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Accuracy of diagnosing early ocular surface squamous neoplasia using methylene blue in Zambia

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Background: Ocular surface squamous neoplasia (OSSN) includes pre-invasive and invasive squamous lesions of the ocular surface. Current diagnosis relies on surgical excision and histology, with potential complications. This study assessed the reliability of methylene blue staining as a non-invasive diagnostic tool for OSSN at the University Teaching Hospitals – Eye Hospital in Lusaka, Zambia.

Aim: This study aimed to evaluate the accuracy of methylene blue staining in diagnosing OSSN.

Setting: The study took place at the University Teaching Hospitals-Eye Hospital, Lusaka, Zambia, between February 2022 and February 2023.

Methods: A cross-sectional study design compared methylene blue staining to histology in diagnosing OSSN. Participants underwent staining before excision biopsy on the same day. Data were analysed using SPSS and SAS OnDemand, employing Chi-square and McNemar's tests for paired diagnostic test comparison.

Results: The study involved 79 participants, with a high OSSN prevalence (62%), primarily among females (62%), with an average age of 40. Ocular surface squamous neoplasia correlated significantly with employment (P = 0.02) and HIV infection (P = 0.03). The most common symptom was nasal conjunctival growth. Duration of symptoms did not consistently indicate disease severity. Methylene blue staining showed 87.8% sensitivity, 83.33% specificity, a positive predictive value of 89.6%, and a negative predictive value of 80.7%.

Conclusion: Methylene blue staining is an effective alternative diagnostic method for OSSN, offering high accuracy. However, it cannot replace histology.

Contribution: This study contributes valued insights into the reliability of methylene blue staining for diagnosing OSSN and delineation of surgical margins during excision biopsy.

Keywords: ocular surface squamous neoplasia; diagnosis; methylene blue; Zambia; accuracy.

Introduction

Ocular surface squamous neoplasia (OSSN) is a term that encompasses a spectrum of pre-invasive and invasive squamous lesions of the ocular surface, which is the cornea and conjunctiva.[1] Clinically, OSSN is characterised by growth along the interpalpebral region of the conjunctiva, occasionally extending to the cornea.[2] Distinguishing early-stage OSSN from benign lesions such as pterygia can be a challenging task.[3] Clinical diagnosis of OSSN is often supported by the presence of feeder vessels, hyperpigmentation, and an irregular mass, which may exhibit various characteristics, including gelatinous, leukoplakic, or nodular.[4] Ocular surface squamous neoplasia includes the clinical continuum of mild, moderate, severe dysplasia, carcinoma *in situ* (CIS), and invasive squamous cell carcinoma (SCC).[5]

The incidence of OSSN is geographically variable and difficult to ascertain accurately, with notably higher rates observed in sub-Saharan Africa.[6] A study conducted in Zimbabwe by Pola et al. in 2003 reported an incidence rate of 3.4 cases per year per 100 000 for males and 3.0 cases per year per 100 000 for females. In Zambia, eye cancers account for 9% of all cancers,[7] with OSSN being the most prevalent ocular malignancy. The OSSN in Zambia poses a significant threat to both visual function and patients' lives.[7]

Prevention, early detection, and timely intervention are crucial for effectively managing OSSN.[8] The current gold standard for diagnosing OSSN involves surgical excision with a no-touch

technique followed by histological examination of the excised specimen.[4] However, surgical excision is associated with long-term complications, including scarring, symblepharon formation, and limbal stem cell deficiency.[9] In addition, it is costly and requires specialist expertise.[9] Unfortunately, this remains the sole diagnostic method employed at the University Teaching Hospitals (UTHs)-Eye Hospital in Lusaka, Zambia.

Various cost-effective and non-invasive diagnostic methods for OSSN, such as anterior-segment optical coherence tomography (AS-OCT), impression cytology, and methylene blue dye staining,[10] have been explored and found promising in global contexts. Early diagnosis of OSSN is helpful as it can act as a basis for the treatment of early lesions with topical chemotherapy such as 5% fluorouracil, mitomycin C or Interferon alpha-2b. Subtle findings from these non-invasive methods help characterise OSSN beyond what clinical examination reveals.[11] The non-surgical diagnostic methods are underutilised in Africa, particularly in regions where the burden of OSSN is highest.

