The use of Lugol's iodine in achieving surgical margins free from dysplasia and invasive carcinoma in squamous cell carcinoma of the oral cavity

A dissertation submitted to the M.G.R. Medical University, Tamil Nadu: in partial fulfillment of the requirement for the M.S. Branch I (General Surgery) examination held in April 2014.

Certificate

This is to certify that the dissertation entitled "**The use of Lugol's iodine in achieving surgical margins free from dysplasia and invasive carcinoma in squamous cell carcinoma of the oral cavity "** is a bonafide work done by Dr. J. Nithyla Rosalynn, post graduate resident in Masters of General Surgery 2011-2014 at the Christian Medical College, Vellore, towards partial fulfillment for the MS General Surgery Branch I final examination held in April 2014.

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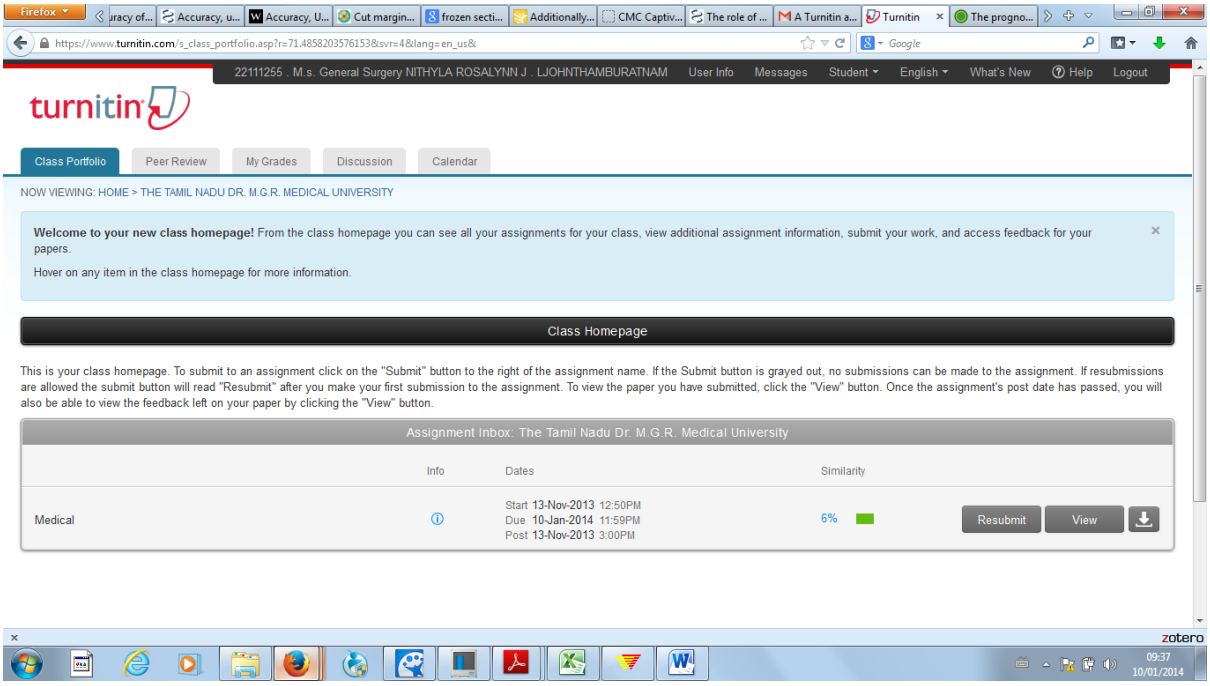
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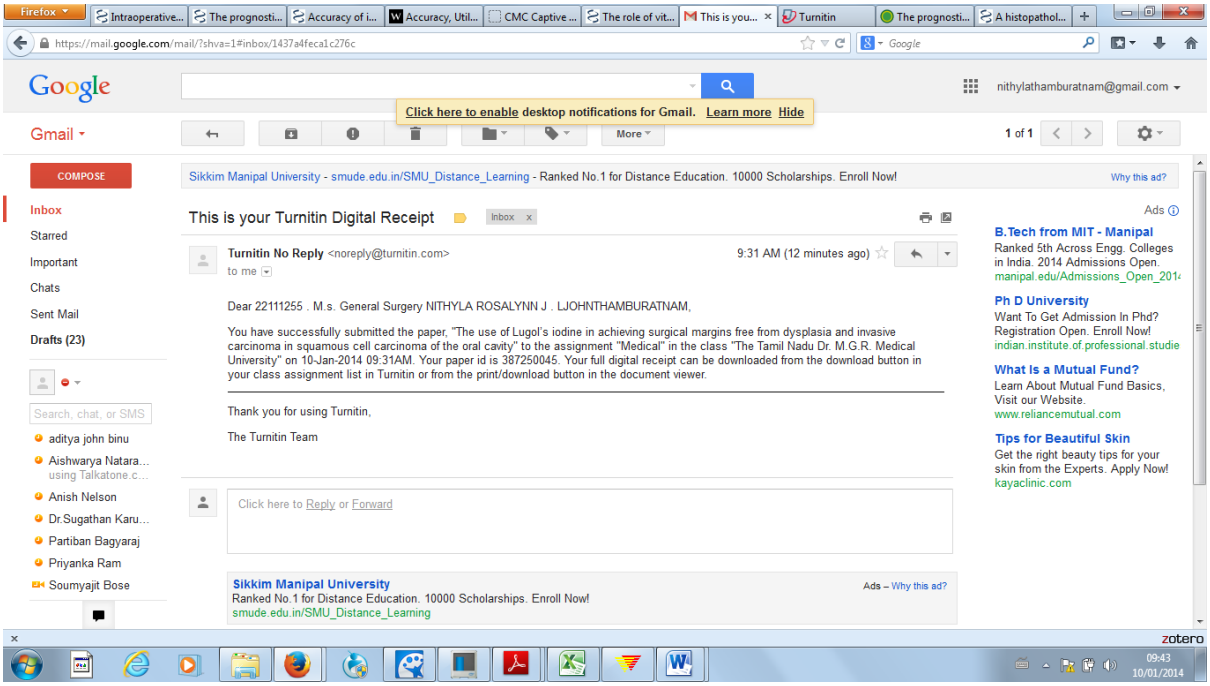
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Abstract

Title of the abstract: The use of Lugol's iodine in achieving surgical margins free from dysplasia and invasive carcinoma in squamous cell carcinoma of the oral cavity

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Degree and subject : M. S. General Surgery

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Aim:

To study the utility of Lugol's iodine in achieving tumour free surgical margins in squamous cell carcinoma of the oral cavity.

Objectives:

(1) To determine whether the use of Lugol's iodine resulted in reduced incidence of involved and close surgical margins, in squamous cell carcinoma of the oral cavity.

(2) To determine whether the use of Lugol's iodine enabled the surgeon to visualize the tumour extent better compared to clinical examination alone.

(3) To study the staining patterns observed following local application of Lugol's iodine in squamous cell carcinoma of the oral cavity.

(4) To look for association between the various staining patterns and the incidence of involved and close resection margins on final histopathology.

Materials and methods:

We evaluated the use of Lugol's iodine in achieving surgical margins free of dysplasia or invasive carcinoma by an observational study of two sets of patients who underwent resection for oral carcinoma between 15.05.10 – 15.06.11 and from 15.09.12 to 15.10.13. The patients in the second group underwent staining with 1.25% Lugol's iodine as per the described procedure. The patterns of staining were noted and the margins on clinical examination and following Lugol's staining were compared. Resection was left to the surgeons discretion. Margin status was compared with the retrospective cohort of 60 patients operated over a similar duration.

Results:

90 patients underwent resection in the study period between 15.09.12 to 15.10.13, of which data from 30 patients was available for analysis . The incidence of close and positive margins decreased significantly (17% vs 37% and 6% vs 38% respectively) in the cohort where Lugol's iodine was used ($p = 0.000$).

Conclusion:

With appropriate patient selection, staining with Lugol's iodine appears to be a promising method of obtaining tumour free resection margins in carcinoma of the oral cavity. However, studies with a larger sample size and randomization are required to further establish it's utility.

Introduction

Head and neck malignancies are the sixth most common of all malignancies worldwide, squamous cell carcinoma accounting for 95% of them. In the Indian subcontinent however, it is overall, the most common cancer. In terms of frequency, it ranks first among the male population and third among the female population. The reason for this increased incidence can be attributed to the habit of chewing paan, khaini and other forms of smokeless tobacco, peculiar to this geographical location, which in addition to alcohol and smoking, has been identified as one of the major risk factors in the development of oral malignancies.

The 5 year survival has been reported to be 58% among the Caucasian population and 45 – 49% in the European population (1). In contrast, Indian statistics cite a 5 year survival of approximately 30%, likely due to the higher proportion of advanced disease at presentation in these patients. Apart from the high mortality rate, oral malignancies pose a significant therapeutic challenge due to the high rate of local recurrence and the incidence of second primaries within the oral cavity, pharynx, oesophagus and respiratory tract.

Many factors have been identified, that are associated with increased likelihood of recurrence. Most of these factors are related to tumour biology, stage of the disease and final histopathology following excision. Of particular note among these, is the presence of close (invasive carcinoma $\leq$ 4mm from the resection

margin) or involved margins (invasive carcinoma at the resection margin), which has been reported to have a significant association with increased local recurrence rates and decreased 5 year survival. The presence an inadequate margin alone, of all the identified adverse risk features, is within the direct control of the surgeon and thereby amenable to modification by variation in treatment strategies.

Furthermore, it is one of the factors which dictate the need for adjuvant therapy. Current management protocols, as described in the NCCN (National Comprehensive Cancer Network) guidelines for management of carcinoma of the oral cavity, state that the presence of close or involved resection margins in any one direction requires the addition of radiation therapy. Hence, in early lesions (stage I, II) where surgical resection alone would normally suffice, the presence of inadequate surgical resection margins necessitates the addition of a second modality of treatment, namely radiotherapy. As radiotherapy to the head and neck region can be administered only a single time, this results in the utilization of a therapeutic modality which could otherwise be held in reserve for recurrences or second primaries.

Additionally, though the use of postoperative radiotherapy administered to patients with close or involved resection margins decreased local recurrence rates, it was unable to match the local recurrence rates of those who had

adequate margins in the first instance. Also, in these instances, although postoperative radiotherapy decreased local recurrence rates significantly, it did not impact five year survival (2), (3). The presence of inadequate resection margins also necessitates prolonged hospital stay due to wound complications, re- excision and additional chemoradiation. In a developing country like India where the burden of this disease is high, the financial implications are likewise, significant.

Various solutions have been sought to decrease the incidence of inadequate surgical resection margins. These include the use of imaging modalities preoperatively, frozen sections to determine adequacy of the margin intra-operatively and the use of an optical spectroscope for better visualization of malignant tissue, to aid complete resection. Of particular interest is the use of vital dyes including toludine blue and Lugol's iodine for staining malignant tissue. These dyes stain malignant tissue a different colour from normal tissue. They can be used as an adjunct to clinical examination in identifying the presence and extent of malignant tissue intra-operatively and to better delineate lesions unclear on clinical examination, thereby improving completeness of resection.

Another attempt at decreasing the incidence of inadequate margins involves excising a wider margin of normal tissue surrounding the malignancy. The

optimal margin of resection has been debated. This poses a significant challenge in oral malignancies, given the limited space and the functional aspects of the oral cavity. A smaller margin of excision leaves to question the adequacy of resection margins, especially after allowing for post excisional and post fixation shrinkage of tissue. A minimum of 1 cm circumferential margin of normal tissue is required to ensure the adequacy of resection margins. However, excision of a larger margin of tissue within the oral cavity will cause greater compromise of oral functions including speech, swallowing, mouth opening and continence of the oral commissure, as well as greater cosmetic deformity. Additionally, given the high proportion of advanced lesions at presentation, larger excision margins will result in larger defects, requiring the employment of increasingly complex options in the reconstruction ladder, for closure of the defect. This will require additional operating time and financial support, which, in a country where resources are limited, becomes an important factor in deciding treatment. The ideal margin, which is a balance between these considerations, is a matter of debate and a consensus as to the optimal margin is yet to be reached.

In our institution, we operate on approximately 60 cases of malignancies of the oral cavity each year. Looking into the incidence of close and involved margins in our institution, it was found to be 44% for close margins and 34% for involved margins. When compared to the incidence rates reported worldwide -

approximately 20 – 43 % for close margins and ranged from 11-32% involved margins (2), (4), (5), (6) - it was clear that further steps were required to decrease this relatively high rate of inadequate margins.

After debating the available options and the feasibility of each in our setting, it was decided to use Lugol's iodine to stain the lesion in a bid to better visualize abnormal tissue and thereby decrease the incidence of inadequate resection margins. Lugol's iodine was preferred due to the wide availability, simple procedure, reproducibility, low cost, few side effects and minimal additional time required. Following staining with Lugol's iodine, the close and involved margin rates were re-examined for change and the observed findings constitute this thesis.

Literature review

Oral malignancies are of particular relevance in the Indian subcontinent, by virtue of sheer numbers. In this geographical location, the incidence is far more frequent compared to the overall global incidence, making it the most common cancer in India. This fact alone indicates the need of further efforts to control and mitigate the effects of this disease. (7)

Furthermore, a significant proportion of malignancies of the oral cavity occurs in people of the lower socioeconomic strata, likely due to the habit of chewing khaini or paan, a potent carcinogen. Compared to Western literature,where patients present early in the course of the disease, in India, a significant proportion of patients tend to present with advanced stages. Hence, therapy frequently involves extensive resection with relatively complicated reconstructive procedures and usually requires additional radiation or chemotherapy. Taking into account the fact that most of these patients are unlikely to be covered by health insurance and that the existing government schemes cannot provide adequate relief, the financial demands of treatment on these patients becomes a significant consideration in their treatment. It is hence of paramount importance that further steps are taken to address this problem.

Anatomy:

The oral cavity extends from the vermillion border of the lips upto the circumvallate papilla, which demarcates the anterior two- thirds of the tongue -

part of the oral cavity - from the posterior thirds, which forms a part of the oropharynx.

It includes the following subdivisions:

- Lip
- Buccal mucosa
- Anterior tongue
- Alveolus
- Floor of mouth
- Retromolar trigone
- Hard palate

Epidemiology:

In 2008, Jemal et al reported the worldwide incidence of malignancies of the oral cavity to be 263,900, causing 128,000 deaths. The geographical distribution of oral cavity malignancies is uneven, with two thirds of all cases occurring in developing countries, predominantly in South East Asia. Reported age standardized incidence rates varied from 9.4 and 5.5 among males and females respectively in South East Asia, to a corresponding 5.1 and 2.8 in Northern Europe (8) . SEER (Surveillance, Epidemiology and End Results program) database statistics of 2013 reported an incidence of 10.8 new cases per

100,000 men and women, causing 2.5 deaths per 100,000 of the population. This comprised 1.4 % of all cancer deaths in the United States of America. In terms of proportion among all cancers, it accounts for 30% of all cancers in India, as opposed to 3% in the UK and 6% in France (International agency for research on cancer WHO).

