EVALUATING USEFULNESS OF 5 – AMINOLEVULINIC ACID INDUCED FLUOESCENCE TO GUIDE BIOPSY OF ORAL CANCERS AND PRE MALIGNANT LESIONS

By

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Dissertation submitted to the

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In partial fulfilment of the requirements for the degree of

MASTER OF SURGERY IN OTORHINOLARYNGOLOGY

Under the guidance of

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TAMAKA, KOLAR

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LIST OF ABBREVIATIONS

TB	⇨	Toludine Blue
MB	⇨	Methylene Blue
LI	⇨	Lugol's Iodine
HpD	⇨	Hematoporphyrin Derivatives
5-ALA	⇨	5 – Aminolevulinic Acid
PpIX	⇨	Protoporphyrin IX
NADH	⇨	Nicotinamide adenine dinucleotide
FAD	⇨	Flavin adenine dinucleotide
AJCC	⇨	American Joint Committee on Cancer
SCC	⇨	Squamous Cell Carcinoma
SIN	⇨	Squamous Intraepithelial Neoplasia
SIL	⇨	Squamous Intraepithelial Lesion
HPV	⇨	Human Papilloma Virus
HIV	⇨	Human Immunodeficiency Virus
HSV	⇨	Herpes Simplex Virus
EBV	⇨	Epstein Barr Virus
PVL	⇨	Proliferative Verrucous Leukoplakia

ALAS	⇨	Aminolevulinic Acid Synthase
ALAD	⇨	Aminolevulinate Dehydratase
UROD	⇨	Uroporphyrinogen decarboxylase
PBGD	⇨	Porphobilinogen Deaminase
PDT	⇨	Photodynamic Therapy
MAL	⇨	Methyl aminolevulinate
HAL	⇨	Hexyl aminolevulinate
BCC	⇨	Basal Cell Carcinoma
BPD	⇨	Benzoporphyrin derivative monoacid
mTMPC	⇨	Meso-tetro-[hydroxyphenyl]-chlorin
mTHPC	⇨	Meso-tetrahydroxyphenylchlorin
FDA	⇨	Food and Drug Administration
EGFR	⇨	Epidermal Growth Factor Receptor
CFD	⇨	Combined Fluorescence Diagnosis
FD	⇨	Fluorescence Diagnosis
i/a	⇨	intra-arterial
HRM	⇨	Heme Regulatory Motif
ORFs	⇨	Open Reading Frames
DNA	⇨	Deoxyribonucleic Acid

OSMF	⇨	Oral Submucous Fibrosis
PEG-chito	⇨	Polyethylene glycol/ chitosan
BB	⇨	Brush biopsy

ABSTRACT

Background:

Prevalence of oral carcinoma is high in Kolar due to the tobacco chewing habits of people. In current days, intake of alcohol and cigarettes/ beedis also show synergistic action causing oral cancer and/ or pre-malignant lesions. Oral cancer is the most common cancer in males and 3rd most common in females in India. The standard diagnostic modality is histopathological examination of the biopsy specimen. 5-Aminolevulinic Acid (5-ALA) induced fluorescence due to Protoporphyrin IX (PpIX) accumulation in malignant cells helps demarcate the extent of tumour (beyond visible margin). This is a non-invasive method of investigation. Also, 5-ALA is a naturally occurring substance and does not have any side effects. It helps in achieving complete tumour clearance at surgery.

Objectives:

- To perform a biopsy from the visible margin of the cancer or pre malignant condition in the oral cavity.
- To perform a biopsy again, if an extension of the margin of the lesion (fluorescence) is seen on visualization under blue light following staining with 5- Aminolevulinic Acid.
- To perform histopathological examination on both the biopsy samples.
- To evaluate whether tumour cells or pre malignant changes could be detected in the second sample.

Methods:

Our study included 50 patients presenting with oral carcinoma/ pre-malignant lesions. Biopsy was taken from visible margin and from fluorescence margin following incubation period of 3 hours post rinsing oral cavity with 5-ALA solution. The specimens were subjected to histopathological examination for detection of malignant cells. Documentation of usefulness of 5-ALA was done by evaluating sensitivity, specificity, positive predictive value and negative predictive value and diagnostic accuracy.

Results:

In our study, out of 50 patients with the age ranging from 33–78 years, female predominance – 4:1 was observed in this region. On staining, extension of fluorescence was seen in 47 cases with a range of 2-13mm., wherein tongue malignancies showed maximum extension under fluorescence.

The diagnostic sensitivity with 5-ALA was found to be 95.74%% and specificity was 100%. The positive and negative predictive value was found to be 100% and 60% respectively. The diagnostic accuracy of 5-ALA was determined to be 96%.

Conclusion:

Biopsy is the standard diagnostic method for detection of oral carcinoma/ pre-malignant lesions. Biopsy taken under guidance – 5-ALA induced fluorescence, aids by determining margin of the lesion accurately owing to Protoporphyrin IX accumulation in subclinical malignant cells. This may improve tumour clearance and improve locoregional control. 5-ALA also has the advantage of being non-invasive, naturally occurring substance with rapid metabolism and no side effects.

KEYWORDS:

Oral carcinoma, pre-malignant lesion, 5-Aminolevulinic Acid, Protoporphyrin IX

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INTRODUCTION

Oral cancer is the 8th most common cancer worldwide. In India, it is the most common cancer in males and 3rd most common in females. Statistics show that squamous cell carcinoma (SCC) [>90%] is the most common type of oral cavity malignancy. It is generally seen in people above 40 years and peaking at around 60 years of age, with male: female ratio being 2:1. The main aetiology for development of oral cavity cancers is tobacco chewing. The increasing use of alcohol and tobacco also play an important synergistic role in the pathogenesis. We get a large number of oral cancer patients in our institution due to tobacco chewing. Oral cancer has the highest risk for developing 2nd primary tumour ['field cancerzation phenomenon']. Pre-malignant lesions such as leukoplakia, erythroplakia, oral submucous fibrosis (OSMF) and lichen planus have a 10%, 30%, 2-5% and 1% chance respectively to turn malignant.

The primary investigation for confirmation of diagnosis is biopsy of the lesion for histopathological examination. In field cancerization, biopsy taken may be from a non- representative area. Biopsy can be made more representative by utilizing supravital stains. Various stains like toluidine blue (TB), methylene blue (MB), lugol's iodine (LI) have been in use. Lately hematoporphyrin derivatives (HpD) and photosensitizers like 5-ALA are being studied for their usefulness.