To date, the accuracy of alternative diagnostic methods, as compared to surgical excision and histopathology, has not been evaluated in Zambia. This study seeks to provide a comprehensive overview of the socio-demographics and clinical characteristics of patients referred for suspected OSSN and scheduled for surgery at UTHs-Eye Hospital. In addition, it aims to determine the specificity, sensitivity, and both negative and positive predictive values (PPVs) of methylene blue staining in the diagnosis of OSSN. Identifying alternative diagnostic approaches for OSSN could lead to quicker diagnoses, expedited referrals for treatment, and the prevention of complications associated with delayed treatment or surgery, ultimately improving patient outcomes and reducing morbidity and mortality.

Materials and methods

This hospital-based, cross-sectional study was conducted at the UTHs-Eye Hospital, a tertiary referral centre for complex ocular conditions in Zambia. The study spanned 13 months, from February 2022 to February 2023.

Participants

Patients referred to the UTHs-Eye Hospital with suspected OSSN were recruited for this study. Eligible participants were consenting adults aged 18 years and older, scheduled for excision biopsy because of suspected OSSN. Exclusion criteria included individuals with a history of prior ocular surgery on the affected eye, those who had previously received topical or systemic chemotherapy for OSSN or any other cancer, and patients with late-stage OSSN (stage 4). The T criteria by the American Joint Committee on Cancer staging for OSSN was used to categorise patients clinically. This staging categorises tumours as follows: Tx Primary tumour cannot be assessed; T0 No evidence of primary tumour; Tis CIS; T1 Tumour (≤5 mm in the greatest dimension) invades through the conjunctival basement membrane without invasion of adjacent structures; T2 Tumour (>5 mm in greatest dimension) invades through the conjunctival basement membrane without invasion of adjacent structures; T3 Tumour invades adjacent structures (excluding the orbit); T4 Tumour invades the orbit with or without further extension; T4a Tumour invades orbital soft tissue without bone invasion; T4b Tumour invades the bone; T4c Tumour invades adjacent paranasal sinuses; T4d Tumour invades the brain. Convenience sampling was utilised, with consecutive patients meeting the inclusion criteria being included. The sample size was calculated using the finite Cochran's formula and determined to be at least 64 participants.

Data collection tools

Data collection involved the use of a researcher's questionnaire or data collection sheet, adapted from a prior OSSN study conducted at UTHs-Eye Hospital. Additional tools included topical lignocaine, topical methylene blue dye, and a Samsung Galaxy A50 smartphone with a 25-megapixel camera.

Procedures

Patients suspected of having OSSN and scheduled for excision biopsy were recruited during their clinic visits. A slit-lamp examination was performed to identify clinical and morphological features of suspicious lesions. Prior to the biopsy in the operating room, a pre-staining photograph of the lesion was taken. The procedure involved applying two drops of 2% lignocaine eye drops to the affected eye, followed by the topical application of methylene blue staining dye for 10 s, which was subsequently washed out with 20 mL of normal saline. A second photograph was captured after staining to document the staining pattern of the lesion. Finally, excision biopsy was performed as scheduled by the researcher or the ophthalmologist. Pathologists and ophthalmologists interpreting the results were blinded to minimise bias. The turnaround time for histology results was 2 weeks.

Data analysis

Data collected were entered into an Excel spreadsheet and analysed using statistical software, including Statistical Package for the Social Sciences (SPSS) and Statistical Analysis Software (SAS) OnDemand. Statistical Package for the Social Sciences was developed by International Business Machines Corporation (IBM), United States and SAS OnDemand was created by Goodnight and North Carolina State University Colleges, United States. A standardised binary diagnosis test (paired samples) was conducted based on methods from NCSS 2020. The sensitivity and specificity of the methylene blue stain were compared to the gold standard (histology) using Chi-square tests, specifically the McNemar's test. Participants' characteristics were presented as frequencies and

percentages, while continuous variables that were normally distributed were expressed as mean or standard deviation. Non-normally distributed continuous variables were represented as median and interquartile range. Frequency and percentage with a 95% confidence interval (CI) were reported for all comparisons.