Asia, particularly India, is home to 57.5% of all head and neck cancers. The annual incidence of head and neck cancers in India alone is greater than 200,000. It is the most common cancer among Indian males, accounting for 30% of all malignancies (74), (75). Among Indian females, it ranks third in order of frequency, following carcinoma of the cervix and breast, and accounts for 16% of all malignancies (9)

Demographic profiling shows that the mean age at diagnosis is 63 years. It occurs more commonly in males, with a male: female – 2.42:1. The burden of this disease falls on the lower and lower middle classes of the population. Histopathological examination shows that 90- 95% of all oral malignancies fall under the category of squamous cell or epitheliod carcinoma. The most common sites of occurrence are the tongue and buccal mucosa. Involvement of the floor of mouth and lip are usually due to contiguous spread from adjacent

areas, as primary malignancies at these sites are relatively less common. In India, 60-80% of patients present with advanced stages of the disease (stage III, IV) as opposed to 40 % of patients in developed countries (10). The differences in the prevalence of use of carcinogenic agents likely accounts for some of the demographic differences, as is evidenced by the use of paan in the Indian subcontinent and the increased use of tobacco and alcohol among males irrespective of geographical location.

Risk factors:

The development of malignancy in the oral cavity is attributed almost exclusively to the major risk factors, which include smoking, alcohol consumption and the use of smokeless tobacco. Other identified risk factors include human papilloma virus infection, consumption of spicy food, poor oral hygiene, periodontal disease and repeated trauma due to the presence of sharp facets on adjacent teeth (11). Hereditary factors have little or no role to play in the etiology and pathogenesis of oral carcinomas. Dietary factors including high vegetable and fish consumption were noted to have a protective effect in sporadic trials.

Heavy cigarette smoking increased the risk of developing cancer to 5 – 25 times that of similarly matched non smokers (12), (13), (14). The relative risk in

smokers was reported to be 6.5 (15). The strength of association between smoking and development of malignancy was directly proportional to the duration of smoking and number of cigarettes per day. Tobacco being the active principle predominantly responsible causing malignancy, there was no difference between those smoking cigarettes, cigars or pipes. Cessation of smoking reduced the relative risk of developing cancer, however, it was equivalent to non smokers only after 20 years.

Alcohol consumption independently increased the risk of developing oral cancer, the relationship being more pronounced in those who consumed larger quantities. It was noted by Stefani et al that regular consumption of 50g of alcohol per day increased the risk of developing oral cavity cancer by 5 - 6 times, compared to those who consumed less than 10g per day (16). Apart from the independent effect in causing cancer, alcohol also showed a synergistic, almost multiplicative effect, when added to smoking (15) (17).

Paan, khaini and gutkha are various forms of smokeless tobacco available in the Indian subcontinent. They contain variable combinations of betel leaves, areca nut, tobacco and slaked lime and are used in different forms, for either chewing or inhalation (18). Both forms are implicated in the development of oral cavity

malignancy and are in part responsible for it's increased incidence in this geographical location (19).

Though tobacco and alcohol were established risk factors for development of malignancies of the head and neck region, investigators noted, however, that despite a decrease in the prevalence of both, the incidence and prevalence rates of oral and oropharyngeal cancer failed to show a corresponding trend. Additional risk factors were sought and this led to the emergence of human papilloma virus (HPV) infection as an added risk factor contributing to carcinogenesis in these sites. The prevalence of HPV in the oral cavity was noted to be 10.1 % in men and 3.6% in women, resulting in an overall prevalence of 6.9% (20). Based initially on the study of epidemiological patterns, followed by molecular evidence, HPV has been causally linked to the development of carcinoma of the head and neck region. HPV DNA was detected in 10% and 37% of oral cavity and oropharyngeal carcinomas, respectively (21) . The predominant subtypes were HPV16, 18 and 33. HPV associated cancers occurred 10 years earlier on an average and were less likely to be associated with second primaries (22).

Premalignant lesions:

Premalignant lesions are, by definition, those lesions which presently do not exhibit histopathological features of invasive carcinoma, but have the potential, over time and in response to risk factors, to develop into overt malignancy. Commonly described premalignant lesions of the oral cavity include leukoplakia, erythroplakia and submucous fibrosis. Each has a different likelihood of developing into overt carcinoma. The malignant potential of other lesions like oral lichen planus is yet to be established beyond doubt. The risk factors implicated in the development of premalignant lesions are broadly the same as those for invasive squamous cell carcinoma.

Leukoplakia is defined as the presence of a white plaque or patch that cannot be classified, either on clinical or histopathological examination, as any other characterized disease process. On histopathology, leukoplakic patches show hyperkeratosis, dysplasia or carcinoma in situ. A small percentage of these patches (3.1%) can contain invasive squamous cell carcinoma, which had been mistaken as a benign premalignant process, as was reported by Waldron et al in their description of a large series comprising 3256 specimens (23). Leukoplakia occurs more commonly in middle aged to older males and is frequently noted over the buccal and gingival mucosa. Variants described include nodular leukoplakia, speckled leukoplakia (with interspersed patches of erythroplakia)

and proliferative verrucous leukoplakia, the last of which is associated with a higher risk of progression to invasive carcinoma (noted in upto 70%) (24). The malignant potential of leukoplakia is reported to range from 15.6 to 39.2%. Lee and colleagues noted among a cohort of patient patients with leukoplakia, after a median 7 year follow up, that the incidence of invasive carcinoma was 31.4%, with an annual incidence of 5.7% (25). Important considerations in the management of leukoplakia include biopsy of suspicious lesions to uncover masquerading malignancy and the use of beta carotenes and 13 – cis retinoic acid, which have been shown to induce regression of these lesions, though conclusive trials are still underway.

In appearance, erythroplakia is the erythematous counterpart of leukoplakia and is manifested as velvety, red lesions that are usually discrete. It is important to distinguish erythroplakia from inflammatory conditions of the oral cavity, which can present in a similar fashion. Though usually asymptomatic, few patients can present with a burning sensation. Erythroplakia is commonly noted in older males, on the floor of the mouth or the lateral border of the tongue. Though less commonly prevalent in comparison to leukoplakia (0.1 – 0.6 % vs 10.3%), it is more likely to be dysplastic. Histopathological examination of a series comprising 65 specimens showed dysplasia in all cases, with half demonstrating invasive carcinoma (26).

Submucous fibrosis is a condition which involves extensive inflammation and fibrosis in the lamina propria and deeper tissues, predominantly affecting the buccal mucosa. This causes trismus, which in addition to affecting the quality of life by limiting oral intake, can also interfere with complete examination and biopsy of the oral cavity, impeding the diagnosis of malignancy. The development of submucous fibrosis is strongly linked to the use of various forms of smokless tobacco, mentioned earlier. A malignant transformation rate of 17% has been reported.

Pathogenesis:

Our current understanding of the pathogenesis of head and neck malignancies owes it's origin to the contribution made by Slaughter, when he proposed his theory of field cancerization. Following histopathological examination of 738 resected specimens containing malignancies of the oral cavity or pharynx, he made the observation that there were multiple areas exhibiting various degrees of dysplasia in the macroscopically normal tissues adjoining the tumour (27)

Further work carried out along these lines involved follow up of dysplastic lesions to observe how many of them progressed to invasive carcinonma, and

comparison of the molecular profiles of synchronously present dysplastic and malignant foci. A malignant transformation rate of 51% (variably reported as 15 – 30% by other investigators) was noted in one study which included 97 dysplastic lesions followed up for a mean duration of 30 months. It was additionally noted on subgroup analysis that the potential for malignant transformation was directly proportional to the degree of dysplasia (28). It was also observed on molecular analysis that the genetic alterations noted in synchronously present dysplastic and malignant foci were not always concordant (29). These findings led to some important conclusions – that dysplasia and malignancy had similar causative factors and that there was a clear progression of certain dysplastic lesions to overt malignancy. It was also demonstrated that adjacent clinically normal tissue was preconditioned as a result of field cancerization and had the potential to later develop second primary malignancies in response to a different set of initiating events, as evidenced by molecularly discordant clones.

From these findings evolved our understanding that the development of invasive carcinoma from premalignant lesions is a multistep process, as described in Vogelstein's hypothesis for colonic cancer. It involves mutations at multiple steps, affecting genes involved in mitosis, cell cycle regulation, cell to cell adhesion, cell signaling pathways, apoptosis and DNA repairs. Progressive

accumulation of these mutations due to different initiating events, results in the development of invasive carcinoma from premalignant and dysplastic lesions.

Implicated genes that are involved in mitogenesis and cell signaling pathways include epidermal growth factor receptor (EGFR), transforming growth factor – alpha (TGF-alpha), HRAS (of the Ras family) and HER2 gene (30), (31), (32). Cell cycle control factors disrupted include cyclin D1 (overexpression) with inactivation of p53, p16 and p14 foci (33), (34), (35) . Mutations of the doc 1 (deleted in oral cancer) gene and genes associated with E – cadherin and matrix metalloproteinases cause disruption of intercellular adhesion and differentiation (36), (37), (38) . Upregulation of Bcl-2 (B – cell leukaemia - 2) and Bcl- xL, two genes involved in the antiapoptotic pathways are also noted in head and neck malignancies (39). Observation of these mutations associated with squamous cell carcinoma of the head and neck is of both prognostic and therapeutic significance.

Pathology:

The most common histological type is squamous cell carcinoma, which accounts for 90 – 95% of all malignancies of the oral cavity. These malignancies are further classified on the basis of differentiation into well

differentiated (> 75% keratinisation), moderately differentiated (25% - 75% kertininzation) and poorly differentiated (<25% keratininzation) types.

Presentation:

Patients commonly present with a growth or ulcer, corresponding to the exophytic or ulcerative forms of the disease. These lesions may be associated with pain and occasionally with bleeding as well. Carcinoma of the alveolus can present as loosening of the teeth. On examination, in addition to exophytic or ulcerative lesions, areas of induration, seen in infiltrative forms can be present. Premalignant lesions may also be seen. Depending on the site, advanced malignancies may present with trismus, difficulty in speech, swallowing or protrusion of the tongue, skin involvement, cervical lymphadenopathy and neuropathy involving the hypoglossal, marginal mandibular and inferior alveolar nerves.

Diagnosis and staging:

Given the accessibility of the oral cavity, diagnosis is usually straightforward and is established by complete physical examination, followed by histopathological examination of wedge biopsies from the edge of suspicious

lesions. Fine needle aspiration cytology comes into play in the presence of palpable neck nodes, either in isolation or in conjunction with an oral lesion.

The utility of imaging modalities – CT or MRI lies mainly in the more accurate identification of the extent of disease - particularly in infiltrative lesions, commonly seen in the tongue and to determine invasion of cortical bone. It is also used in the identification of cervical nodal metastasis, detection of second primaries and distant metastatic disease. While CT scan is superior for detection of infiltration of bone, MRI scanning provides better soft tissue resolution and is preferred for visualization of perineural invasion, intracranial extension and accurate delineation of tongue tumours. The role of PET CT scan is limited to the detection of metastasis and occult primary malignancy in patients presenting with cervical lymph node metastasis. NPL scopy (nasopharyngolaryngoscopy) is done routinely in some centres for detection of second primary malignancies.

Oral malignancies are most commonly staged according to the widely accepted AJCC (American Joint Committee for Cancer) classification, as mentioned in the 7^{th} edition of their handbook, which is as follows:

T1 - $\leq$ 2 cm

T2 - 2–4 cm

T3 - > 4 cm

T4a – Lip - through cortical bone, inferior alveolar nerve, floor of mouth, skin

- Oral cavity - through cortical bone, deep/ extrinsic muscle of tongue, maxillary sinus, skin of face

T4b - Masticator space, pterygoid plates, skull base, encasing internal carotid artery

N1 - Ipsilateral single $\leq$ 3 cm

N2 (a) - Ipsilateral single 3–6 cm

(b) - Ipsilateral multiple $\leq$ 6 cm

(c) - Bilateral, contralateral $\leq$ 6 cm

N3 - > 6 cm

M0 – No distant metastasis

M1 – Distant metastasis present

Stage 0 - Tis N0 M0

Stage I - T1 N0 M0

Stage II - T2 N0 M0

Stage III - T3 N0 M0

T1, T2, T3 N1 M0

Stage IVA - T1, T2, T3 N2 M0

T4a N0, N1, N2 M0

Stage IVB - Any T N3 M0

T4b Any N M0

Stage IVC - Any T Any N M1

Treatment :

Current treatment protocols as defined by the NCCN guidelines recommend that for early stage tumours (stage I, II), either radiotherapy or surgery can be offered as curative modalities, though surgery is usually preferred in most centres, as radiation can thus be reserved for recurrences, or in case of adverse prognostic factors on final histopathology.

In advanced stages, namely stages III and IVA, combined surgery and radiation is advised. Radiation protocol includes 66 – 74 Gy administered as 5 fractions per week according to the conventional schedule. Intensity modulated radiation therapy is preferred. Adjuvant therapy in cases where the surgical resection margin is involved with tumour includes chemotherapy in addition to radiation. Chemotherapeutic agents commonly used include cisplatin, 5 fluorouracil and drugs belonging to the taxane group.

Prognosis:

As can be expected, the prognosis, specifically local recurrence and five year survival rates are progressively worse in direct proportion to the stage of the disease. On surveying records of patients with oral cancer from 1996 – 2003, the SEER program calculated 5 year survival to be 82.8%, 51.8% and 27.8% for patients with local, regional and distant disease respectively.

Adverse prognostic factors:

Adverse prognostic factors that dictate the addition of chemoradiation include involved surgical resection margins (mucosal, soft tissue and bone) and extracapsular nodal spread of disease. Other established indications for radiation include N2 or N3 disease, pT3, pT4 disease, perineural, vascular or lymphatic invasion and positive level 4 or level 5 nodes.

Apart from these factors, it has also been proposed that the pattern of invasion of the tumour front seen on histology may have an impact on prognosis. Spiro et al described 4 patterns of invasion as follows; Grade 1—invasion in a broad "pushing" front with well-delineated border; Grade 2—invasion at the advancing edge in the form of solid cords, bands or strands; Grade 3—invasion in small groups or cords of infiltrating cells; Grade 4—marked cellular

dissociation in small groups and/or single cells (40). The latter two patterns were associated with poor prognosis. On the basis of this, a histological assessment score (combining pattern of invasion, lymphocytic response and perineural invasion) for treatment and prognosis has been developed (41).