Vital staining assists in:

- Choice of biopsy site
- Demarcation of lesion margin
- Follow up of premalignant lesion (after treatment) [E]

5-aminolevulenic acid [ALA] induced fluorescence is a non invasive method to detect malignant lesions due to preferential protoporphyrin accumulation. This method is superior as it allows for early detection of malignancy, the actual border demarcation, which cannot be accurate by the naked eye, guiding biopsy especially in field cancerization and also helping in surgery so that no malignancy is left behind by taking an adequate margin. 5-ALA also has the advantage of being a naturally occurring substance. It can be locally applied or taken systemically. Rapid metabolism of photosensitizing products occurs and clears from the system within 24 hours.

It has been documented in literature that 5-ALA induced protoporphyrin IX (PpIX) has high specificity for neoplastic tissue. 5-ALA also has the advantage of causing no major side effects and no cosmetic deformity. By demarcating the margins of tumour, it helps in adequate resection of tumour and thereby decreases rate of recurrence and mortality. The main drawback of 5-ALA is that it is expensive and is not easily available in our country.

OBJECTIVES OF THE STUDY

- To perform a biopsy from the visible margin of the cancer or pre malignant condition in oral cavity.
- To perform a biopsy again, if an extension of the margin of the lesion (fluorescence) is seen on visualization under blue light following staining with 5- Aminolevulinic Acid.
- To perform histopathological examination on both the biopsy samples.
- To evaluate whether tumour cells or pre malignant changes could be detected in the second sample.

REVIEW OF LITERATURE

Head and neck cancer is the 5th most common malignancy worldwide.[1] An upward trend is seen in morbidity and mortality rates of squamous cell carcinoma (SCC) of oral cavity in industrialized areas.[1] Majority of cancers are preceeded by precancerous lesions, like, leukoplakia, lichen planus, erythroplakia, OSMF which have a risk of malignant transformation.[2] Small, harmless looking areas of induration or localized morphological changes representing early lesions of oral mucosa are difficult to detect within innocuous tissue by clinical examination.[3]

EMBRYOLOGY

The stomatodeum bounded by brain above and pericardial sac below becomes apparent at 4th week of intra-uterine life. The breakdown of buccopharyngeal membrane causes mouth to become continuous with developing pharynx.[4]

Mesodermal condensation in lateral wall and floor of pharynx gives rise to branchial arches which differentiate to produce cartilaginous bar, branchial musculature and branchial arch artery with each arch receiving an afferent and an efferent nerve supply, post and pre-trematic nerve supply.[4]

The mandibular processes arising from lateral aspects of developing head fuse by 6th week in midline and the maxillary processes arising as buds from mandibular processes, grow forwards and meet with lower end of nasal septum and its contralateral side in the midline. Fusion of maxillary processes separates primitive nasal cavity from primitive oral cavity.[4]