Results

Sociodemographic characteristics and associated risk factors

Table 1 presents the sociodemographic characteristics and associated risk factors of the 79 participants in the study. The median age of the participants was 40 years, with an interquartile range of 31–52 years, and ages ranging from 19–76 years. Among the participants, 62.0% were female, and 38.0% were male. The most common employment status was self-employment (36.7%), followed by employed (25.3%), unemployed (17.7%), retired (11.4%), and students (11.4%). Only a small percentage reported drinking alcohol (15.2%), and even fewer reported smoking (1.3%). Regarding HIV status, 39.2% of participants were HIV-positive, with 86.7% of them being on highly active antiretroviral therapy (HAART). Past medical history revealed that 7.6% had hypertension, 1.3% had asthma, and 39.2% had HIV, while 51.9% had no significant medical history. None of the participants reported a family history of any cancer or ocular cancer.

TABLE 1: Sociodemographic features and associated risk factors of ocular surface squamous neoplasia (N = 79).

Characteristic	*n*	%
Age	40	(31–52) 19–76†
Gender		
Female	49	62.0
Male	30	38.0
Employment status		
Employed	49	62.0
Unemployed	14	17.7
Retired	9	11.4
Student	7	8.9
Alcohol		
Yes	12	15.2
Smoking		
Yes	1	1.3
HIV		
Negative	48	60.8
Positive	31	39.2
HAART		
Yes	26	86.7
PMHx		
Asthma	1	1.3
HIV	31	39.2
HTN	6	7.6
None	41	51.9
Family history of OSSN		
No	79	100.0
Family history of ocular cancer		
No	79	100.0

HAART, highly active antiretroviral therapy; PMHx, past medical history; OSSN, ocular surface squamous neoplasia; HTN, hypertension.

†, Median interquartile range.

Characteristics of ocular surface squamous neoplasia versus non-ocular surface squamous neoplasia patients

Table 2 compares the characteristics of patients with OSSN and non-OSSN. Among the 79 participants, 49 were diagnosed with OSSN, while 30 were classified as non-OSSN. The prevalence of OSSN in this study was 62.0%. The OSSN patients had a median age of 36 years (interquartile range [IQR]: 29–48), while non-OSSN patients had a median age of 46 years (IQR: 38–57), and this age difference was statistically significant (P = 0.008). There was no significant difference in gender distribution between the two groups (P = 0.10). Employment status showed a significant difference (P = 0.019), with a higher percentage of non-OSSN patients being employed (43.3%) compared to OSSN patients (14.3%). HIV status also revealed a significant difference (P = 0.033), with 49.0% of OSSN patients being HIV-positive compared to 23.3% of non-OSSN patients, resulting in an odds ratio of 3.1 (95% CI: 1.0–10.0). Past medical history (PMHx) showed significant differences in the prevalence of asthma (P = 0.014) and hypertension (P = 0.014), with a higher percentage of non-OSSN patients having a history of

TABLE 2: Characteristics of patients with ocular surface squamous neoplasia versus non-ocular surface squamous neoplasia.

Characteristic	OSSN, N = 49		Non-OSSN, N = 30		*P*	Unadjusted odds ratio	95% CI
	n	%	*n*	%			
Age	36	29–48	46	38–57	0.008	-	-
	-	19–76†	-	24–74†	-	-	-
Gender							
Female	34	69.4	15	50.0	0.100	0.5	0.2 – 1.3
Employment status							
Employed	7	14.3	13	43.3	0.019	-	-
Unemployed	11	22.4	3	10.0	-	-	-
Self-employed	21	42.9	8	26.7	-	-	-
Retired	4	8.2	5	16.7	-	-	-
Student	6	12.2	1	3.3	-	-	-
Alcohol							
Yes	6	12.2	6	20.0	0.400	0.6	0.1 – 2.4
Smoking							
Yes	1	2.0	0	0.0	-	-	-
HIV							
Negative	25	51.0	23	76.7	0.033	3.1	1.0 – 10.0
Positive	24	49.0	7	23.3	-	-	-
HAART							
Yes	20	83.3	6	66.7	0.400	2.4	0.3 – 19.4
PMHx							
Asthma	1	2.0	0	0.0	0.014	-	-
HTN	1	2.0	5	16.7	-	-	-
None	23	46.9	18	60.0	-	-	-
HIV	24	49.0	7	23.3	-	-	-
Family history of any cancer							
No	49	100.0	30	100.0	-	-	-
Family history of ocular cancer							
No	49	100.0	30	100.0	-	-	-

OSSN, ocular surface squamous neoplasia; CI, confidence interval; HAART, highly active antiretroviral therapy; PMHx, past medical history; HTN, hypertension.