Apart from patients with close or involved margins, it was also noted that 16 – 32% of patients with negative resection margins also developed local recurrence, with a corresponding decrease in survival (3),(2). It was hence hypothesized that tissue that appears phenotypically normal (on histopathology) may harbour genotypic abnormalities which may predict local recurrence, causing an impact on treatment and prognosis. Using molecular markers, it was noted that these margins exhibited multiple mutations of various genetic loci (as mentioned earlier) particularly those coding for p53, p16 and eIF4e (a translation factor) (42), (43) . On further evaluation, it was found that apart from being a more sensitive tool for the detection of malignancy, these markers were also found to be predictive of recurrence and survival. However, trials including molecular markers in the treatment and prognosis of oral cancer are still underway and their routine use is not a part of standard clinical practice yet.

Challenges in management:

Preservation of function:

There are specific challenges involved, peculiar to the management of malignancies of the oral cavity. The small area, combined with its functional importance in terms of speech and swallowing, are a particular test to the skill of a reconstructive surgeon. While oncological principles of resection cannot be compromised, mucosal continuity of the oral cavity has to be ensured to permit oral feeding.

Reconstruction:

In those patients who present with advanced stages of the disease, resection often results in a defect in the overlying skin as well, which also needs to be addressed. Additionally, the cosmetic outcome of these procedures cannot be ignored. For the above mentioned reasons, a microvascular free tissue transfer would be the ideal procedure for reconstruction of large defects, however the expertise and expense involved is often prohibitive. As these patients are generally nutritionally compromised, wound healing is not optimal. In patients undergoing operation for recurrence, prior radiation worsens wound healing, increasing the chances of flap reated complications. Flap failure, infection or necrosis can result in an orocutanous fistula which, besides delaying initiation of oral nutrition, prolonging hospital stay and delaying rehabilitation, may require additional corrective procedures.

Local and regional recurrence:

Recurrence following curative therapy is another complication frequently encountered in the management of oral cancery. Recurrence can take the form of either local recurrence at the resection site or region recurrence in the neck. Local recurrence rates have been variably reported as 10 - 16%, with locoregional recurrence upto 21% (44), (45) depending on the mean follow up period. Important predictors of recurrence include close or involved surgical resection margins and pattern of invasion of the tumour front. It has also been suggested that neodjuvant chemotherapy may play a role in recurrence, as the deep margin can be difficult to judge and may lead to inadequate tumour clearance (44). Locoregional recurrence is clearly associated with reduced 5 year survival rates and carries an overall poor prognosis. Three year cancer specific survival rates of 27.2 % – 64.3 % have been reported, depending on the clinical stage of the recurrence and the expression of molecular markers, particularly EGFR (46).

Synchronous, second primary tumours:

The observation that tumour biology is king is decidedly apparent in oral malignancies where, as a result of field cancerization, the incidence of second primary tumours is increased. A large retrospective study by Day et al noted that in head and neck cancers, second primaries developed at the rate of 3.7% per year, with a relative risk nearing 20 for oral cavity cancers at 3 years

following treatment (47). Rogers et al noted a second primary rate of 7% (45). The incidence rate varies according to definition, however most investigators prefer to use the criteria set down by Warren and Gates in 1932 (76). Second primaries can be either synchronous (detected within 6 months of diagnosis of the primary tumour) or metachronous (detected after 6 months from diagnosis of the primary tumour). They are distinguished from the primary tumour by the presence of 2 cm of normal intervening mucosa and a time interval of 3 years. These definitions have, however, been called into question following the advent of molecular typing. Newer concepts include second field tumours (as opposed to second primary tumours) and premalignant cell migration, which may in future have a therapeutic and prognostic impact (48).

Importance of negative surgical resection margins:

It has been clearly demonstrated that both close and involved margins are associated with higher rates of local recurrence and poorer 5 year survival rates. Loree et al noted local recurrence rates of 36% in patients with close or involved margins, as opposed to 18% in those with pathologically adequate margins. On follow up, they found that the 5 year survival rate in the former group was 52%, compared to 60% noted in those with adequate margins (2). Binahmed et al reported that the 5 year survival rates in patients with clear, close and positive margins were 69%, 58% and 38% (4) . Both these findings

were statistically significant. This emphasized the importance of obtaining adequate surgical resection margins.

In addition, of all the identified adverse prognostic factors, the surgical resection margin was the only alterable factor. As per the NCCN guidelines, the presence of close margins (invasive carcinoma $\leq$ 4cm from the edge of the specimen) warrants the addition of radiation and positive margins (invasive carcinoma at the edge of the resection specimen) mandates either re- excision or additional chemoradiation. It has also been noted that though recurrence rates decreased following adjuvant radiation therapy, it still didn't compare to those who had adequate margins initially. There was no significant improvement in 5 year survival following adjuvant radiotherapy for management of positive resection margins (2).

The worldwide incidence of close and involved margins varied. In order to assess the magnitude of the problem in our institution in comparison with international statistics, our institutional records of 50 consecutive patients undergoing curative resection for malignancies of the oral cavity prior to June 2011 were reviewed. It was found that 34% of our patients had involved margins, in contrast to 32% in Glasgow, 22% in Manitoba and 11% in New

York. The incidence of close margins was 44%, compared to a similar figure from Manitoba and 21% from New York (2), (49), (5). Review of international literature does not show a consensus regarding the definition of involved or positive margins. In the series in our institution and Manitoba, involved margins referred to the presence of invasive carcinoma at the resection margin. The series from New York included patients with dysplasia at the surgical margins as well, while the series from Glasgow, the involved margins group consisted exclusively of patients with dysplasia at the resection margins.

Methods explored to address the problem:

Various options have been explored to aid achieving negative surgical resection margins. They include pre operative measures such as imaging in addition to clinical examination, for better visualization of the extent of the tumour and intra operative measures, including the use of vital stains, optical spectroscopy, frozen section examination of the margins and increasing the width of surrounding normal tissue excised.

Increasing distance of normal tissue to be excised:

The adequate amount of normal tissue that is required to be excised circumferentially surrounding the tumour is controversial. A retrospective

study comparing the distance of clear surgical margins with 5 year survival by Nason et al noted significantly improved 5 year survival rates as the distance of normal tissue increased. They concluded that in their study cohort, which included various groups of patients - from involved margins upto 5 mm tumour free margins - each additional millimetre of tumour free tissue contributed to increased five year survival (49). The optimum tumour free margin taken routinely in our centre was 1 cm. This was corroborated by McMahon et all, who concluded that beyond 1cm macroscopic margin, there was no additional survival benefit. They suggested that inadequate histopathological margins and recurrence in these patients was a result of poor tumour biology, rather than inadequate resection (50).

However, there is notable discordance between the size of the resection specimen in situ and on histopathological examinaiton. Studies in canine models showed mean shrinkage of 30.7% for lingual mucosa and 47.3 % for buccal mucosa, with maximum shrinkage following resection and additional shrinkage following fixation in formalin (36). Beaumont and Haines noted a 46% reduction in longitudinal diameter with a mean reduction of 4.82 mm (77). They also concluded that shrinkage occurred mostly after resection, with formalin fixation contributing to 30% or less of the overall shrinkage. Current

NCCN guidelines recommend excision of 1.5 – 2 cm of normal tissue circumferentially around grossly malignant tissue.

Intraoperative frozen sections:

A large retrospective review from Mayo clinic including 24,880 pathology reports from general surgery specimens, reported a diagnostic accuracy rate of 97.8% for intraoperative frozen sections (52). Considering head and neck malignancy specimens alone, a similar accuracy rate (98.3%) with a sensitivity and specificity rate of 88.8% and 98.9% has been noted (53). It was noted that in approximately 67% of patients, resection margins were adequate using clinical examination alone (54). Additionally, though high sensitivity and specificity rates have been noted, the improvement in overall outcome in terms of local recurrence and survival is yet to be proved beyond doubt. It was noted that in approximately 67% of patients, resection margins were adequate using clinical examination alone.

Optical spectroscopy:

Spectroscopy is the study of the interaction between matter and radiated energy. Fluorescence spectroscopy analyzes the fluorescence from a particular sample. The property of certain cellular and extracellular components (endogenous

fluorophores) - notably mitochondria, lysosomes, collagen and elastin- to emit absorbed light when excited by radiation of certain wavelengths is called autofluorescence, and is a property of normal tissue. Changes in cellular components and extracellular matrix by a malignant process leads to alterations in the endogenous fluorophore concentration and distribution. This is manifest as a loss of fluorescence, or variation in the intensity of autofluorescence, which can be measured by various devices and can be indicative of neoplastic transformation (55), (56). Compared to direct visualization of autofluorescence, which is subjective and investigator dependent, analysis of digital fluorescence images yields more reliable results (57). Loss of autofluorescence has also been used intraoperatively to identify the extent of tumour and to direct the margin of resection (58).

Diffuse reflectance spectroscopy is based on the principle that different cellular and extracellular components have distinct refractive indices and tend to scatter light differently. Hence, when light of a certain wavelength is incident on tissue, different reflective or scatter spectra are noted depending on the composition of the tissue. This scatter spectrum can be analyzed by various fibreoptic probes and other devices. Given the changes in tissue composition associated with neoplasia, malignant tissue can be differentiated from normal tissue by various types of reflection spectroscopy. Analysis of scatter spectra

using white light, ultraviolet light and the interaction of gold impregnated nanoparticles have been described to better delineate malignancies in the oral cavity (59), (60) .

Vital stains – Lugol's iodine:

To identify malignancy, vital stains commonly used in clinical practice include toludine blue and Lugol's iodine. The sensitivity and specificity of toluidine blue was noted to be 0.925 and 0.632, while that of Lugol's iodine was 0.875 and 0.842, respectively (61). Due to low specificity and limited utility in dysplasia, toludine blue is not preferred (62), (63).

Lugol's iodine is a combination of iodine (I_2), potassium iodide (KI) and distilled water, which in various proportions forms the different percentages of the solution available (10%, 5%, 3%, 1.25%). 5% Lugol's iodine contains 5 g of iodine mixed with 10 g potassium iodide and 85 ml distilled water. The total iodine content of this solution is 150 mg/ml. It penetrates living tissue and binds to glycogen found in superficial epithelial cells, staining normal tissue mahogany brown or black. Due to increased utilization of glycogen by malignant tissue (Warburg effect) associated with unchecked cellular proliferation, malignant cells contain less glycogen and therefore appear as

ayellow, pale, unstained lesion (64), (65). Based on this principle, malignant or dysplastic tissue can be identified from normal tissue.

Lugol's iodine was primarily used in the treatment of toxic goitre. As a vital dye, it gained widespread popularity as the Schiller's test, to identify dysplastic and malignant lesions in the cervix. It's use in the upper aerodigestive tract began with identification of dysplastic lesions in the oesophagus during endoscopy (66). This was later employed in the oral cavity, as a simple test for diagnosis of suspicious lesions and for taking directed biopsies. It was also shown in one study that different shades of discolouration after application of Lugol's iodine was associated significantly with the varying degrees of dysplasia. Based on this observation, the investigators hypothesized that it was possible to develop a colorimetric scale correlating with histopathology, that would enable macroscopic discrimination of the various degrees of dysplasia by comparing the shade of the unstained lesion with a colour scale (67) .

Apart from phenotypical studies, genotypical studies involving measurement of p53, PCNA (proliferating cell nuclear antigen) levels and telomerase activity, which are associated with malignancy in tissues, also revealed good correlation between Lugol's unstained areas and the presence of malignancy (68), (69) . Later studies were directed towards the use of Lugol's iodine to better identify margins. Kurita et al compared the distance between the boundary of the

tumour noted following the application of Lugol's iodine, with the location of the proliferative tumour front on final histopathology. They noted a mean difference of 0.81 mm ± 0.64 mm (mean ± standard deviation), showing that the margin of the lesion on examination with Lugol's iodine correlated well with the histopathological tumour front (70).

The wide availability, ease of use, rare occurrence of side effects and modest cost of Lugol's iodine makes it a promising option to employ, in a bid to reduce the incidence of close or positive surgical resection margins. However, Lugol's iodine cannot be used in keratinized epithelial surfaces and the normal epithelium in these tissues contains only small amounts of glycogen. Hence, in these regions, normal tissue also appears unstained and cannot be differentiated from malignant tissue.

Other methods:

Other methods including brush cytology, improved imaging with MRI for better determination of the extent of the lesion preoperatively have also been used with varying success.

Aims and Objectives

Aim:

To study the utility of Lugol's iodine in achieving tumour free surgical margins in squamous cell carcinoma of the oral cavity.

Objectives:

(1) To determine whether the use of lugol's iodine resulted in reduced incidence of involved or close surgical margins, in squamous cell carcinoma of the oral cavity.

(2) To determine whether the use of Lugol's iodine enabled the surgeon to better visualize the tumour extent compared to clinical examination alone.

(3) To study the staining patterns observed following local application of Lugol's iodine in squamous cell carcinoma of the oral cavity.

(4) To look for association between the various staining patterns and the incidence of involved and close resection margins on final histopathology.

Materials and Methods

Following approval by the institutional review board, after taking informed consent, all consecutive eligible patients operated from 15.09.12 to 15.10.13 (13 months) were enrolled in the study. No method of randomization or blinding was used.

Inclusion criteria:

(1) Biopsy proven squamous cell carcinoma.
(2) Primary malignancies of the alveolus, buccal mucosa, anterior tongue and floor of mouth.
(3) Resectable tumours.
(4) Age > 18 years.

Exclusion criteria:

(1) Known allergy to iodine.
(2) Severe trismus noted preoperatively.
(3) Pregnancy.

Procedure:

- Intra-operatively, on examination under anesthesia, the extent of the tumour on clinical examination was determined and marked. The proposed boundary of resection including a 1 cm circumferential margin of normal tissue was marked using diathermy.

- Mucus secretions were removed by irrigating the oral cavity with 30 ml of 5% carbocisteine syrup, left for 1 minute.
- The entire oral cavity was irrigated with 1.25% Lugol's iodine taken in a 10ml syringe.
- Excess Lugol's iodine was removed using suction.
- Irrigation with Lugol's iodine was repeated until the mucosa distant to the malignancy stained dark brown.
- The adequacy and pattern of staining with Lugol's iodine was noted.
- The extent of the tumour following the application of Lugol's iodine was compared for any discordance and the resection boundary was revised where deemed appropriate.
- Resection was carried out according to the surgeon's discretion, by either

  - following the initial plan, guided by clinical margin alone,

  - increasing the resected area as indicated following the use of Lugol's iodine, or

  - additional stripping of the mucosa in cases where there were large areas of abnormal mucosa indicated by Lugol's iodine, but not identified on clinical examination .
- The resected specimen was oriented, fixed in formalin and sent for histopathological examination.