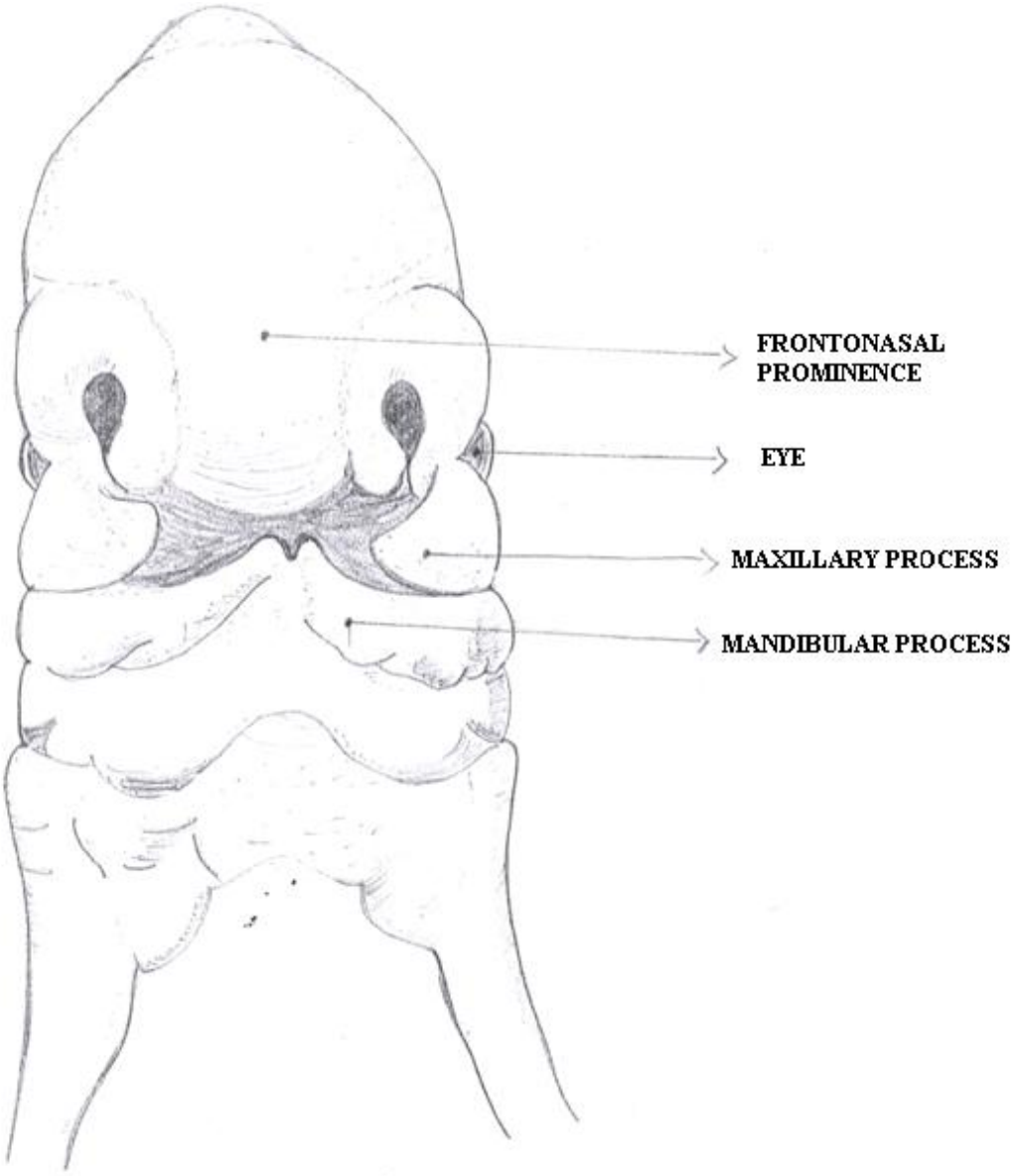


Figure 1 : EMBRYOLOGY – 4th WEEK OF INTRA-UTERINE LIFE

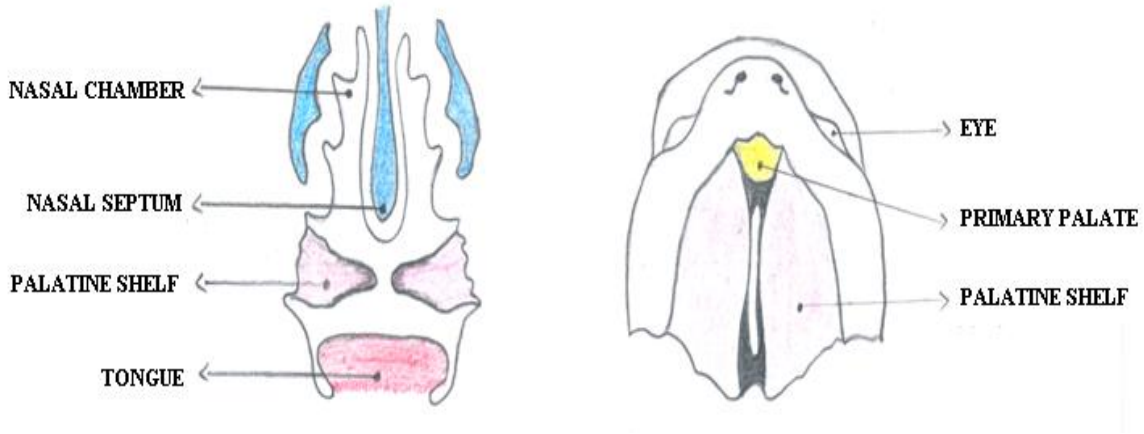


Figure 2 : DEVELOPMENT OF PALATE

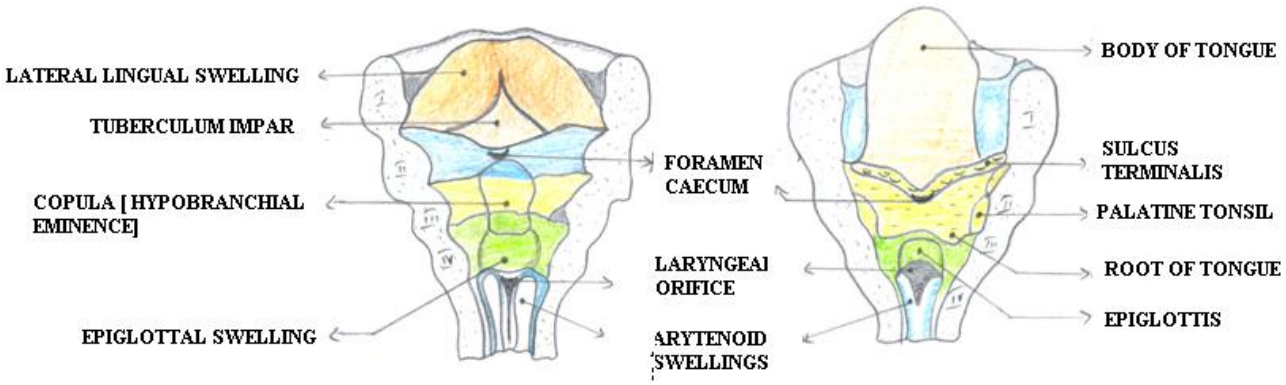


Figure 3 : DEVELOPMENT OF TONGUE

Development of Tongue

The anterior ($2/3^{rd}$) of tongue arises from mandibular arches from paired eminences and tuberculum impar and posterior ($1/3^{rd}$) part arises from hypobranchial eminence. This grows forward over second arches to become continuous with anterior part. Sulcus terminalis lies posterior to site of union of the two parts. Foramen caecum is the small median pit in dorsum of tongue.[4]

Mucosal cover of body of tongue arises from 1^{st} arch tissue and its sensory innervations from lingual branch of mandibular division of trigeminal nerve. The 3^{rd} arch nerve – glossopharyngeal nerve provides sensory innervations to posterior $1/3^{rd}$ of tongue. Some amount of tissue between the above two parts are supplied by 7^{th} nerve. Gustatory function is by Chorda tympani branch of Facial nerve.[4]