†, Median interquartile range.

hypertension. There were no significant differences in family history of any cancer or family history of ocular cancer between the two groups.

Clinical presentation of ocular surface squamous neoplasia

Table 3 outlines the clinical manifestations of OSSN in the 79 participants. The most common presenting complaint was nasal conjunctival growth, reported by 93.7% of participants, followed by foreign body sensation (29.1%), tearing (6.3%), pain (5.1%), poor vision (1.3%), and itchiness (1.3%). The duration of symptoms varied, with 34.1% experiencing symptoms for at least 12 months, 15.2% for 3 to 6 months, 30.4% for less than 3 months, and 20.3% for more than a year. The majority of participants (88.6%) had not previously received treatment for the suspected OSSN lesion in the affected eye. Feeder vessels were observed in 59.5% of participants and adhesion to the sclera was noticed in 11.4% of participants. Pigmentation characteristics included brown (39.2%), white (21.5%), pink (8.9%), black (1.3%), and red (1.3%), with 27.8% of participants having no abnormal pigmentation. One participant had a previous lesion in the same eye.

TABLE 3: Clinical presentations of ocular surface squamous neoplasia (N = 79).

Characteristic	*n*	%
Clinical symptoms		
Conjunctival growth	74	93.7
FB sensation	23	29.1
Tearing	5	6.3
Pain	4	5.1
Poor vision	1	1.3
Itchiness	1	1.3
Duration of symptoms		
0–3 months	24	30.4
3–6 months	12	15.2
6–12 months	27	34.1
≥ 12 months	16	20.3
Previous treatment		
Artificial tears	2	2.5
Dexamethasone eye drops	1	1.3
Gentamycin drops	1	1.3
Chloramphenicol eye drops	1	1.3
TEO	1	1.3
Unknown	3	3.8
None	70	88.6
Lesion in same eye before		
Yes	1	1.3
Abnormal pigment		
Black	1	1.3
Brown	31	39.2
None	22	27.8
Pink	7	8.9
Red	1	1.3
White	17	21.5
Feeder vessels		
Present	47	59.5
Adhesion to sclera		
Present	3	11.4

FB, foreign body; TEO, tetracycline eye ointment.

Ocular surface squamous neoplasia diagnosis by histology and methylene blue staining

In a study involving 79 patients, the diagnosis of OSSN was established through a combination of histological examination and methylene blue staining. The findings, as outlined in Table 4, provide an overview of the frequencies of various diagnostic methods used in this research. All 79 cases underwent histological assessment, resulting in the identification of specific conditions within the cohort. Notably, one case (1.3%) was diagnosed as conjunctival intraepithelial neoplasia (CIN)-1, five cases (6.3%) as CIN-2, 11 cases (13.9%) as CIN-3, eight cases (10.1%) as CIS, two cases (2.5%) exhibited epithelial inclusion cysts, 26 cases (32.9%) were pterygia, two cases (2.5%) were diagnosed as pyogenic granuloma, and 24 cases (30.4%) were identified as SCC. According to the study's findings, a substantial portion of the patients received a diagnosis of OSSN, with 49 individuals (62.0%) falling into this category, while 30 patients (38.0%) received a non-OSSN diagnosis. The clinical staging of all 79 cases revealed the following distribution: 38.0% at stage T0, 13.9% at stage T1, 11.4% at stage T2, 5.1% at stage T3, and 31.6% at stage Tis. Furthermore, each of the 79 patients had a defined histologic grade, with 30.4% (24) classified as premalignant cases, 30.4% (24) as malignant cases, and 39.2% (31) as benign instances. Methylene blue staining was employed in all 79 cases to aid in the diagnostic process. Of these, 31 cases (39.2%) yielded negative results, while 48 cases (60.8%) displayed positive staining. A positive methylene blue test resulted in a distinct royal blue stain on the tissue, as depicted in Figure 1a, while negative cases did not take up the stain, as illustrated in Figure 1b.

TABLE 4: Frequencies of ocular surface squamous neoplasia and non-ocular surface squamous neoplasia by clinical staging and histological grade (N = 79).