The primary outcome measured was the incidence of close and involved margins in this group of patients, designated group B. The margin status in these patients was noted and compared with a retrospective cohort of similar patients (60 patients operated from 15.05.10 – 15.06.11), designated group A

The results were tabulated and subject to statistical analysis. Pearson's chi square test/ Fischer's exact test was used as applicable, to identify significant association.

Procedure and interpretation of staining with Lugol's iodine

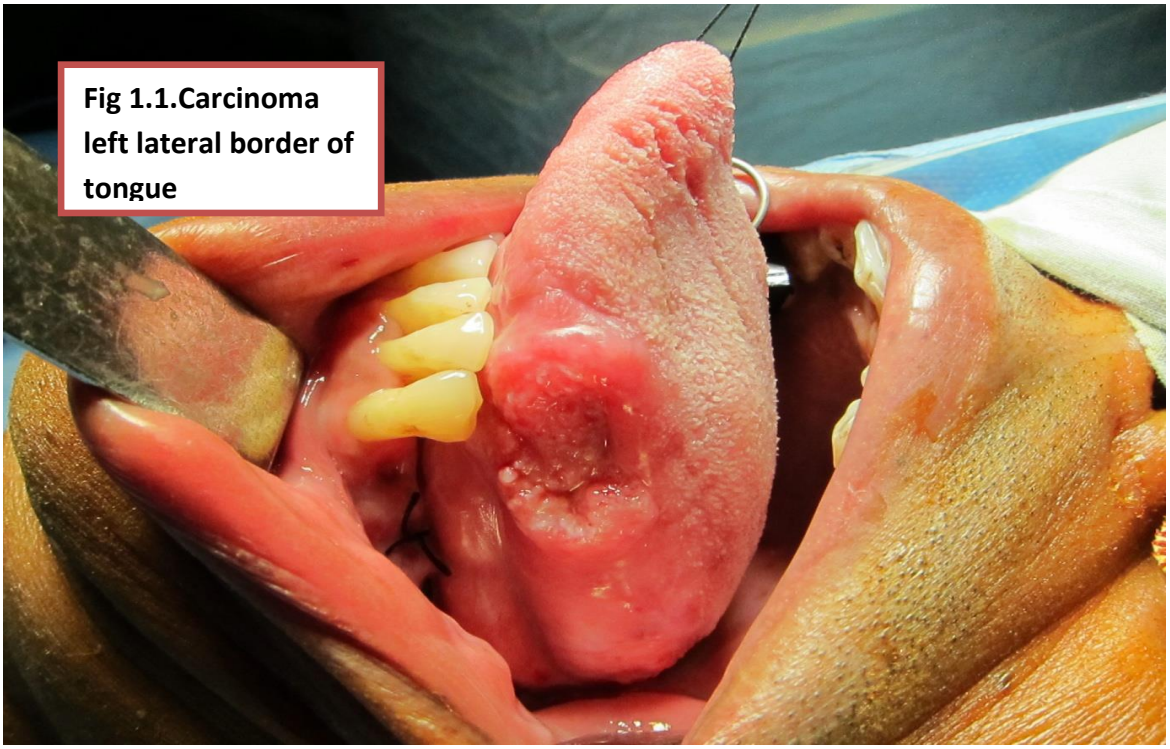


Fig 1.1.Carcinoma left lateral border of tongue

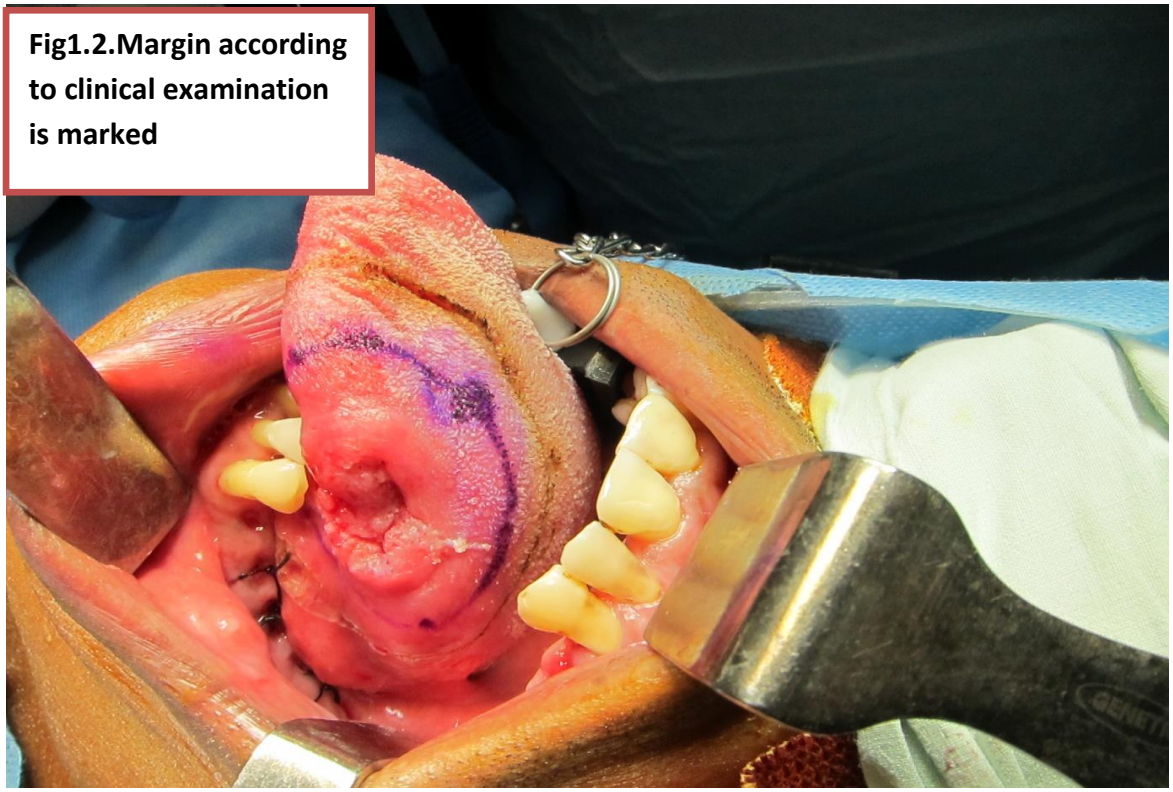


Fig1.2.Margin according to clinical examination is marked

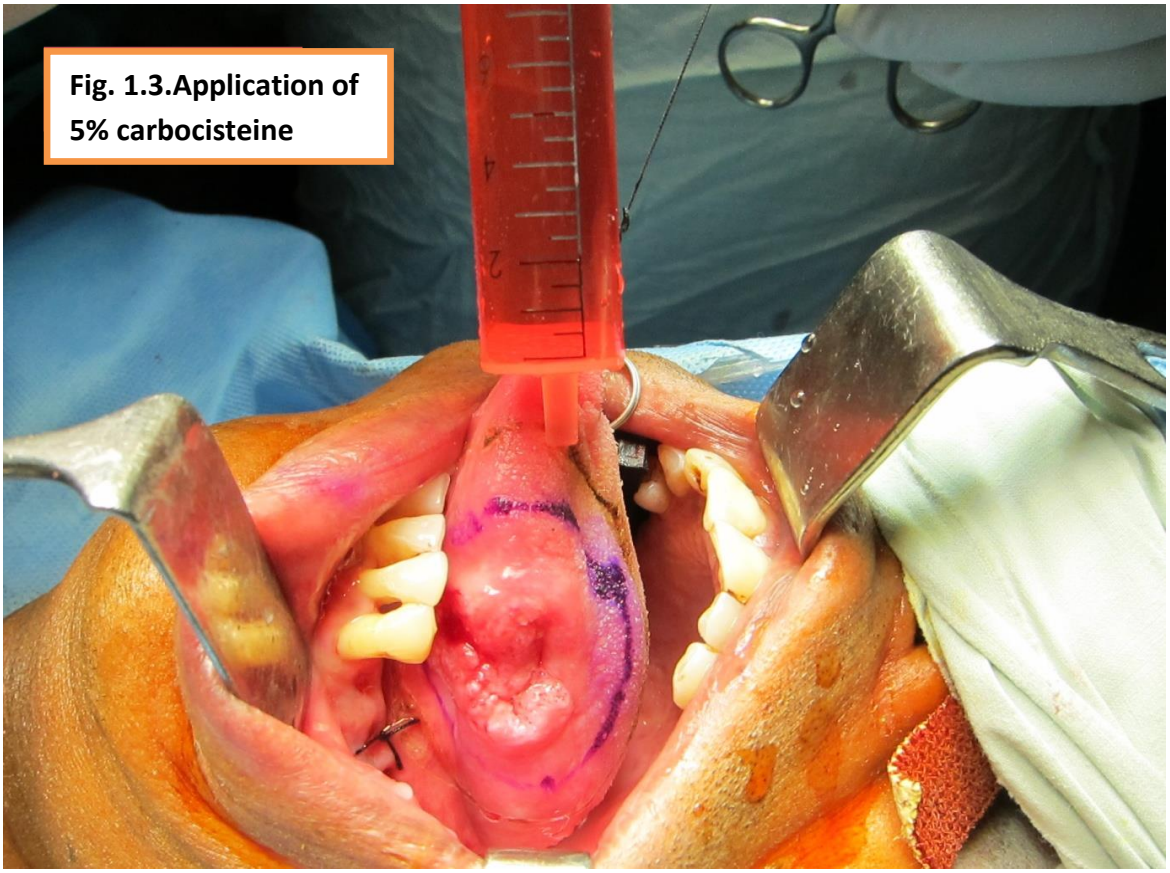


Fig. 1.3.Application of 5% carbocisteine

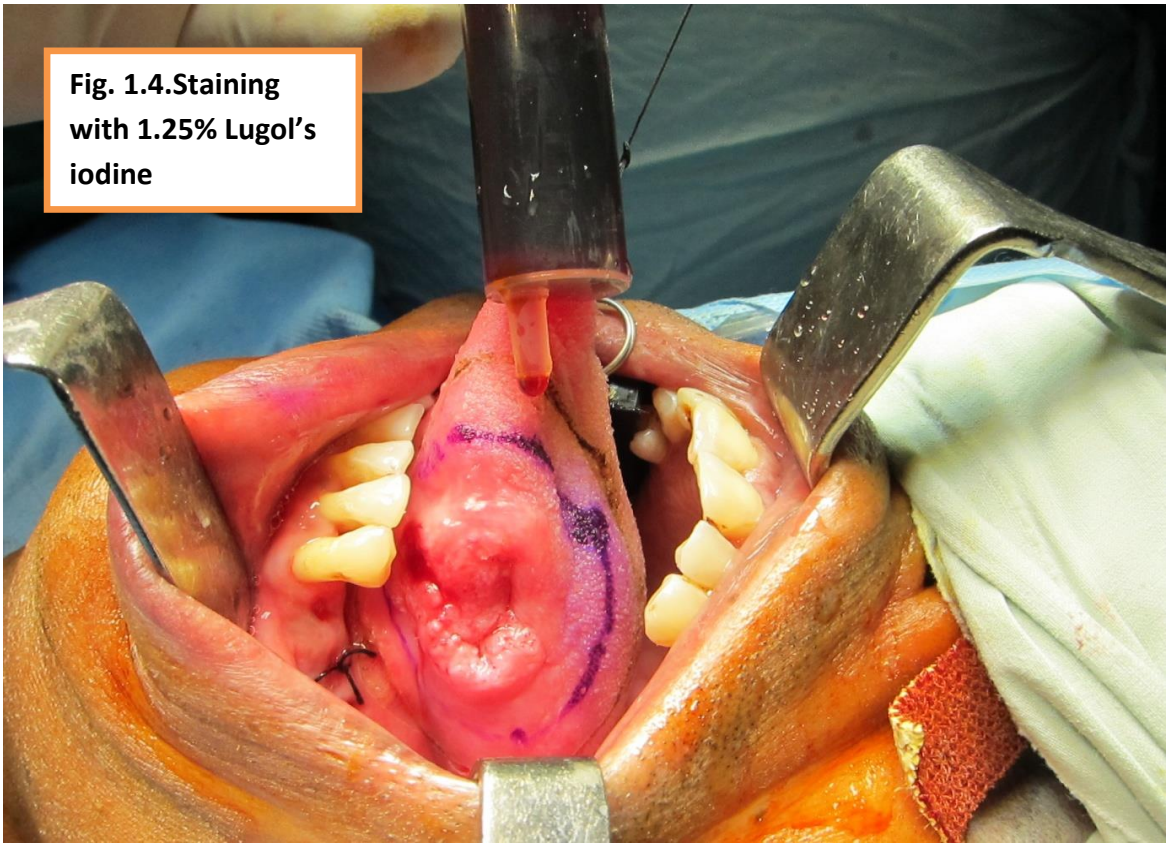


Fig. 1.4.Staining with 1.25% Lugol's iodine

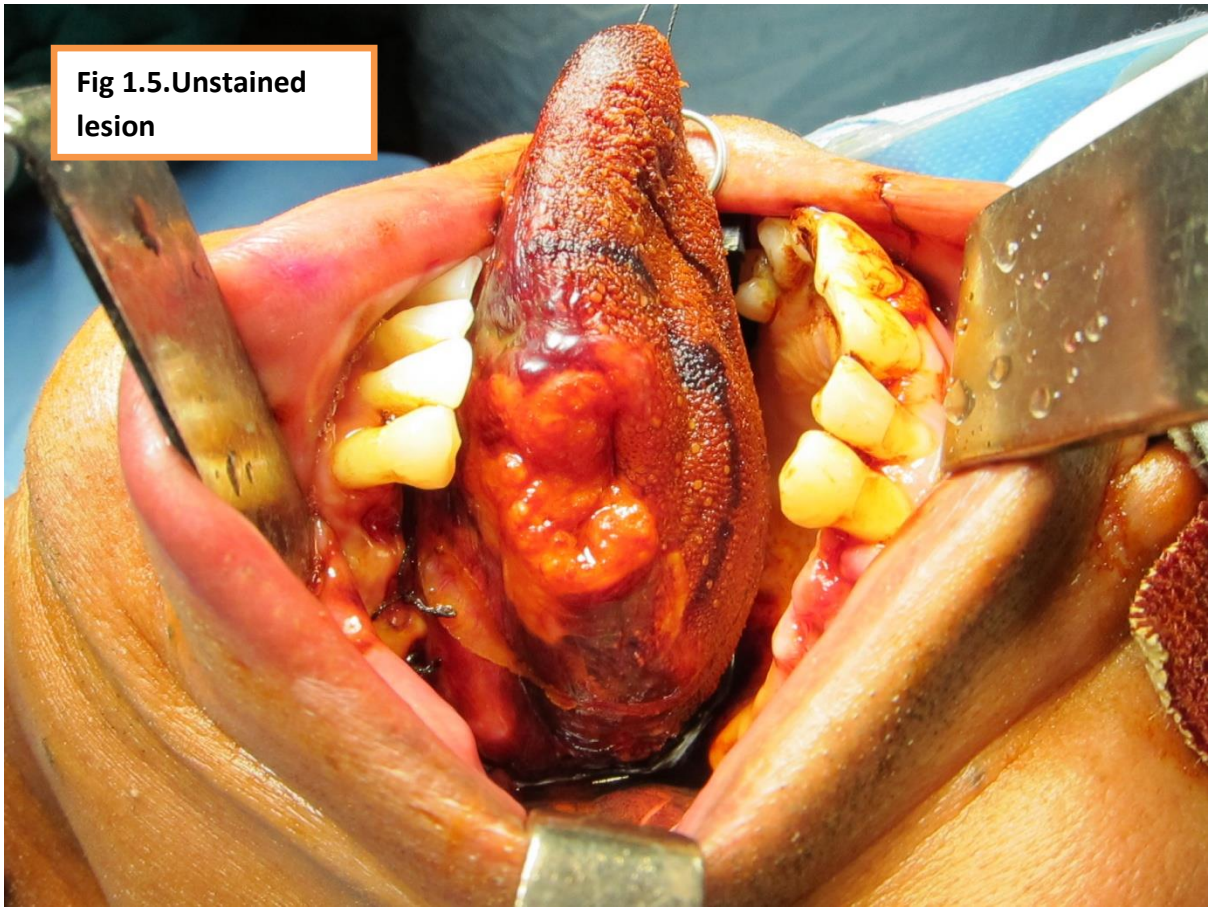


Fig 1.5.Unstained lesion

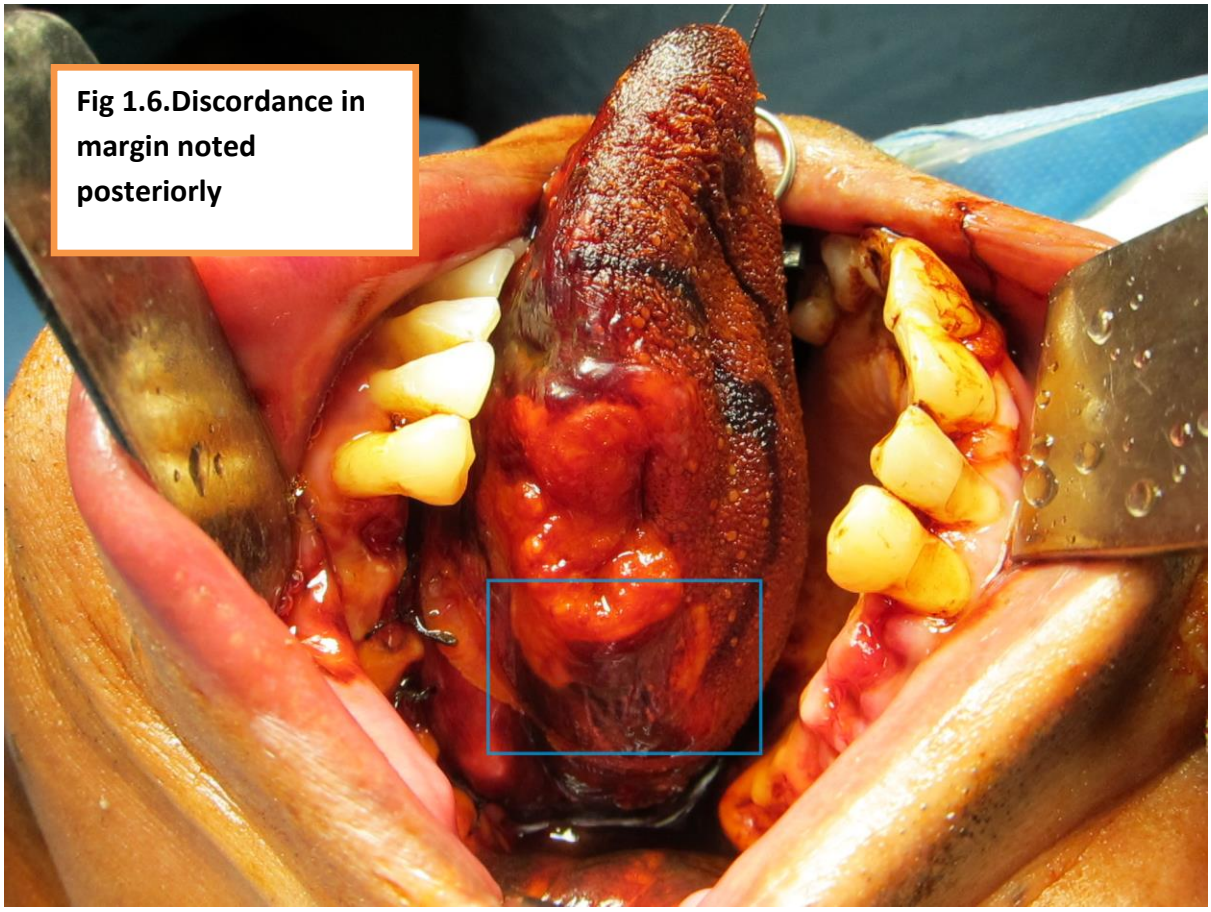


Fig 1.6.Discordance in margin noted posteriorly

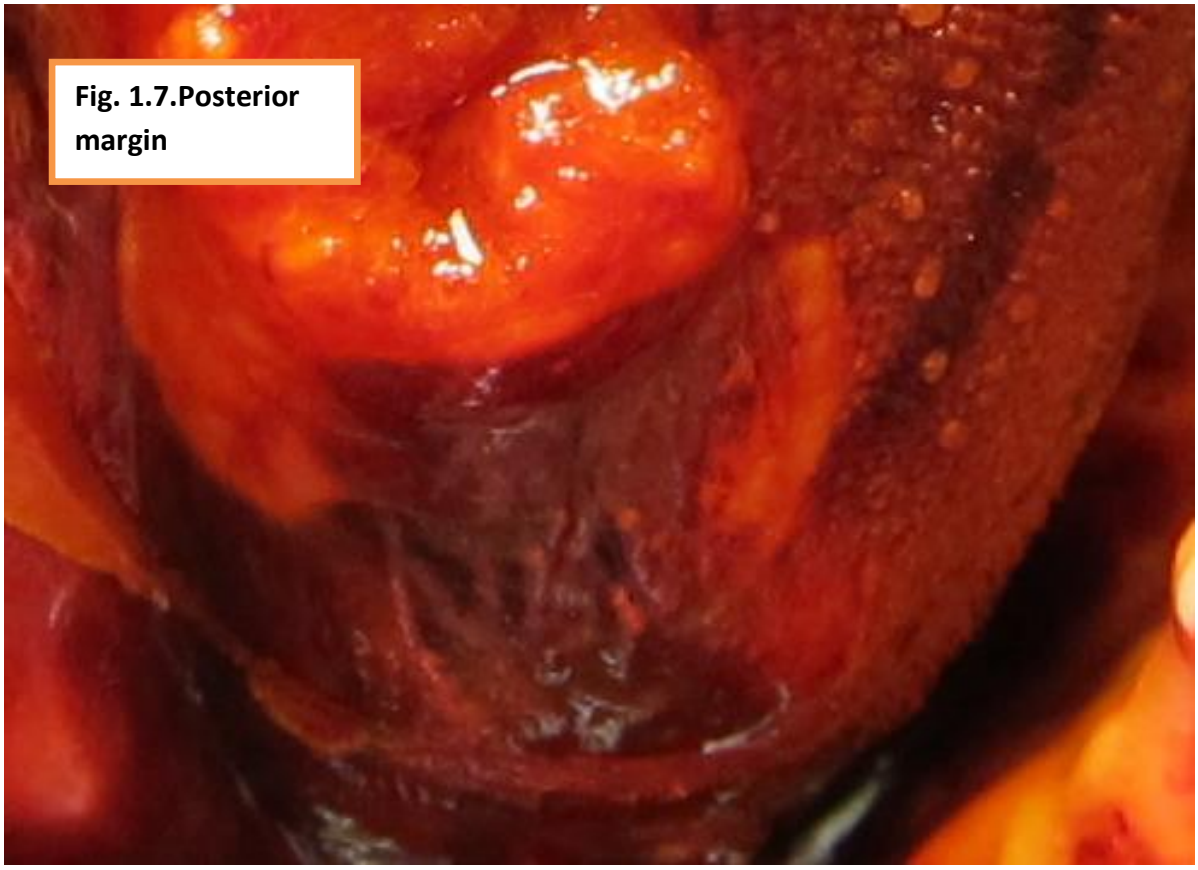


Fig. 1.7.Posterior margin

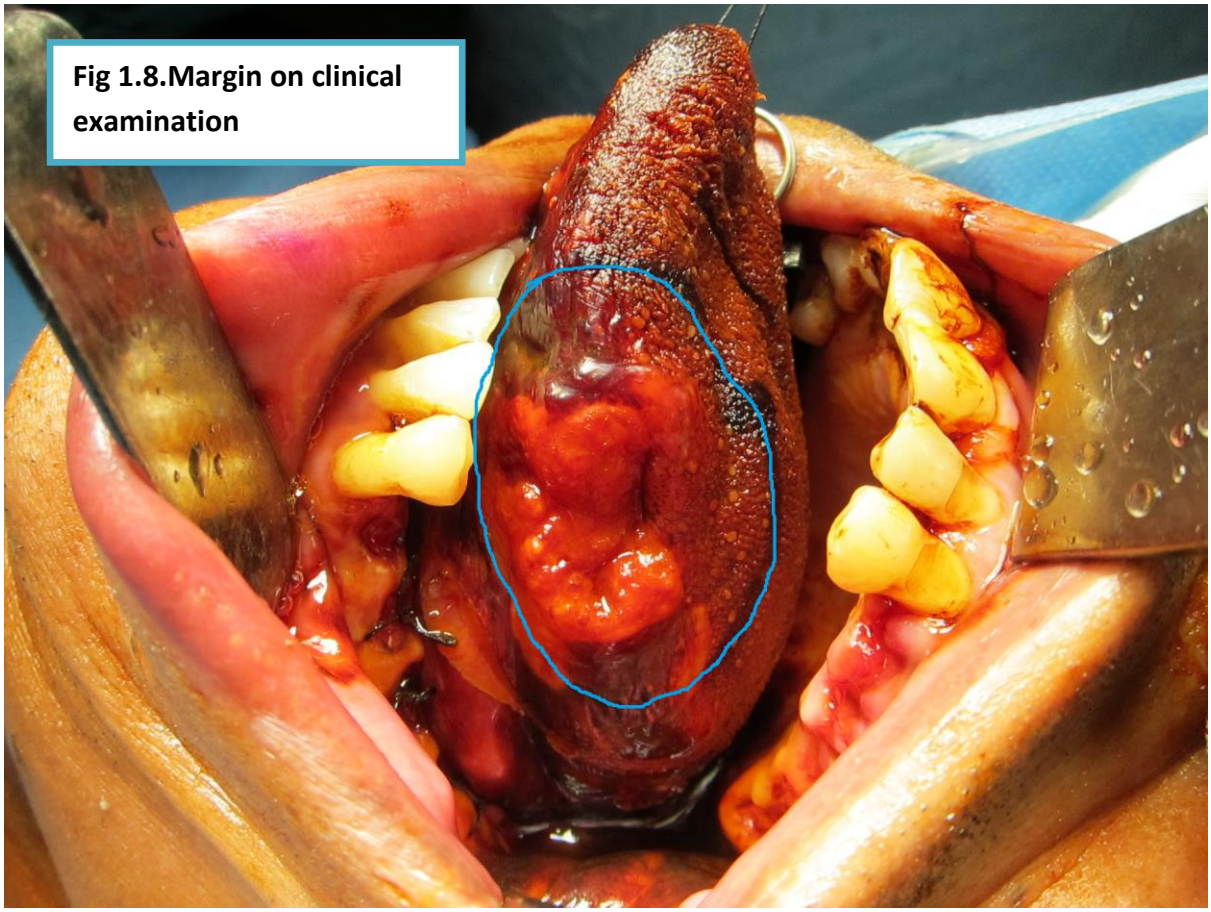


Fig 1.8.Margin on clinical examination

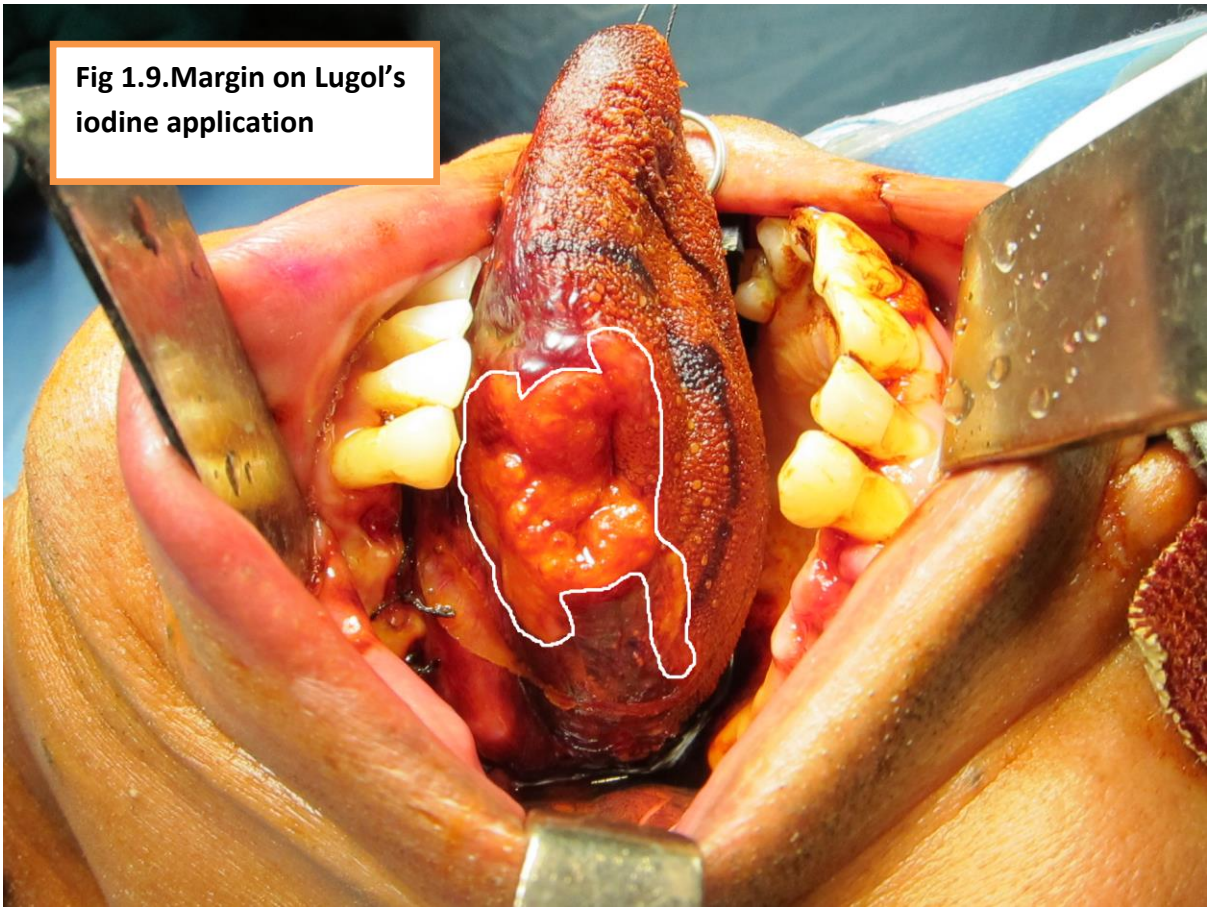


Fig 1.9.Margin on Lugol's iodine application

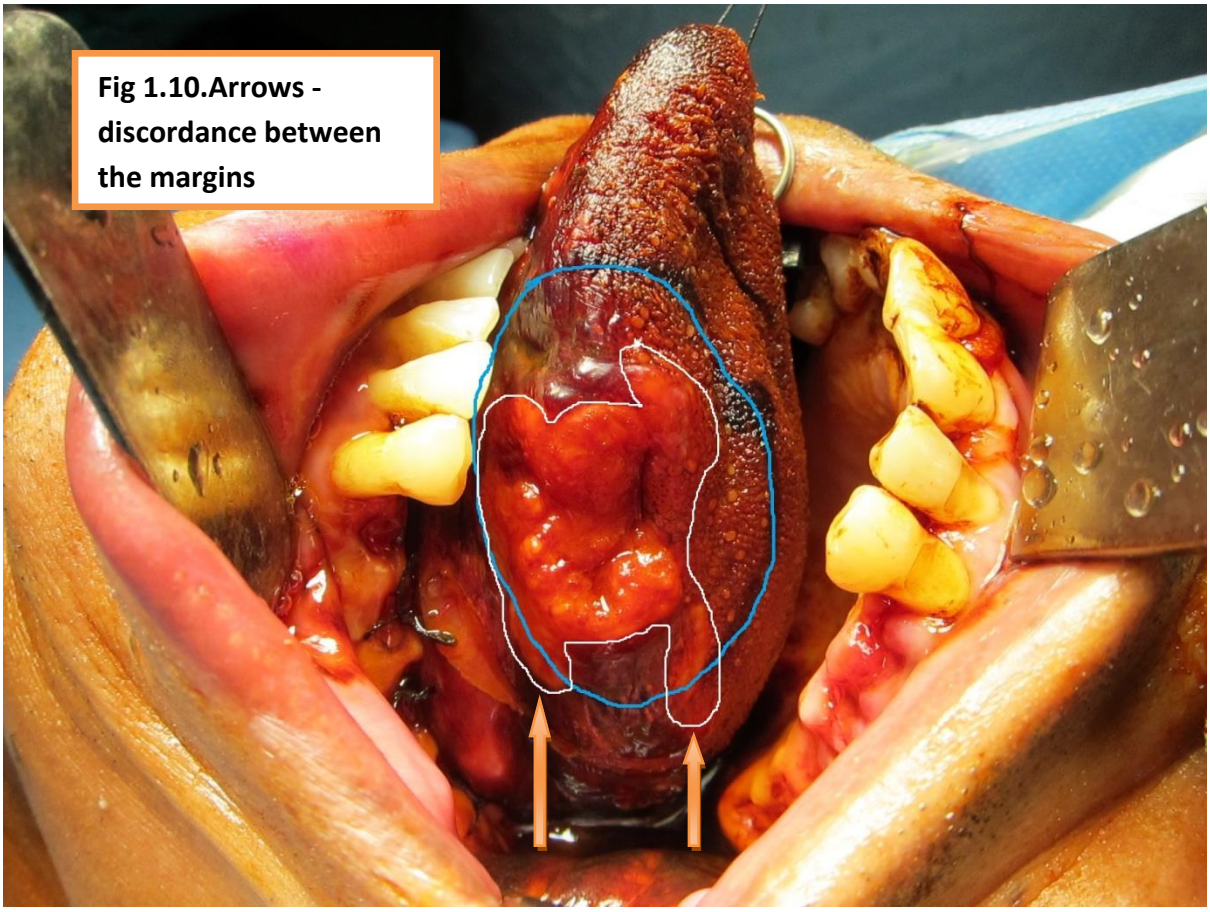


Fig 1.10.Arrows - discordance between the margins

Results and Analysis

A total of 90 patients with squamous cell carcinoma of the oral cavity underwent curative resection from 15.09.12. to 15.10.13. Of these, 86 patients fulfilled the requisite eligibility criteria. Consent could not be obtained from 24 of these patients, hence 62 patient underwent the abovementioned procedure. Of these, adequate data was unavailable for 32 patients, hence a total of 30 patients were finally included in the study for analysis.

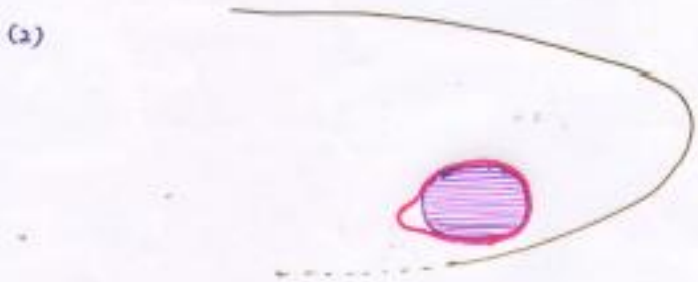
Patterns of staining:

- Pattern 1: The margins on clinical examination and following staining with Lugol's iodine were concordant.
- Pattern 2: The margins on clinical examination and following staining with Lugol's iodine were discordant. The Lugol's margin exceeded the clinical margin in a particular direction.