ANATOMY OF ORAL CAVITY

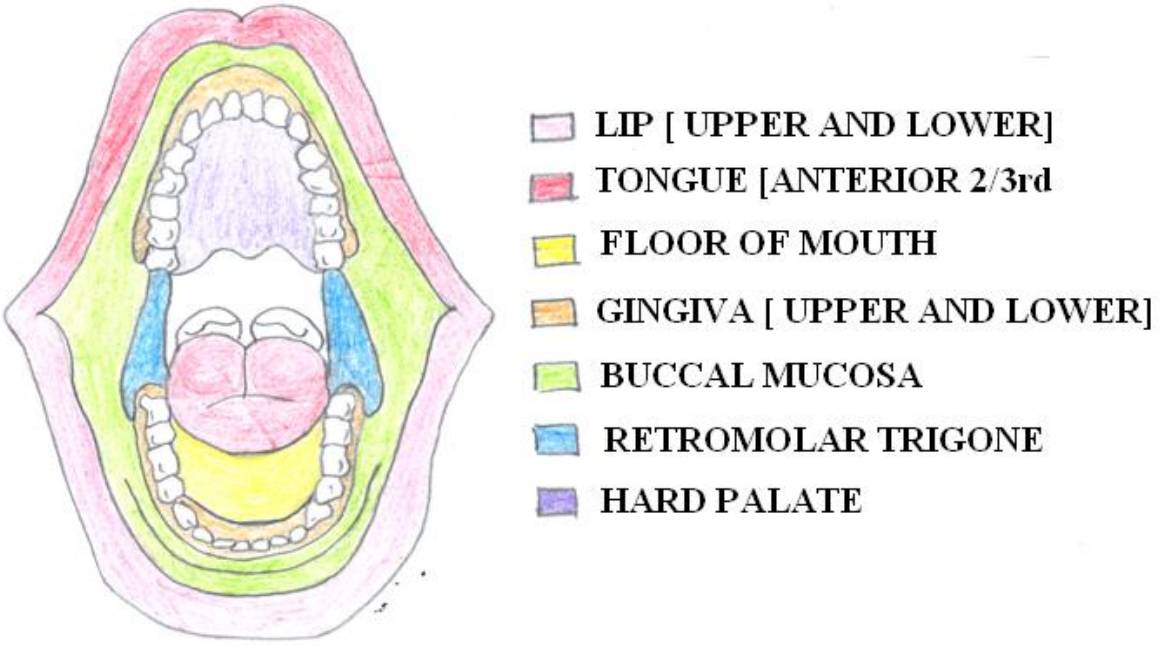


Figure 4 : ANATOMY OF ORAL CAVITY

Mouth is divided into vestibule and oral cavity proper. Extending from lips anteriorly, upto anterior pillars posteriorly, superiorly the palate, inferiorly floor of mouth and laterally buccal mucosa and retromolar trigone.[5]

Functions of the mouth – mastication and ingestion of food. Phonation being a secondary function.[5]

Cheek and oral mucosa

Cheek is lined on outer surface by skin and on inner surface by mucosa which is adherent to buccinators. Cheek contains a variable amount of buccal fat pad of Bichat, fibrous connective tissue, vessels, nerves, small salivary glands. Fordyce's spots, opening of parotid duct and

linea alba seen on the mucosal side. Oral mucosa is divided into lining, masticatory and specialized mucosae.[5]

Vascular supply to the cheek is by the Buccal branch of External Maxillary artery [Facial artery]. Nerve supply is by cutaneous branch of Maxillary division of Trigeminal nerve and buccal branch of Mandibular division of Trigeminal nerve.[5]

Oral cavity extends from vermilion border of lips to a plane bound by circumvallate papillae of tongue inferiorly and junction of hard and soft palate superiorly.[6]

Buccal mucosa

The inner mucosal lining extends from line of occlusion of lips to attachment of mucosa to alveolar ridge and pterygomandibular raphe. Lymphatic drainage is to the parotid, submandibular and submental nodes which inturn drain to upper deep cervical nodes or the facial nodes. Drainage from the latter group of lymph nodes is to the submandibular nodes.[6]

Lip

Lip consists of vermilion surface or the portion in opposition with the other lip. Labial mucosa is smooth, shiny and the underlying glands produce small elevations.[5,6] Vascular supply is from superior and inferior labial branches of Facial artery.[6] Nerve supply of upper lip is infraorbital branch of Maxillary nerve and of lower lip is mental branch of inferior dental nerve. Motor nerve supply of upper lip and orbicularis oris is buccal branch of facial nerve and of lower lip is cervical branch of facial nerve and marginal mandibular nerve.[6] Lymphatic drainage of upper lip: buccal and parotid nodes drain to facial nodes which inturn drains into submandibular nodes and later into upper deep cervical nodes. Lower lip : submandibular nodes drains into upper deep cervical nodes. Central portion of

lower lip : submental nodes drains into submandibular and then to upper deep cervical nodes or to level 3 (omohyoid) group of nodes.[6]

Alveolar ridges

Consist of alveolar processes of mandible and maxilla with covering mucosa adherent to periosteum. Nerve supply of lower alveolus is from branches of mandibular nerve and of upper alveolus is branches of maxillary nerve. Lymphatic drainage is to submental and submandibular nodes.[6]

Floor of mouth

It is a horse shoe shaped space lying beneath tongue and above mylohyoid diaphragm.[5,6] Extends from inner aspect of lower alveolar ridge to base of anterior fauces. Frenulum divides it into two parts. The opening of submandibular and sublingual ducts are seen here.[6] Main muscles of floor of mouth are mylohyoid and geniohyoid.[5] Lymphatic drainage is to submental and submandibular nodes.[6]

Hard palate

Formed by horizontal plate of palatine bone and palatine processes of maxilla with extension from inner surface of superior alveolar ridge anteriorly to posterior edge of palatine bone posteriorly.[5,6] There is a strong adherence of mucosa to periosteum and this in turn to bone by Sharpey's fibres. A number of mucous glands are present between mucosa and periosteum. The lining epithelium is keratinized stratified squamous with regional variations like ortho or para keratinized.[6] Greater palatine artery provides the vascular supply. Nerve supply by greater palatine branch of Maxillary nerve supplying palate upto the incisive foramen. The premaxillary area (between incisors and incisive foramen) is supplied by

nasopalatine branches. Lymphatic drainage occurs from retropharyngeal nodes to deep cervical nodes.[6]

Oral Tongue

Tongue is a highly muscular organ of deglutition, taste & speech. It is divided into oral (pre-sulcul) & pharyngeal (post-sulcal) parts by a 'V' shaped sulcus terminalis.[5]

Anterior 2/3rd (oral) part develops from the lingual swellings of mandibular (1st) arch and tuberculum impar. It is freely mobile & extends from anterior to circumvallate papillae, with apex touching incisors, lateral margins touching teeth and gums and the dorsum in relation to hard & soft palates and consists of nonvillous undersurface.[5,6]

It is lined by keratinizing stratified squamous epithelium.[6] Foliate papillae lie in front of palatoglossal arch and the filiform, fungiform, circumvallate papillae lie over the dorsum. The ventral surface is smooth, pink to purplish & connected to floor by lingual frenulum. Lingual vein lies lateral to frenulum and Plica fimbriata lies lateral to the vein.[5] Mucous & serous glands are mainly concentrated under the tip and sides.[6]