Characteristics	*n*	%
Methylene blue		
Negative	31	39.2
Positive	48	60.8
Histology diagnosis		
CIN-1	1	1.3
CIN-2	5	6.3
CIN-3	11	13.9
CIS	8	10.1
Epithelial inclusion cyst	2	2.5
Pterygium	26	32.9
Pyogenic granuloma	2	2.5
SCC	24	30.4
OSSN versus non OSSN		
Non-OSSN	30	38.0
OSSN	49	62.0
Clinical staging		
Tis	25	31.6
T0	30	38.0
T1	11	13.9
T2	9	11.4
T3	4	5.1
Histologic grade		
Benign	31	39.2
Premalignant	24	30.4
Malignant	24	30.4

CIN, conjunctival intraepithelial neoplasia; CIS, carcinoma *in situ*; SCC, squamous cell carcinoma; T, tumour; OSSN, ocular surface squamous neoplasia.

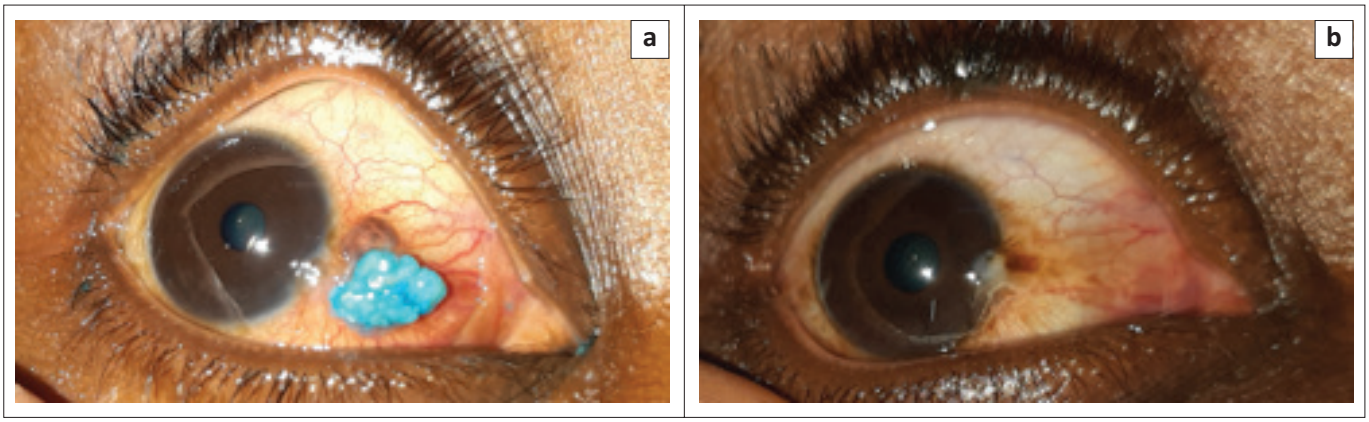

FIGURE 1: (a) Ocular *surface squamous neoplasia* conjunctival lesion taking up methylene blue dye, staining royal blue. (b) A non-ocular surface squamous neoplasia (pterygium) conjunctival lesion not taking up the dye.

TABLE 5: Methylene blue versus histology in diagnosing ocular surface squamous neoplasia.

Methylene Blue	OSSN versus non OSSN (histology)		
	OSSN	NON OSSN	Total
Positive	43	5	48
Pct	54.43	6.33	60.76
Row pct	89.58	10.42	-
Col pct	87.76	16.67	-
Negative	6	25	31
Pct	7.59	31.65	39.24
Row pct	19.35	80.65	-
Col pct	12.24	83.33	-
Total	49	30	79
Percentages	62.03	37.97	100.00

Col, column; Pct, percentage; OSSN, ocular surface squamous neoplasia.

TABLE 6: Sensitivity and specificity of methylene blue.

Statistic	Estimate	s.e.	95% CI
Prevalence	54.430	-	-
Odd ratio	35.833	-	9.912–129.541
Sensitivity	0.878	0.047	0.786–0.969
Specificity	0.833	0.068	0.700–0.967
Positive predictive value	0.896	0.044	0.809–0.982
Negative predictive value	0.806	0.071	0.667–0.945
McNemar's test for methylene blue versus histology			
$\chi^2 = 0.091$	-	-	-
$df = 1$	-	-	-
$P = 0.763$	-	-	-
Kappa coefficient for methylene blue versus histology	0.706	0.082	0.546–0.867

s.e., standard error; CI, confidence interval.