- Pattern 3: The margins on clinical examination and following staining with Lugol's iodine were vastly discordant . The Lugol's margin greatly exceeded the clinical margin in three or more directions, resulting in a diffuse unstained lesion.

Fig 2.1 – Patterns of staining noted following application of Lugol's iodine

Pattern 1 – Lugol's margin = clinincal margin

Pattern 2 – Lugol's margin > clinical margin in a particular direction



Pattern 3 – Lugol's margin >> clinical margin (diffuse unstained lesion)

Demographic profile of patients

Variable		15.05.10- 15.06.11 n = 60 (GroupA)	15.09.12- 15.10.13. n = 30 (Group B)
Age	<40	7 (12%)	6 (20%)
	40-60	33 (55%)	15 (50%)
	>60	20 (33%)	9 (30%)
Gender	Male	43 (72%)	16 (53%)
	Female	17 (28%)	14 (47%)
Site	Alveolus	9 (15%)	3 (10%)
	Buccal mucosa	19 (32%)	5 (17%)
	Tongue	26 (43%)	20 (67%)
	Others	6 (10%)	2 (6%)
Smoking	Yes	14 (23%)	5 (17%)
	No	46 (77%)	25 (83%)
Alcohol	Yes	11 (18%)	3 (10%)
	No	49 (82%)	27 (90%)
Paan	Yes	33 (55%)	13 (43%)
	No	27 (45%)	17 (57%)

Variable		15.05.10-15.06.11 n = 60 (Group A)	15.09.12-15.10.13 n = 30 (Group B)
Prior operation at the same site	Yes	8 (13%)	1 (3%)
	No	52 (87%)	29 (97%)
Prior chemotherapy	Yes	12 (20%)	6 (20%)
	No	48 (80%)	24 (80%)
Prior radiation at same site	Yes	3 (5%)	1 (3%)
	No	57 (95%)	29 (97%)
Clinical T stage	1	10 (17%)	6 (20%)
	2	19 (32%)	16 (53%)
	3	9 (15%)	2 (7%)
	4	20 (33%)	6 (20%)
	x	2 (3%)	0 (0%)
Clinical N stage	0	20 (34%)	16 (54%)
	1	26 (43%)	7 (23%)
	2	14 (23%)	7 (23%)
Clinical TNM stage	I	8 (13%)	5 (17%)
	II	7 (12%)	9 (30%)
	III	17 (28%)	7 (23%)
	IV	26 (44%)	9 (30%)
	Indeterminate	2 (3%)	0 (0%)

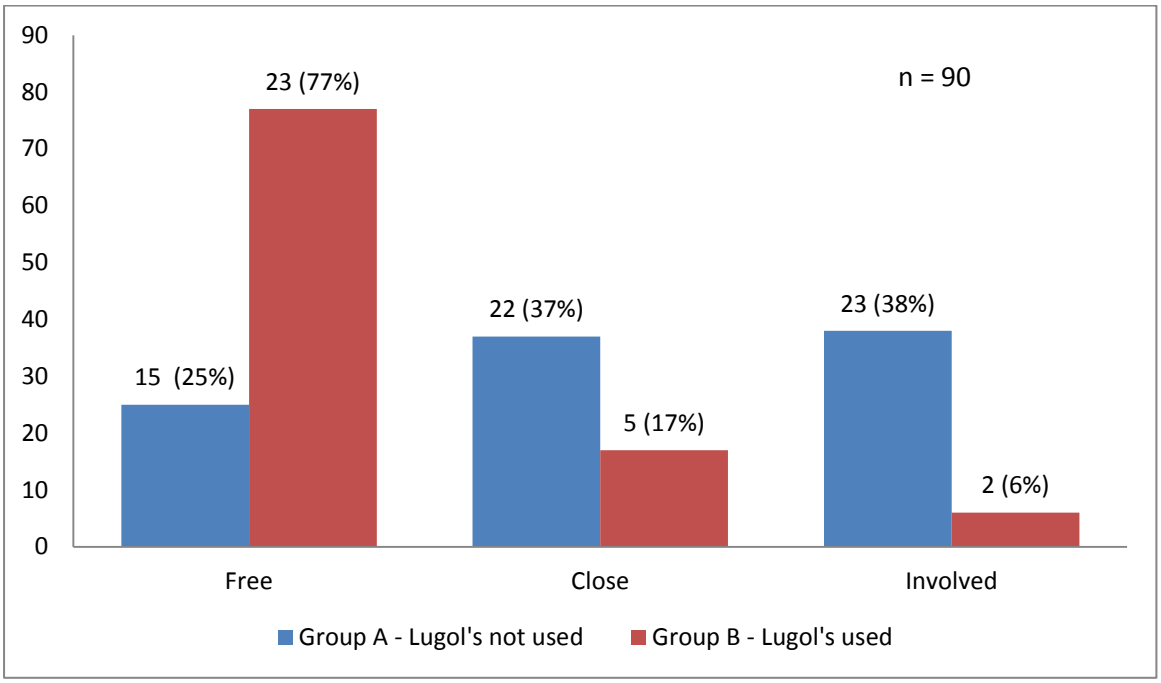
Variable		15.05.10-15.06.11 n = 60 (Group A)	15.09.12-15.10.13 n = 30 (Group B)
Pathological T stage	1	25 (42%)	15 (50%)
	2	12 (20%)	10 (33%)
	3	4 (7%)	2 (7%)
	4	19 (31%)	3 (10%)
Pathological N stage	0	36 (60%)	19 (64%)
	1	7 (12%)	4 (13%)
	2	11 (18%)	4 (13%)
	3	0 (0%)	1 (3%)
	x	6 (10%)	2 (7%)
Differentiation	Well	25 (42%)	7 (23%)
	Moderately	32 (53%)	22 (74%)
	Poorly	3 (5%)	1 (3%)
