (aOR = 10.14, 95% CI: 1.00–102.50,  $p = 0.050$ ). The magnitude of association, while substantial, is lower than some extreme estimates, possibly reflecting the increasing coverage and effectiveness of antiretroviral therapy (ART) in Ethiopia. The Kenyan study demonstrated that while ART use reduces the risk compared to untreated HIV, the odds remain significantly elevated (OR = 19.16 for HIV with ART use) (10).

In the Gondar study, HIV prevalence among OSSN cases was 22% (26), comparable to the 20% observed in our OSSN cases. The Ugandan study reported that OSSN was more common in

females (59.1%), which may reflect the higher HIV prevalence among women in that population (32). The Malawi clinicopathological review similarly noted a strong association with HIV, with OSSN presenting at younger ages in HIV-positive individuals (36).

The biological mechanism involves HIV-induced immunosuppression leading to loss of immune surveillance against HPV-infected cells and dysregulated apoptosis of neoplastic cells. HPV infection has been identified as a co-factor, with a meta-analysis reporting an OR of 2.64 for HPV in OSSN (8). The synergistic effect of HIV and HPV in OSSN pathogenesis explains the high burden of disease in regions with dual epidemics.

This study highlights an inverse relationship between educational attainment and OSSN risk. Illiteracy was higher among OSSN cases (32.5%) compared to non-OSSN cases (10.3%), while college education was more common in non-OSSN cases (44.8%) than OSSN cases (22.5%). In multivariate analysis, college education demonstrated a strong protective trend (aOR = 0.30, 95% CI: 0.08–1.08,  $p = 0.065$ ), though it did not reach conventional statistical significance, likely due to limited sample size.

This social gradient likely operates through different pathways. Education enhances health literacy, including awareness of sun protection measures and early healthcare-seeking for suspicious lesions. It also influences access to preventive resources, such as sunglasses, hats, and sun-protective clothing.

The finding that age 40-60 years independently predicted OSSN (aOR = 4.23) after adjusting for outdoor exposure and other factors suggests that age-related factors beyond cumulative UV exposure contribute to OSSN risk. These may include age-related immune senescence, cumulative duration of HPV infection, and reduced DNA repair capacity. Interestingly, age >60 years was not significantly associated with increased risk compared to those under 40, possibly due to the small number of participants in this age group ( $n=11$ ) or survival bias wherein individuals with aggressive disease may not survive to older ages.

The mean age of OSSN patients in our study (47.9 years) is comparable to the South African study where median age was 44 years (34) and the Kenyan study where the mean age was 42.1 years (10). The relatively young age of OSSN patients in sub-Saharan Africa compared to high-

income countries reflects the additional burden of HIV-related immunosuppression, which accelerates OSSN development by a decade or more.

In contrast to several other cancers, our study found no statistically significant association between smoking and OSSN. Only 7.5% of OSSN cases and 0% of non-OSSN cases had a history of smoking, and the overall smoking prevalence in our sample was low (4.29%). This is consistent with the broader literature on OSSN, where smoking has not been consistently identified as a major risk factor. The primary etiological drivers remain UV radiation and viral co-factors like HIV and HPV. The low smoking prevalence in our population limited statistical power to detect any association, and future studies with larger samples and higher smoking prevalence may be needed to definitively address this question.

## **7. Limitations and strengths**

The cross-sectional design limits causal inference, as exposure assessment and outcome determination occurred simultaneously. The relatively modest sample size (n=70) restricted statistical power, particularly for subgroup analyses and less prevalent exposures. In addition, exposure assessment relied on self-report, which may be subject to recall bias and measurement error, especially for variables like daily outdoor time. The hospital-based sampling may limit generalizability to community populations with milder or asymptomatic disease. Furthermore, this study did not collect data on several clinically relevant variables including duration of symptoms, chief complaints, recurrence status, prior treatments, viral load and CD4 count for HIV. Future studies should incorporate these variables to provide a more comprehensive clinical profile of OSSN patients.

## **8. Conclusion**

This study demonstrates a high prevalence of OSSN among patients presenting with ocular surface lesions at SPHMMC, with prolonged outdoor exposure and middle age identified as independent risk factors. The findings reinforce the central role of ultraviolet radiation in OSSN pathogenesis while highlighting the potential protective effect of higher education. Although limited by sample size and cross-sectional design, these results contribute valuable insights to the sparse literature on OSSN in Ethiopia and similar settings. The identified risk factors provide a foundation for targeted prevention strategies, emphasizing sun protection for high-risk occupational groups and enhanced clinical suspicion in middle-aged patients with significant sun exposure.

## **9. Recommendations**

Clinical and public health strategies should prioritize sun protection education for high-risk groups, particularly individuals engaged in outdoor occupations exceeding six hours daily and those in the 40–60-year age bracket. Healthcare providers should incorporate brief ocular surface examinations into routine care for these populations, with particular attention to individuals presenting with conjunctival lesions. Integration of OSSN awareness into existing community health programs, occupational safety guidelines, and HIV care protocols would enhance early detection and timely referral. Additionally, provision of affordable sun-protective equipment, such as wide-brimmed hats and UV-blocking sunglasses, should be explored through workplace safety initiatives or public health campaigns, especially for agricultural and outdoor workers.

Further prospective, population-based studies with larger sample sizes and longitudinal follow-up are recommended to better establish causal relationships and refine risk estimates. Future investigations should employ more objective measures of ultraviolet exposure, such as personal dosimetry or geospatial mapping, and explore molecular markers to understand the interplay between environmental exposure, immunodeficiency, and genetic susceptibility in OSSN pathogenesis.

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## **Annex.1**

### **Advisors' Thesis Approval Sheet**

#### **Declaration**

I hereby declare that this Graduate Study Program thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this proposal have been duly acknowledged.