Main action of the intrinsic muscles – Superior & Inferior Longitudinal , Transverse, Vertical, is to alter shape the of tongue. Extrinsic muscles bear attachment to base – Genioglossus (mandible & hyoid) making up the bulk of tongue, Hyoglossus (hyoid), Styloglossus (styloid process) & Palataglossus (hard palate). These muscles stabilize the tongue. Midline of tongue is a tough fibrous septum making it an avascular plane. Blood supply to the tongue is mainly by branches of lingual artery. Tip of tongue drained by deep lingual vein and the rest by lingual vein.[6] Hypoglossal nerve supplies all muscles except palatoglossus which is supplied by pharyngeal plexus. The sensory supply is by lingual nerve and chorda tympani provides taste sensation. Lymphatic drainage of tip is via submental nodes to level III (middle deep jugular). Rest of the oral tongue is drained by submandibular

nodes. The unique feature of tongue is that the lymph from one side might reach lymph nodes of contralateral side of neck.[6]

EPIDEMIOLOGY

Worldwide estimate of oral cancer detection each year is 4,05,000 cases with 2/3rd occurring in developing countries. India, Sri Lanka, Pakistan, Bangladesh, Hungary & France have the highest rates with the former 4 accounting for 30% of newly detected cases and seen more commonly in men.[6]

Carcinoma of buccal mucosa accounts for 40% of oral cancers in South East Asia.[7] 85% cases occur >50 years of age, except in developing countries where onset is earlier due to tobacco/ pan chewing habits. In India, the male : female ratio is said to be 4:1. Floor of mouth accounts for 18-33% of oral cancers and seen more frequently in men in 6th-7th decade. 22-39% of oral carcinomas arise in the tongue, most commonly in middle 1/3rd and in lateral aspect preceding ventral aspect. 90% are >40 yrs of age & male : female ratio decreasing.[7]

Retromolar trigone incidence in oral cancers is 6 - 7% and is more common in males. Incidence of carcinoma in Maxillary alveolus is 3.5 – 6.5% & hard palate is 1 – 3%. Oral cancers are more common in males except in hard palate carcinomas where precedence in females is more due to reverse smoking .[7] Mandibular cancers account for 7.5 – 17.5 % of oral cancers. Ratio for mandibular : maxillary alveolus cancers is 3:1 & is more common in males.[7]

Table 1 : Site distribution[6]

Country	Anterior tongue (%)	Floor of the mouth (%)	Buccal mocosa (%)	Alveolar ridges (%)	Hard palate (%)
United kingdom	36	46	46	46	46
USA(40)	36	35	10	16	3
France(41)	22	68	7	2	1
India(42)	22	4	43	18	3.5

ETIOLOGY

Tobacco

>90% of patients with oral cavity cancer have a history of smoking.[6] Polycyclic hydrocarbons & nitrosamines are a couple of the over 30 carcinogens tobacco contains.[7] 34,000 of the estimated 56,000 annual cases are tobacco induced.[6] Alcohol & tobacco has a synergistic action.[4] There is an increased risk of oral carcinoma in pipe & cigar smokers. The extract of black tobacco cigarettes is more carcinogenic than blonde tobacco cigarettes. 'Bidi' smoking is linked to oral commissure & tongue carcinoma. 'Chutta' causes hard palate carcinoma. 'Pan chewing' causes alveolobuccal carcinoma – lime lowers pH which accelerates release of alkaloids from both tobacco & areca nut.[6,7] 'Khaini' (mixture of lime & tobacco) causes predisposition to carcinoma in inferior gingivolabial sulcus.[6] Toombak, contains high levels of nitrosamines & its associated carcinogens have a high prevalence of p53 protein aberration.[7] Marijuana, according to Memorial Sloan Kettering Cancer Centre, has a 2.6 risk in comparison to non users. Release and absorption of a range of carcinogens – polycyclic aromatic hydrocarbons, benzopyrene, phenols, phytosterols, acids, terpenes are associated with its consumption.[7]

Photo 1 : VARIOUS FORMS OF TOBACCO

Alcohol

Alcohol by itself is not a carcinogen but potentiates the action of other carcinogens. Possible mechanisms of carcinogenesis :

- The cellular permeability for carcinogens is increased due to its solvent property.
- The constituents of alcoholic beverages may induce carcinogenic effects.
- Acetaldehyde – immediate metabolite of ethanol will cause a damaging effect on cells
- Regular intake of alcohol results in upregulation of enzymes of cytochrome P450 system which causes activation of procarcinogens into carcinogens.
- Causes chromosomal damage due to decreased activity of Deoxyribonucleic acid (DNA) repair enzymes.
- Causes reduction in T cell number, decreased mitogenic activity and macrophage activity leading to impaired immunity.
- Suppresses appetite as it is high in calories and metabolism is further impaired due to liver disease resulting in nutritional deficiencies and thus lowering resistance to cancer.

Drinkers show a higher predilection to buccal carcinoma & lateral tongue cancer than other parts of tongue when compared to non-drinkers.[7]

Nutritional factors

According to literature, 15% of oral and pharyngeal cancers are attributed to dietary deficiency. High fruit and vegetable intake due to increased antioxidants or free radical scavenging decreases the risk. Vitamin A, C & E have a reduced risk of head & neck cancers.[7]

Chronic irritation

Poor orodental hygiene, painful or loose fitting dentures, mouthwash containing alcohol, syphilis are associated with increased risk.[6,7]

Pre cancerous lesions

Leukoplakia, erythroplakia, OSMF, lichen planus predispose to development of carcinoma.[7]

Genetic & Immunological

Alcohol is associated with p53 mutations.[6] Conditions associated with increased risk is Li-Fraumeni Syndrome where p53 mutation is seen. Increased chromosomal fragility & defect in DNA repair causes increased susceptibility to cancer in conditions like Fanconi's Anaemia, Bloom Syndrome, Ataxia telangiectasia.[6,7] Patients undergoing bone marrow or organ transplant show increased incidences of oral or skin cancers.[7]

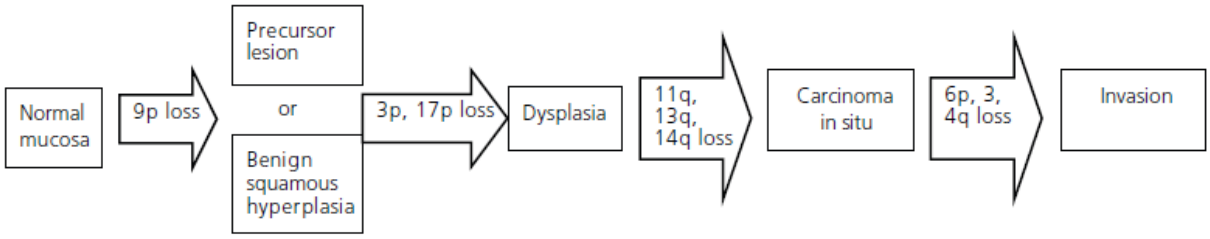


Figure 5 : GENETIC PROGRESSION OF HEAD AND NECK CANCERS[8]