Table 5 presents a contingency table detailing the results of methylene blue staining for two distinct patient groups: those diagnosed with OSSN and those without the condition. This table provides a comprehensive breakdown, including the frequency, percentage, row percentages, and column percentages of staining outcomes for each group. According to the data presented in Table 6, the methylene blue staining test demonstrates an estimated sensitivity of 87.8% and specificity of 83.3%. Moreover, the test's negative predictive value (NPV) is calculated to be 80.7%, while the PPV stands at 89.5%. Further analysis utilising McNemar's test, as detailed in Table 6, indicates no significant difference between the diagnostic performance of methylene blue staining and histology in identifying OSSN cases ($\chi^2 = 0.091$, $P = 0.763$).

Discussion

This cross-sectional study aimed to assess the accuracy of methylene blue staining in diagnosing OSSN among patients referred for suspected OSSN and booked for excision biopsy at UTHs-Eye Hospital in Lusaka, Zambia. The study also provided insights into the prevalence, sociodemographic characteristics, clinical features, and associated risk factors of OSSN among the study participants.

The study reported a prevalence of OSSN among the study population of 62.0%, which is consistent with findings from a study in Tanzania.[12] This high prevalence underscores the significance of OSSN as a common ocular malignancy in the region. The study participants had a median age of 40 years, with a wide age range. This age distribution aligns with previous research on OSSN patients.[13,14] Notably, the study revealed a higher proportion of female participants (62.0%), which is consistent with some prior studies.[3,15] This gender difference may be attributed to differences in health-seeking behaviour and adherence to follow-up appointments between men and women in Zambia, as discussed in the study by Ngoma. According to Ngoma, in Zambia:

> [*A*] woman may make several trips to health facilities taking other members of the family for treatment, but when she is sick, she may be too tired to go to the clinic, or she may postpone the visit due to other pressing needs of the family. By the time she seeks medical care, it may be too late.[16]

Thus, more women than men would be expected to attend follow-up clinics and any additional examination recommended.

The study found a significant association between employment status and OSSN ($P = 0.02$), with a higher percentage of OSSN patients being self-employed. This association could be related to prolonged sun exposure during outdoor occupations, suggesting that occupational factors may contribute to OSSN risk. This finding aligns with the Zambia labour force survey report, which indicated

a significant informal employment sector.[17] Surprisingly, the study reported a low prevalence of alcohol use and smoking among participants, which contrasts with some previous studies associating OSSN with these risk factors.[15] However, this discrepancy might be because of the small sample size or variations in lifestyle factors among the study population.

The study identified a statistically significant association between HIV infection and OSSN ($P = 0.03$), with nearly half of the OSSN patients being HIV-positive. This finding is consistent with some prior research.[18] However, the study did not find evidence that HAART had a protective effect against OSSN development. The effect of HAART on HIV-related malignancies such as OSSN has not yet been studied. Masanganise et al. suggested that:

> The effect of HAART on HIV related malignancies like OSSN has not yet been studied, the evidence suggests that in patients who develop the malignancy prior to commencing treatment, HAART does not interrupt tumour growth or even its recurrence.[19]

Hence, emphasising the need for further investigation in this area.

It is important to notice that OSSN can present with a variety of symptoms. Conjunctival growth (93.7%) was the most common clinical presentation among OSSN patients, followed by foreign body sensation (29.1%). This trend has been previously seen in other studies.[20] The study also highlighted the variability in symptom duration; a significant proportion of participants (34.1%) experienced symptoms for at least 12 months. This was consistent with what was found in India, where the mean duration of symptoms was nearly a year.[21] It is essential to notice that the duration of symptoms does not necessarily correlate with the severity of the condition, as seen in this study, as it was observed that someone with a more advanced case of OSSN could have had symptoms for a shorter period than someone with a less advanced case. Early diagnosis and intervention remain crucial to prevent complications associated with delayed treatment.

Regarding previous treatment history, most participants (88.6%) reported to have not received treatment for OSSN in the affected eye. Only a small proportion of participants had received some form of treatment for their symptoms before presenting for the study. It is important to observe that the fact that only a small proportion had received some form of treatment before presentation, this limits the ability to conclude the effectiveness of previous treatments.