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Name

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Signature

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Date

#### **Approved by:**

This is to certify that the thesis entitled “Prevalence and associated factors of OSSN at Saint Paul's Hospital Millennium Medical College, Ethiopia, 2023. has been carried out by Dr. Bisrat Amsalu (Ophthalmology Resident)) under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department for defense.

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Name of advisor

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Signature

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Date

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Name of co-advisor

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Signature

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Date

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Name of co-advisor

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## Annex 2. Questionnaire

I, Dr. Bisrat Amsalu, an ophthalmology resident, am conducting a research study on the prevalence and associated factors of ocular surface squamous neoplasia (OSSN) at SPHMMC. It is important to note that there will be no financial benefit or harm to you by participating in this study. However, the results of the study may have potential benefits for both you and the broader adult population in the future.

Rest assured that the confidentiality of your identity and data will be strictly maintained throughout the entire duration of the study. Your personal information will be handled with the utmost care and will only be accessible to authorized members of the research team. Your privacy and anonymity are of the utmost importance to us.

Thank you!

Signature of participant \_\_\_\_\_

Part one: Sociodemographic variables

| S.no. | Variable           | Coding category                                                                              |  |
|-------|--------------------|----------------------------------------------------------------------------------------------|--|
| 1.    | Sex                | A. Male<br>B. Female                                                                         |  |
| 2.    | Age                | ----- Years                                                                                  |  |
| 3.    | Place of residency | A. Urban<br>B. Rural                                                                         |  |
| 4.    | Educational level  | A. Illiterate<br>B. Can read and write<br>C. Primary<br>D. Secondary<br>E. College and above |  |
| 5.    | Occupation         | A. Farmer<br>B. Civil servant<br>C. Daily laborer                                            |  |

|  |  |                                                                                             |  |
|--|--|---------------------------------------------------------------------------------------------|--|
|  |  | D. Housewife<br>E. Merchant<br>F. Student<br>G. Un employed<br>H. Retired<br>I. Other ----- |  |
|--|--|---------------------------------------------------------------------------------------------|--|

#### Part two: Presenting History

| S.no | Variable                              | Category                                                                                                                 |  |
|------|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--|
| 1    | Average time spent outdoors every day | .....                                                                                                                    |  |
| 2    | Smoking                               | <input type="checkbox"/> Current smoker<br><input type="checkbox"/> Quit smoking<br><input type="checkbox"/> Non smoking |  |
| 3    | Previous ocular surgery               | A. Yes<br>B. No                                                                                                          |  |
|      | If Yes                                | A. Pterygium Excision<br>B. Corneal Transplantation<br>C. Cryotherapy<br>D. Conjunctival Biopsy                          |  |

#### Part three: Systemic diseases

| S.no | Variable                   | Category                                                    |  |
|------|----------------------------|-------------------------------------------------------------|--|
| 1.   | RVI status                 | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |  |
|      | If Yes, Use of ARVs        | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |  |
|      | If Yes, Recent CD4 Count   | -----                                                       |  |
| 2    | DM                         | A. Yes<br>B. No                                             |  |
|      | If Yes, Recent HgA1C level | -----                                                       |  |
| 3    | Organ transplant recipient | A. Yes<br>B. No                                             |  |
| 7    | Any autoimmune diseases    | A. Yes<br>B. No                                             |  |
|      | If Yes, Specify            | -----                                                       |  |
| 8    | Any Systemic Malignancy    | A. Yes<br>B. No                                             |  |
|      | If Yes, Specify            | C.                                                          |  |
| 9    | Allergy/Atopic eczema      | <input type="checkbox"/> Yes<br><input type="checkbox"/> No |  |

Part Four: Clinical Measurements and Histology/Chart Review

| Clinical Features                                                                                                                                           | OD | OS | OU |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------|----|----|----|
| 1. Visual Acuity                                                                                                                                            |    |    |    |
| 2. IOP                                                                                                                                                      |    |    |    |
| 3. Lesion                                                                                                                                                   |    |    |    |
| I. Appearance                                                                                                                                               |    |    |    |
| A. Papillomatous<br>B. Leukoplakic<br>C. Gelatinous                                                                                                         |    |    |    |
| II. Location                                                                                                                                                |    |    |    |
| A. Nasal<br>B. Temporal<br>C. Nasal, temporal and superior<br>D. All quadrants of the globe                                                                 |    |    |    |
| III. Site of involvement                                                                                                                                    |    |    |    |
| A. Bulbar conjunctiva<br>B. Limbus<br>C. Limbus and conjunctiva<br>D. Cornea<br>E. Bulbar conjunctiva, Limbus and Cornea<br>F. Whole globe, orbit & eyelids |    |    |    |
| IV. Procedure Done                                                                                                                                          |    |    |    |
| A. Excision alone<br>B. Cryotherapy                                                                                                                         |    |    |    |

|                                                                  |  |  |  |
|------------------------------------------------------------------|--|--|--|
| C. Conjunctival Auto graft<br>D. Photodynamic therapy<br>E. None |  |  |  |
| 4. Size in clock hours                                           |  |  |  |
| 5. Pigmentation                                                  |  |  |  |
| A. Yes<br>B. No                                                  |  |  |  |
| 6. Feeding vessels                                               |  |  |  |
| A. Yes<br>B. No                                                  |  |  |  |
| 7. Local metastasis                                              |  |  |  |
| A. Yes<br>B. No                                                  |  |  |  |
| 8. Histology                                                     |  |  |  |
| A. CIN                                                           |  |  |  |
| B. CIS                                                           |  |  |  |
| C. SCC                                                           |  |  |  |
| D. Pterygium and CIN                                             |  |  |  |
| E. Pterygium and CIS                                             |  |  |  |