Viruses

Carcinogenesis can be caused by Human Papilloma Virus (HPV), Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV) & Epstein Barr Virus (EBV)[7]

- **HPV :** 30 – 100% verrucous carcinomas are caused by HPV. HPV is strongly associated with carcinoma of tonsil in oropharynx. HPV 16, 18 (high risk) are seen to be associated with pre malignant lesions and SCC. HPV afflicted cancer patients are usually young, non-smokers & non-drinkers. HPV positive cancers have a better

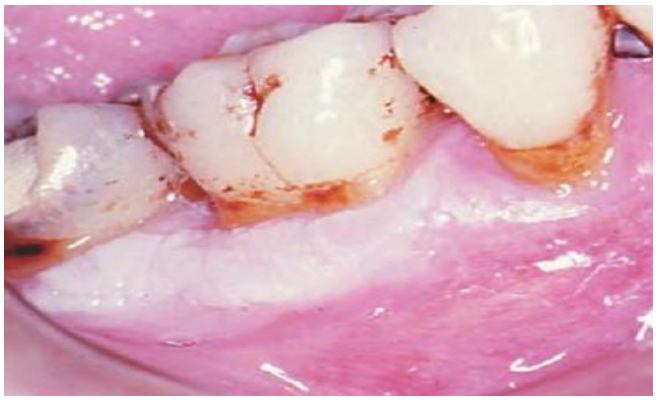
prognosis. E6 & E7 open reading frames (ORFs) bind to and inactivate the tumour suppressor genes p53 & pRb respectively allowing uncontrolled cell proliferation which results in genomic instability & cellular transformation.[4] HPV is associated with oral cancer in13%-15%of patients.[7]

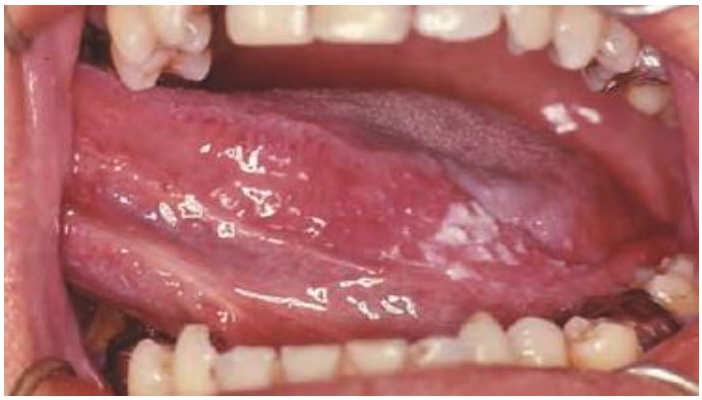
- **HIV :** Immunodeficiency status predisposes cancer patients to present at an advanced stage. 5% of head and neck cancer patients had associated HIV.[7]

- **HSV :** HSV antibodies have been found in patients who are smokers and more so in those who have oral cancers. Also, HSV type protein has been detected in 42% with oral cancers.[7]

PREMALIGNANT LESIONS

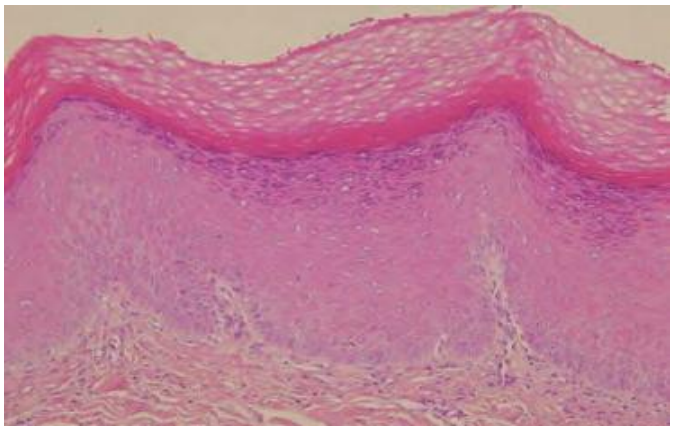
Leukoplakia

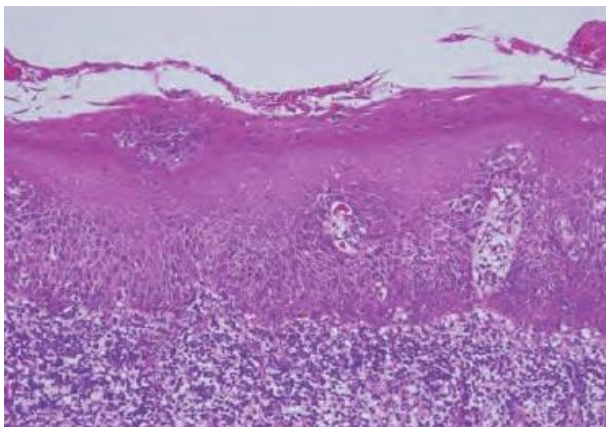




Over lower alveolus **Over lateral border of tongue**

Figure 6 : LEUKOPLAKIA : over lower alveolus and lateral border of tongue[9]





Homogenous **Non-Homogenous**

Figure 7 : HISTOPATHOLOGY OF LEUKOPLAKIA : homogenous and non-homogenous[9]

"White patch or plaque that cannot be characterized clinically or pathologically as any other disease." Leukoplakia prevalence worldwide is 2%.[6] 4 – 38 % are idiopathic and have high risk of malignant transformation.[8] This white lesion is most often related to increase in surface keratin layer. It most commonly affects buccal and alveolar mucosa and lower lip.[10] These are of two types – homogenous and non homogenous.