Reconstruction	Primary closure	25 (42%)	12 (40%)
	Minor reconstructive procedure	6 (10%)	1 (3%)
	Major rotational flap	18 (30%)	14 (47%)
	Free tissue transfer flap	11 (18%)	3 (10%)

Approximately half the patients fell in the 40 – 60 age group. A greater proportion was male patients was noted in group A. The most common sites of malignancy were the tongue, followed by the buccal mucosa. Only 20 % of patients gave history of smoking . A similar number consumed alcohol. The most common risk factor was chewing paan, which was noted in half the population. 20% of patients underwent neoadjuvant chemotherapy. Approximately 10% underwent prior operation at the same site, while nearly 5% underwent prior radiation.

In group A, 72% of patients presented with advanced disease (stage III, IV) as opposed to 53% in group B. The most common clinical T stages were T2 and T4. Most patients had clinically node positive disease. Histopathological examination showed 62% of patients had T1, T2 disease in group A, with no cervical nodal metastasis in 60% of the population. A higher proportion of early pathological T lesions were noted in group B. Most tumours were moderately differentiated. The most common method of reconstruction employed was a rotational flap. A higher proportion of rotational flaps, compared to free tissue transfer flaps were used in group B.

The proposed reconstruction plan was not altered in any patients following the use of Lugol's iodine. No patients had known allergy to Lugol's iodine and none had any side effects related to it's use. The average additional operating time due to the use of Lugol's iodine was 10 minutes.

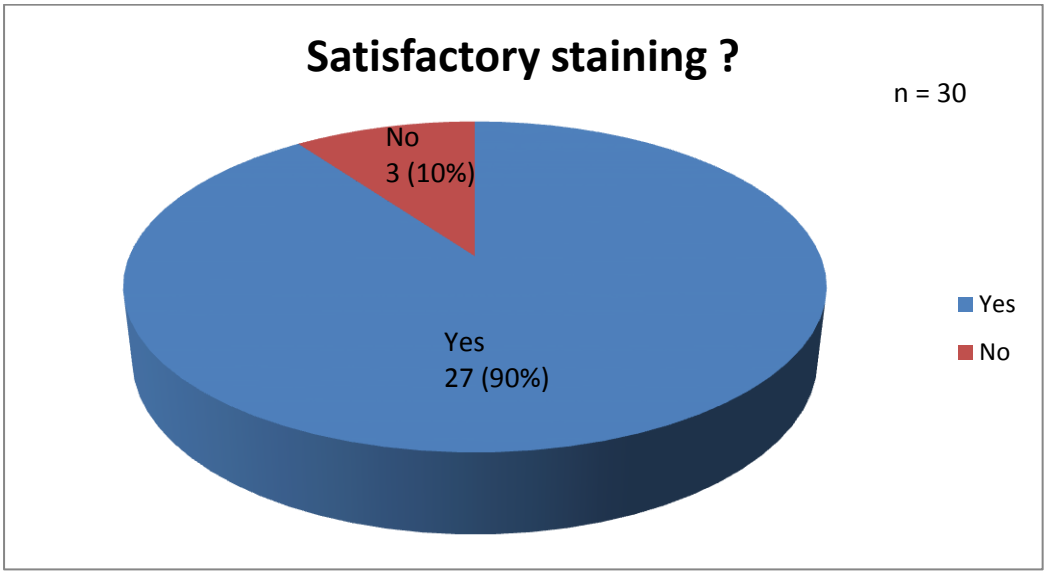
Fig 2.2.Association between margin status and the use of Lugol's iodine (by percentage)



In the group where Lugol's iodine was used, there was a significant decrease in the incidence of close and involved resection margins (17% and 6% in group B as opposed to 37% and 38% in group A). p = 0.000

Lugol's study

Fig 2.3.Did staining with Lugol's iodine aid better visualization of the tumour (surgeon's subjective assessment)?