Various clinical findings were also reported, including pigmentation patterns, feeder vessels, and adhesion to the sclera. Brown pigmentation was the most common finding present in 39.2% of participants, followed by white pigmentation (21.5%), pink pigmentation (8.9%), black pigmentation (1.3%), and red pigmentation (1.3%). Additionally, 27.8% of participants did not have abnormal pigmentation. These findings align with those from other African studies[18] and highlight the invasive nature of OSSN in some cases.[15]

All the study participants underwent a histological diagnosis. The histological grade showed that most cases were malignant, followed by premalignant, and 39.2% were benign. The clinical stage of the disease was identified in all cases, with 31.6% of patients having Tumour *in situ* (Tis), which indicates CIS, the earliest stage of cancer. In addition, 30.4% had SCC, the most advanced stage of OSSN. The findings are not consistent with most of the previous studies; for example, a study done in Zambia showed invasive OSSN as the most frequent diagnosis.[15] Our explanation for this inconsistency could be that only participants with clinical stages one to three were enrolled in this study.

Methylene blue staining showed good sensitivity (87.8%) and specificity (83.3%) in diagnosing OSSN, with a high PPV and NPV. This suggests that methylene blue staining can serve as a reliable alternative to histology, offering a quick and non-invasive diagnostic method for OSSN ($\chi^2 = 0.091$, $P = 0.763$), and simple Kappa coefficient estimated to be 0.706 suggesting that both methods have similar performance in diagnosing OSSN; hence, methylene blue staining can be a reliable alternative to histology in diagnosing OSSN and can also be used during surgery to delineate a lesion and guide margins for excision.[20] The findings differ from Steffen et al., who conducted a prospective study of 75 participants and described a sensitivity of 97.0%, a specificity of 50.0%, a PPV of 60.0%, and an NPV of 96.0%;[10] our exclusion criteria could have resulted in these differences.

The findings of this diagnostic cross-sectional study had a number of significant ramifications for Zambia's clinical practice and public health. The association between self-employment and OSSN points to the necessity for sun protection and occupational safety measures, particularly for people working or cooking from outside. The significant association between OSSN and HIV infection necessitates the development of programmes that consider teaching upcoming clinic staff on how to routinely assess HIV-positive patients to detect these lesions early. Last but not least, methylene blue staining's promising diagnostic accuracy suggests that it has the potential to be a reliable alternative for histology, particularly in resource-restricted contexts where access to histological analysis is curtailed. By using this approach, OSSN patients may receive diagnosis and therapies more quickly.

This study has a number of limitations that should be noticed despite its conclusions. Firstly, the relatively small sample size may limit the generalisability of the results to Zambia. A bigger and more varied sample would give a more accurate representation of OSSN in Zambia. Secondly, the study's selection bias is introduced by its reliance on patients who were referred for excision biopsy at the UTH-Eye hospital. This is because the study does not include cases that did not end up UTH.

To gather knowledge about the health habits of people working and cooking under ultra violet rays, a qualitative study will be essential to identifying the underlying causes of late admission and gender-based disparities in OSSN diagnosis. Methylene blue staining should be used as a diagnostic tool in various healthcare settings and areas based on the practicality and cost-effectiveness of doing so, according to economic evaluations. In places where excision biopsy and histology are performed, it should be used to delineate margins for excision biopsy.

Conclusion

This study contributes valuable insights into the prevalence, sociodemographic features, clinical characteristics, and diagnostic accuracy of methylene blue staining for OSSN in Zambia. The findings support the use of methylene blue staining as an effective and practical diagnostic tool for OSSN, particularly in resource-limited settings where histology may not be readily available. Early diagnosis and intervention remain crucial in managing OSSN and improving patient outcomes. Further research is warranted to explore the role of HAART in OSSN prevention and to validate the utility of methylene blue staining in larger populations.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

A.M. designed the study, wrote the protocol, and wrote the first draft of the manuscript. S.N.S. performed the statistical analysis. P.D.J. analysed and reported the histology specimens. P.D.J., P.M., and E.M.M. managed and supervised the whole study from proposal write up to manuscript submission.

Ethical considerations

An application for ethical clearance was made to the University of Zambia Biomedical Research Ethics Committee and ethics consent was received on 30 September 2021. The ethics approval number is NHREB00013/30/09/2021.

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Data availability

The data that support the findings of this study are not openly available because of human data and are available from the corresponding author, A.M., upon reasonable request.

Disclaimer

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