Homogenous/ Leukoplakia simplex : Is the most common variety. These are homogenous, sharply circumscribed, thickened, whitish areas broken up by longitudinal fissures. Almost always hyperorthokeratotic, but, can be hyperparakeratotic on histology.[6]

Non homogenous : Are predominantly white or white and red lesions that are either nodular, speckled or verrucous. The former two show association with severe dysplasia and candida infection. On microscopy – hyperkeratosis, acanthosis, parakeratosis, widening of rete pegs, dyskeratosis and carcinoma in situ is seen. Nodular lesion appears raised, rounded, with red and/ or white excrescences.[6,10] The latter has a warty surface and is associated with dysplasia and develop into squamous cell carcinoma or verrucous carcinoma. 1% undergo malignant transformation, the percentage being more in non homogenous type. 15 – 30% of cases with dysplasia develop malignancy.[6]

Proliferative verrucous leukoplakia (PVL) : This type of leukoplakia has a high risk of malignant transformation. It transforms from a homogenous solitary patch to an exophytic, diffuse or multifocal, progressive and irreversible lesion. Affects middle aged people, female : male :: 4 : 1. Most commonly affects buccal mucosa. Histological features varies from benign keratotic lesion to verrucous hyperplasia and finally any of the 3 forms of squamous cell carcinoma – verrucous, conventional or papillary types. High rate of

recurrence is seen as it has a widespread growth.[6] They have a very high rate of malignant transformation of 60 – 100%. [8]

Risk factors for malignant transformation :- [6]

- Females
- Long duration of leukoplakia
- Site – tongue or floor of mouth
- Leukoplakia in non smokers
- > 2cm. size
- Non homogenous type
- Presence of dysplasia

Erythroplakia

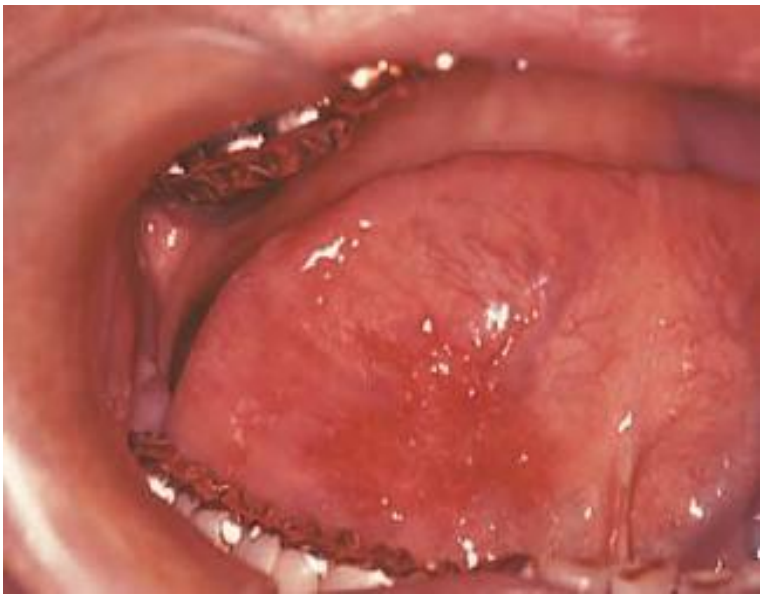


Figure 8 : ERYTHROPLAKIA : over ventral aspect of tongue [9]

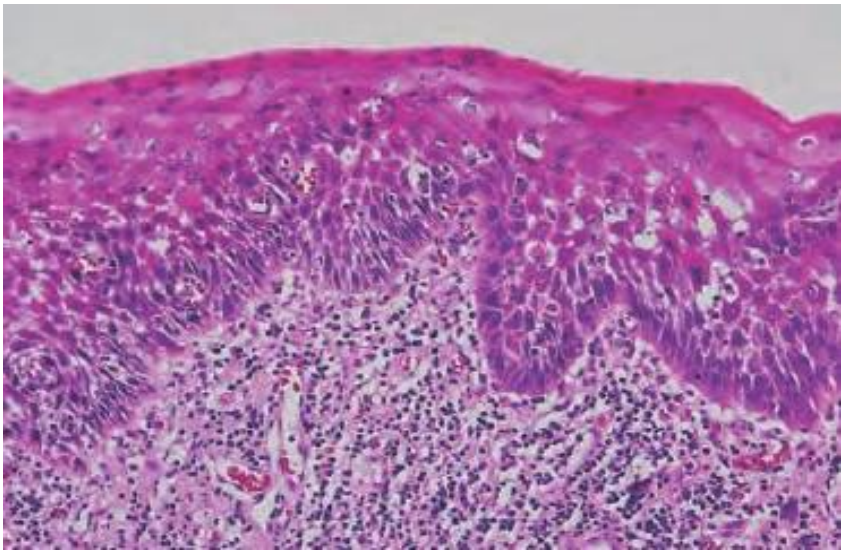


Figure 9 : HISTOPATHOLOGY OF ERYTHROPLAKIA [9]

"Bright red velvety patch that cannot be characterized clinically or pathologically as being caused by any other condition."[6] It appears as a red macule or plaque with a soft, velvety texture, quite sharply demarcated and regular in colouration. Seen more commonly in older men and more so after 6th decade. Most frequently affected sites are floor of mouth, ventral and lateral tongue, retromolar trigone and soft palate. Erythroleukoplakia / speckled mucosa are lesions which are intermixed with white areas and behave similar to a pure oral erythroplakia lesion. Red appearance of erythroplakia is related to increase in subepithelial blood vessels, lack of surface keratin and thinness of epithelium.[10] Prevalence of erythroplakia is 0.05% and erythroplakia aneuploidy rate is 68%. Erythroplakia has a 20-35% chance of malignant transformation.[6]

Oral submucous fibrosis

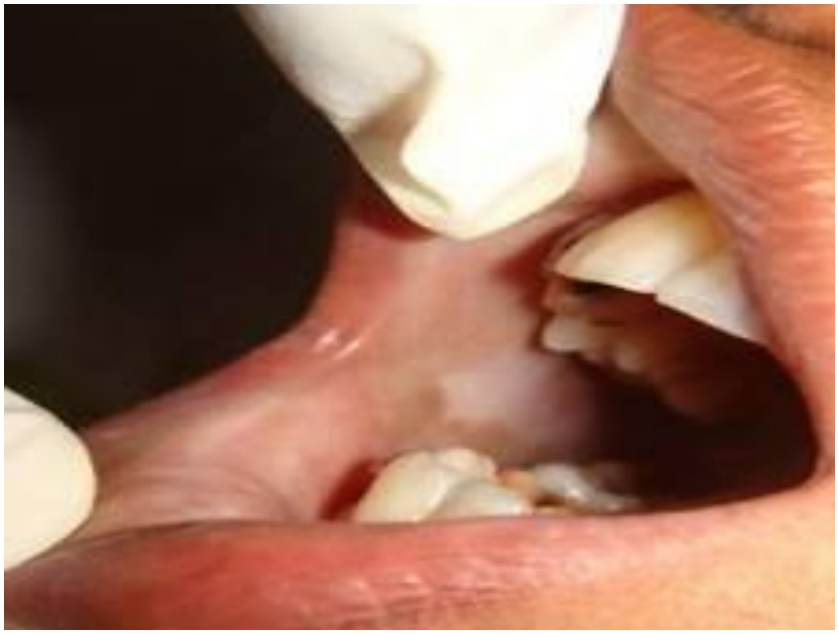


Figure 10 : ORAL SUBMUCOUS FIBROSIS : over buccal mucosa [8]