In 90% of patients, the tumour was better defined following application of Lugol's iodine.

Fig 2.4.Distribution of the patterns of staining following application of Lugol's iodine

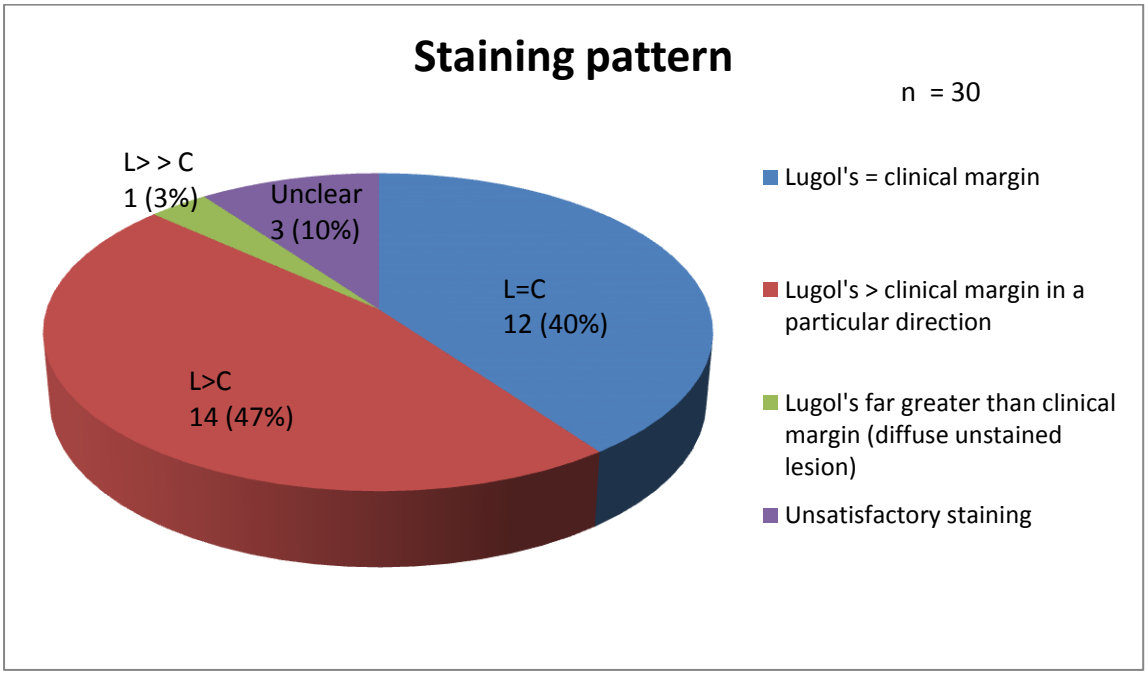


Fig 2.5.Was planned resection margin changed following the application of Lugol's iodine?

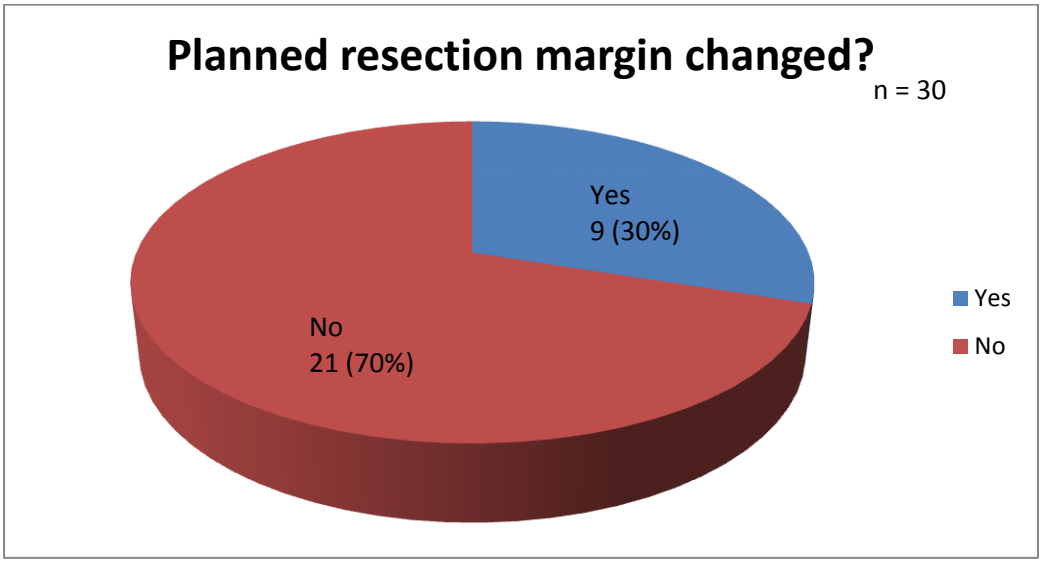


Fig. 2.6.Association between margin status and changing resection margin based on staining with Lugol's iodine (pattern 2 only , n=14)

Was resection changed according to lugol's margin?	Free margins	Close margins	Involved margins	Total
Yes	7	1	1	9
No	4	1	0	5
Total	11	2	1	14

In half the population in group B (15 patients – patterns 2,3), the extent of the lesion as determined by Lugol's iodine was larger compared to the margin on clinical examination. The resection margin was extended in 9 patients (30% of group B), as guided by the Lugol's margin. All these patients showed the second pattern of staining, where the Lugol's margin exceeded the clinical margin in a particular direction.

Fig 2.7.Distribution of margin status in patients with staining pattern where Lugol's = clinical margin (pattern 1)

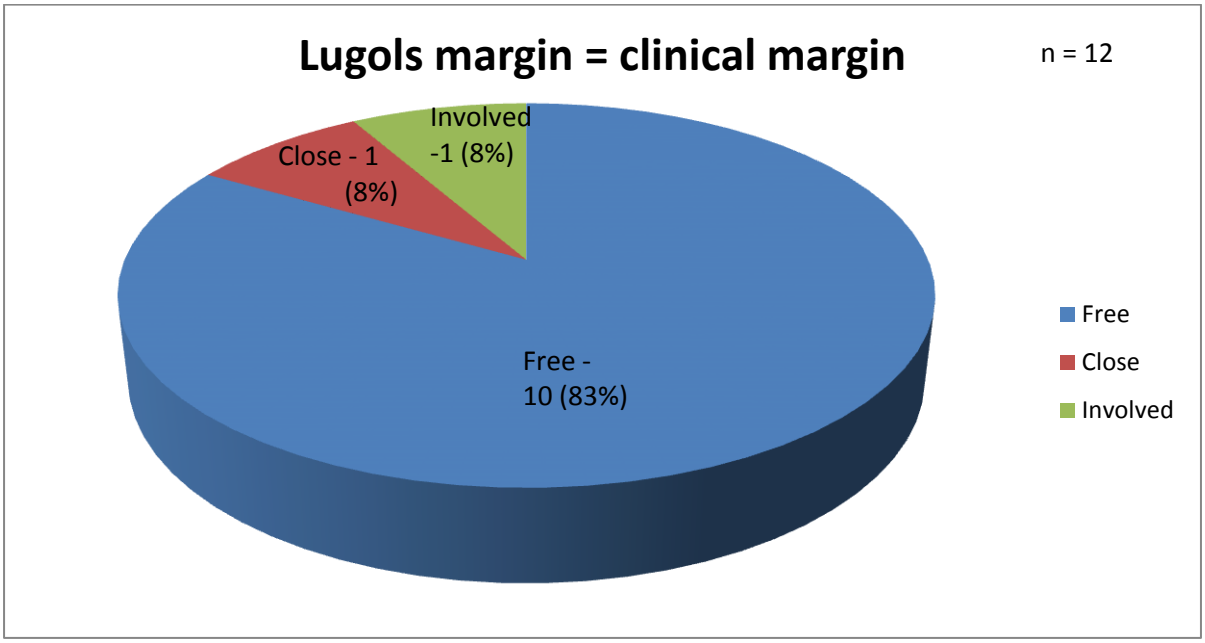
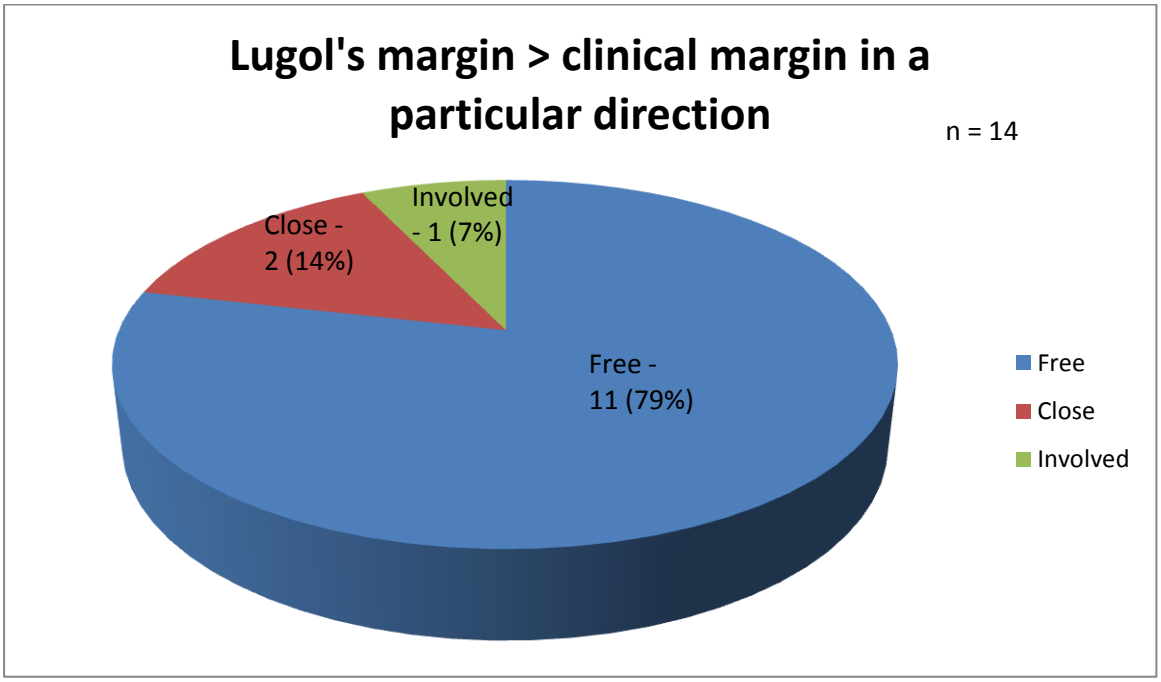
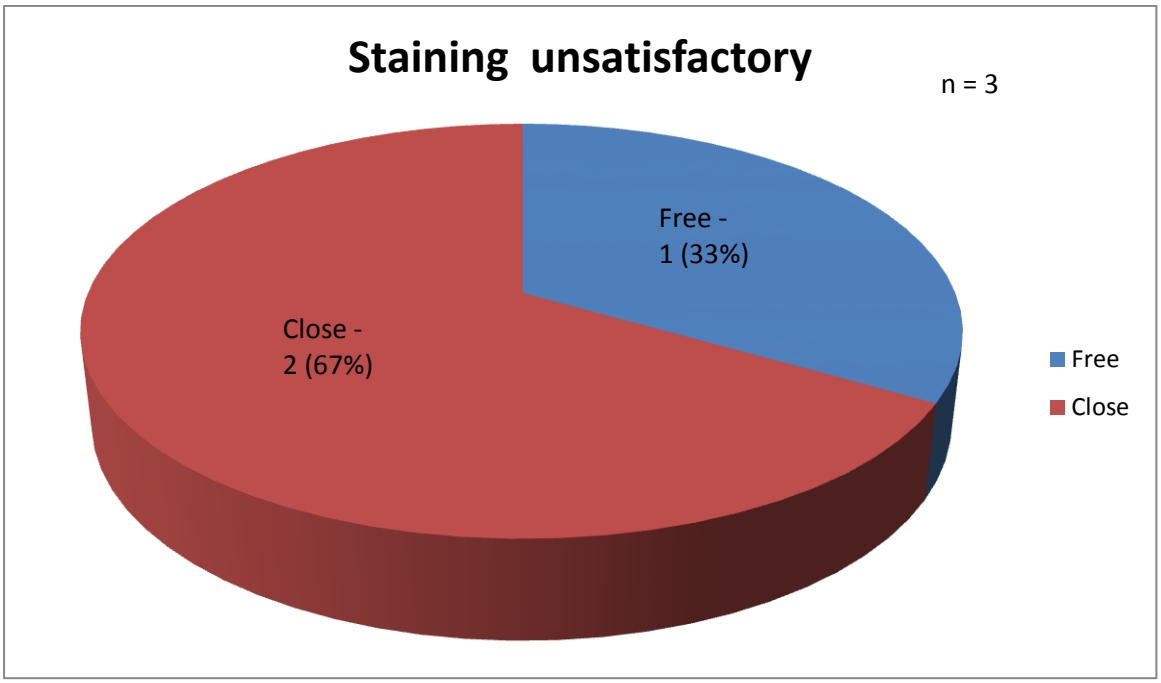


Fig. 2.8.Distribution of margin status in patients with staining pattern where Lugol's margin > clinical margin in a particular direction (pattern 2)



In both patterns 1 and 2 , a majority of patients (approximately 80%) had free margins on final histopathology.

Fig 2.9.Distribution of margin status in patients with staining pattern where staining with Lugol's iodine was unsatisfactory in identifying the lesion



In the 3 patients in whom staining with Lugol's iodine failed to delineate the lesion, resection was carried out according to the clinical margin. Final histopathology did not show involved margins in any of these patients.

In the 1 patient in whom Lugol's margin far exceeded the clinical margin (pattern 3), resection was carried out as per the clinical margin and final histopathology showed free margins.

Discussion

In view of the high incidence, oral malignancies are of particular relevance in India. The presence of invasive carcinoma at or within 5 mm of the resection margins, besides being a major determinant in further treatment and prognosis of the disease, is also one of the few modifiable factors associated with the disease.

Besides the presence of invasive carcinoma at or close to resection margins, the presence of dysplasia has also been noted to have an adverse effect on prognosis. Malignant transformation rates of 6% for mild dysplasia, 18% for moderate dysplasia and 39% for severe dysplasia have been reported (71). A meta analysis investigating the rate of malignant transformation reported 10.3% for mild and moderate dysplasia, compared to 24.1% for severe dysplasia and carcinoma in situ (72). Another factor to be borne in mind is that resection margins include mucosal, soft tissue and bony components. In their series of 301 patients including 70 patients with involved resection margin, Woolgar et al observed that mucosal margins were involved only in 11% of patients, as opposed to bony margin involvement in 14% and deep soft tissue margin involvement in 87% (6). Hence, three dimensional clearance is essential. Additionally, the development of local recurrence inspite of adequate resection

margins, even in early lesions indicates that phenotypically normal tissue may harbour mutations that can progress to dysplasia and overt carcinoma.

Of the different methods explored to address the problem of close and involved resection margins, most - including the use of frozen sections, MRI scanning and spectroscopy- significantly increase operating time or expenditure. Lugol's iodine holds particular appeal due to the wide availability, speed and ease of use, limited cost and few side effects. Additionally, Lugol's iodine has been shown to identify dysplasia (70) and has also been shown to correlate well with molecular markers of malignancy like telomerase and p53 alterations (68), (69).

Though the use of Lugol's iodine in the detection and diagnosis of oral malignancies has been well described, few studies report it's utility in guiding resection and decreasing the incidence of close and involved resection margins. McMahon et al reported that it helped decrease the incidence of dysplasia at and close to resection margins, from 32% in a retrospective control group to4% in the Lugol's study group (5). A randomized control study of the same is in progress.

Our institutional experience showed a significant reduction in the incidence of close and involved margins (17 % and 6%) following the use of Lugol's ioidine, compared to a retrospective cohort (37% and 38%). On comparing the clinical margin with the Lugol's iodine margin, three patterns were noted, as described. The association between each of the different patterns and margin status was difficult to interpret, as the numbers in each category were few.

In 3 patients, the lesion was not clearly delineated. All 3 were tongue lesions, where the lesion was small on inspection and the predominant portion of the lesion was palpable rather than visible. This likely represents subepithelial disease, which cannot be identified by Lugol's iodine.

Lugol's iodine was primarily useful in 7 patients (23%) showing pattern 2 staining, in whom the resection was increased according to the Lugol's iodine margin and resulted in free resection margins. Lugol's was also helpful in better defining the lesion in patients showing pattern 1 staining. Though the extent of resection remained the same, the operative surgeons were of the opinion that the lesion showed up more distinctly following staining, thereby facilitating resection. Additionally, staining with Lugol's iodine resulted in an irregular lesion as opposed to a smooth contoured lesion, as generally marked

on clinical examination. Making allowance for this geographic pattern while deciding the resection margin may have improved completeness of resection.

In 53% of the population in group B (16 patients – 12 showing pattern 1, 4 showing pattern 2, 1 each showing pattern 3 and unsatisfactory staining with Lugol's iodine), clinical examination alone was enough to ensure adequate margins. This was comparable to similar data noted by Byers and other investigators in studies comparing the utility of clinical margin alone with the addition of frozen sections (54), (73). .

Lugol's iodine failed to detect malignancy in 2 patients (7%), who had involved margins on histopathology. Of these, 1 patient showed pattern 1 staining, where both clinical examination and Lugol's iodine examination failed to accurately delineate the lesion. In the second patient, the anterior extent of resection was increased axxording to the Lugol's margin (pattern 2), however final histopathology showed involvement of the medial and lateral margins.

Lugol's iodine cannot be used for improvement of soft tissue resection margins. It cannot be used in heavily keratinized epithelium and is of limited utility in the delineation of infiltrative lesions.

Limitations:

The prinicipal limitation of this study was the small number of patients available for final analysis, making determination of trends and their significance difficult. Additionally, histopathological diagnosis of those areas discordant on clinical and Lugol's examination was unavailable and resection was left to the surgeon's discretion . It was hence difficult to determine the strength of association between improvement in margin status and staining with Lugol's iodine.

Conclusion

Conclusion:

With appropriate patient selection, staining with Lugol's iodine appears to be a promising method of obtaining tumour free resection margins in carcinoma of the oral cavity. However, studies with a larger sample size and randomization are required to further establish it's utility.

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(literature review - challenges in management - second primary)

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PROFORMA

Date: Name: H. No.:

Age: Sex: Study ID No.:

Known history of iodine allergy:

Smoking: Alcohol: Paan use:

Previous treatment:

If yes, when - surgery :

- radiotherapy :

Premalignant/ inflammatory lesions:

Anatomical site:

Clinical stage:

Surgeons subjective assessment:

(Please circle either Y- yes or N – no)

(1) Was the staining with Lugol's adequate, did it demarcate any specific region to be malignant (as opposed to inadequate staining with unclear, ill defined borders) – Y/ N

(2) Was the planned resection margin modified following the use of Lugol's iodine? – Y/ N

If no, please skip question 3.

(3) If yes - (a) in how many directions (please circle one option) -

1/ 2/ 3/ 4

(b) which direction (please circle as many as indicated by previous answer) – anterior/ posterior/ medial or inferior/ lateral or superior

(4) Was the plan of reconstruction of the defect changed as a result of a larger area being resected following the use of Lugol's iodine? - Y/N

(5) What was the additional time added to the surgery as a result of the use of Lugol's iodine (approximately) ?

(6) Were there any adverse reactions noted following the use of Lugol's iodine

(a) immediately - Y/N

(b) at the end of the surgery Y/N

(c) at the end of the patient's hospital stay? Y/N

(if yes, please specify)

(7) Were there any new unstained areas, otherwise unidentified on clinical examination? Y/N

If yes,(a) how many such areas

(b) what was the location – in continuity with the tumour/ in different anatomical subsite

(8) Which scenario did the staining pattern fit into ? 1/ 2/ 3

(9) Are there any other comments?

Patient information sheet

You are invited to participate in a study that researches the use of Lugol's iodine in cancers of the mouth.

Cancers in the interior of the mouth tend to occur repeatedly, inspite of treatment. Surgery for these cancers aims at complete removal of the cancer. In cases where the cancer is not removed completely by surgery, the chances of the cancer occurring again in the same place is higher. This leads to the requirement for additional treatment and increased risk of death. Various studies have shown that Lugol's iodine is useful in identifying and marking out areas of cancer. If the area to be removed during surgery is decided following marking using Lugol's iodine instead of according to what is seen and felt to be the edge of the cancer, the chances of completely removing the cancer is greater. This will lead to decreased chances of the cancer returning in the same region, decreased need for further treatment in some cases and decreased risk of death.

Lugol's iodine is a solution containing potassium iodide and iodine. It is used in a variety of diseases and depending on the disease , it can be either ingested or used on the surface of the body. In this study, Lugol's iodine is used as it can differentiate between cancerous and normal regions when applied on the surface of certain parts of the body. When used in this fashion, it has occasionally caused allergic reactions and ulcers.

In this study, the procedure will be as follows. The surgeon will see and feel the cancer and mark out what is felt to be the limit of the cancer. 5% Carbocysteine syrup will be applied on the inner surface of the entire mouth. This will remove the saliva and secretions on the inner surface of the mouth. This will be followed by application of 1.25% Lugol's iodine and normal saline alternately till the regions far away from the cancer turns dark brown or black. The area of the cancer will appear yellow. This colour difference is used to mark out the cancer from the surrounding normal tissues. The cancer is cut 1 centimetre away from the margin decided by the surgen as the edge of the cancer. A proforma is filled out based on observations made during the operation. The removed cancer will be sent for examination under a microscope. This will show whether any cancer cells have been left behind in the body. Comparing this report with the information in the proforma and records of our past patients, we can determine whether Lugol's iodine served it's purpose or not.

In the rare case of unpleasant side effects caused by the drug, further treatment of the side effect including the expenses incurred for it will be managed by the treating unit. Participation is entirely voluntary and you can withdraw from the study at any time. Information obtained during this study will remain confidential. The decision to take part in the study or not will not influence any further treatment you receive at this hospital.

CONSENT TO TAKE PART IN A CLINICAL TRIAL

Study Title: The use of Lugol's iodine in achieving surgical margins free from dysplasia and invasive carcinoma in squamous cell carcinoma of the oral cavity

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____

_____, son/daughter of _____

(Please tick boxes)

Declare that I have read the information sheet provide to me regarding this study and have clarified any doubts that I had. [ ]

I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]

I understand that I will receive free treatment for any study related injury or adverse event but I will not receive and other financial compensation [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]

I understand that my identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree to take part in this study [ ]

Name:

Signature:

Date:

Name of witness:

Relation to participant:

SNO	DATE	FIELD1	FIELD2	NAME	HNO	HNO1	AGE	SEX	IALLERGY	SM	ALC	PAAN	*PSX*	*PCH*	*PRT*	*SITE*
1	19	9	12	umarani	231347	f	6	2	2	2	2	1	*2*	*1*	*2*	
2	21	9	12	fuljan	237787	f	6	2	2	2	2	1	2	1	2	
3	3	10	12	raghupathi	895262	c	7	1	2	1	2	2	2	2	2	
4	17	10	12	vengatesan	237865	f	2	1	2	1	2	2	2	1	2	
5	7	12	12	karfu	281076	f	4	1	2	2	1	1	2	1	2	
6	19	12	12	parvadham	356110	f	6	2	2	2	2	1	2	2	2	
7	2	1	13	nirmala	285641	f	4	2	2	2	2	2	1	1	1	
8	4	1	13	sheikh	372065	f	3	1	2	2	2	1	2	2	2	
9	1	1	13	sundaraj	346406	f	4	1	2	2	1	1	2	2	2	
10	16	1	13	rose	220413	f	5	2	2	2	2	2	2	2	2	
11	27	3	13	mohammad	337931	f	4	1	2	1	2	2	2	2	2	
12	24	4	13	chengamma4	449653	f	4	2	2	2	2	1	2	2	2	
13	1	5	13	akilesh	454653	f	3	1	2	2	1	2	2	2	2	
14	29	5	13	sandhya	427236	f	5	2	2	2	2	2	2	2	2	
15	31	5	13	basanti	486633	f	6	2	2	2	2	1	2	2	2	
16	14	6	13	shilpi	489741	f	4	2	2	2	2	2	2	2	2	
17	19	6	13	unnikrishn	494788	f	3	1	2	2	2	2	2	2	2	
18	3	7	13	dilip	494718	f	6	2	2	2	2	2	2	2	2	
19	10	7	13	moidu	488070	f	5	1	2	1	2	2	2	2	2	
20	9	8	13	subramanya	726057	d	6	1	2	2	2	2	2	2	2	
21	23	8	13	samuel	47550	a	8	1	2	2	2	2	2	2	2	
22	7	11	12	vittal	231798	f	6	1	2	1	2	2	2	2	2	
23	12	10	12	reba	296228	f	4	2	2	2	2	1	2	2	2	
24	10	10	12	kalyani	253918	f	5	2	2	2	2	2	2	1	2	
25	2	10	13	vijaya	640922	f	5	2	2	2	2	1	2	2	2	
26	2	10	13	raghavan	659353	f	4	1	2	2	2	1	2	2	2	
27	2	10	13	sujoy	650735	f	3	1	2	2	2	1	2	2	2	
28	11	10	13	rajab	663420	f	3	1	2	2	2	2	2	2	2	
29	23	10	13	manickam	663172	f	5	2	2	2	2	2	2	2	2	
30	16	10	13	niranjan	668369	f	4	1	2	2	2	1	2	2	2	

SNO	DATE	FIELD1	FIELD2	NAME	HNO	HNO1	AGE	SEX	IALLERGY	SM	ALC	PAAN	PSX	PCH	PRT	SIE
1	19	9	12	umarani	231347	f	6	2	2	2	2	1	2	1	2	1
2	21	9	12	fuljan	237787	f	6	2	2	2	2	1	2	1	2	2
3	3	10	12	raghupathi	895262	c	7	1	2	1	2	2	2	2	2	5
4	17	10	12	vengatesan	237865	f	2	1	2	1	2	2	2	1	2	3
5	7	12	12	karfu	281076	f	4	1	2	2	1	1	2	1	2	3
6	19	12	12	parvadham	356110	f	6	2	2	2	2	1	2	2	2	3
7	2	1	13	nirmala	285641	f	4	2	2	2	2	2	1	1	1	3
8	4	1	13	sheikh	372065	f	3	1	2	2	2	1	2	2	2	3
9	1	1	13	sundaraj	346406	f	4	1	2	2	1	1	2	2	2	3
10	16	1	13	rose	220413	f	5	2	2	2	2	2	2	2	2	3
11	27	3	13	mohammad	337931	f	4	1	2	1	2	2	2	2	2	3
12	24	4	13	chengamma4	449653	f	4	2	2	2	2	1	2	2	2	2
13	1	5	13	akilesh	454653	f	3	1	2	2	1	2	2	2	2	3
14	29	5	13	sandhya	427236	f	5	2	2	2	2	2	2	2	2	3

15	31	5	13	basanti	486633	f	6	2	2	2	2	1	2	2	2	3
16	14	6	13	shilpi	489741	f	4	2	2	2	2	2	2	2	2	1
17	19	6	13	unnikrishn	494788	f	3	1	2	2	2	2	2	2	2	3
18	3	7	13	dilip	494718	f	6	2	2	2	2	2	2	2	2	3
19	10	7	13	moidu	488070	f	5	1	2	1	2	2	2	2	2	3
20	9	8	13	subramanya	726057	d	6	1	2	2	2	2	2	2	2	3
21	23	8	13	samuel	47550	a	8	1	2	2	2	2	2	2	2	3
22	7	11	12	vittal	231798	f	6	1	2	1	2	2	2	2	2	1
23	12	10	12	reba	296228	f	4	2	2	2	2	1	2	2	2	3
24	10	10	12	kalyani	253918	f	5	2	2	2	2	2	2	1	2	2
25	2	10	13	vijaya	640922	f	5	2	2	2	2	1	2	2	2	2
26	2	10	13	raghavan	659353	f	4	1	2	2	2	1	2	2	2	3
27	2	10	13	sujoy	650735	f	3	1	2	2	2	1	2	2	2	3
28	11	10	13	rajab	663420	f	3	1	2	2	2	2	2	2	2	5
29	23	10	13	manickam	663172	f	5	2	2	2	2	2	2	2	2	3
30	16	10	13	niranjan	668369	f	4	1	2	2	2	1	2	2	2	2