Submucous fibrosis is characterized by mucosal fibrosis. It can be graded into early and late forms. **Early forms** present with burning sensation which exacerbates on intake of spicy food. On examination, oral mucosal ulceration or vesiculation, blanching of mucosa and 'leathery' mucosa which shows thickened, firm tissue with a wrinkled surface are seen. In **late presentations,** there is appearance of fibrous bands within mucosa. Patient experiences mastication and phonation difficulties. On examination, trismus, narrowing of oropharyngeal isthmus with distortion of uvula and 'woody' feel to mucosa and tongue are seen. Histological features – epithelial atrophy with metaplasia of non keratinized areas to para or ortho keratinization with varying degrees of dysplasia. Basement membrane is thickened. There is a marked reduction in connective tissue vascularity inversely proportional to increased collagen density.[9]

It is seen predominantly in the Asian community owing to areca nut and betel quid chewing habit.[3,5] Alkaloids present in it – arecoline and arecaidine its active metabolite cause fibroblast proliferation and increased collagen synthesis. Tannin inhibits collagenases and reduces collagen degradation. The high copper content in areca nut stimulates fibroblast proliferation. It further induces OSMF formation by increased cytokine levels – Interleukin-1, Interleukin-6, Transforming growth factor-Beta, Platelet derived growth factor and Basic fibroblast growth factor in lamina propria. Rate of malignant transformation is 2-5%.[9]

Lichen planus

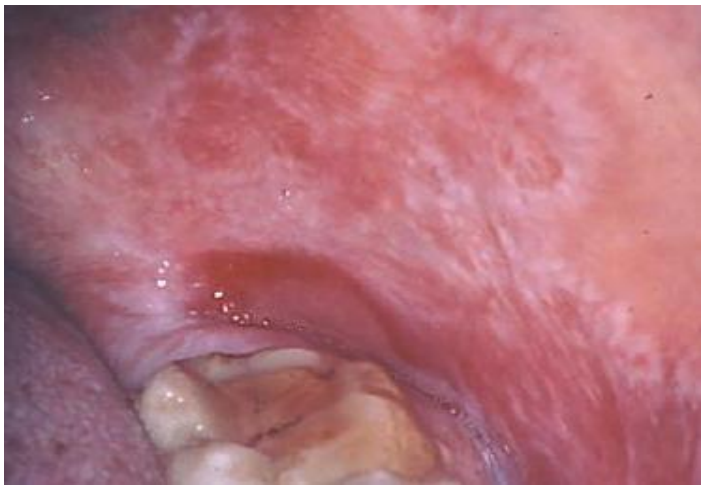


Figure 11 : LICHEN PLANUS : over buccal mucosa and alveolus [9]

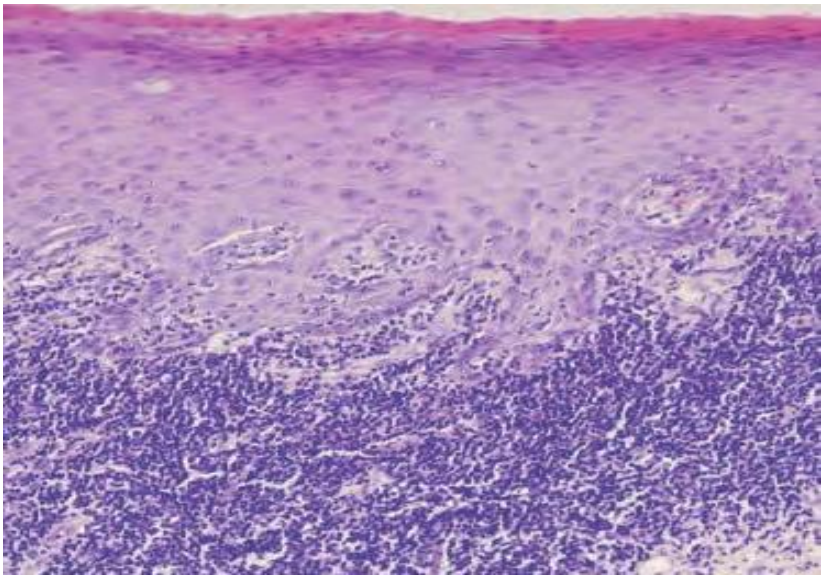


Figure 12: HISTOPATHOLOGY OF LICHEN PLANUS[9]

Is a chronic inflammatory mucocutaneous disease affecting oral cavity, hair, nails. Oral cavity lesions are usually persistent with no scope of spontaneous resolution. There is a possible link with autoimmunity as the histological appearance shows T lymphocytes attacking the basal epithelium.[11]

Females are affected more than males and people over middle age affected more commonly. These white lesions are often symmetrical and mainly appear as a striae, interspersed with well-defined, small, elevated papules. The other types being confluent plaques and homogenous lichen planus. In some, they lead to painful intractable oral ulceration. These lesions are well demarcated, raised and appear tessellated due to the intersecting grooves. Striated areas can most times be associated with atrophic areas with redness due to mucosal thinning without ulceration. If erosions are seen they present as shallow, irregular ulcers covered by slightly raised, yellowish fibrinous slough.[11]

Most common site of involvement is posterior part of buccal mucosa followed by lateral border of tongue. Gingiva is also often involved by atrophic lichen planus.[11]

These white lesions show parakeratosis or hyperorthokeratosis with a prominent granular cell layer. In the superficial corneum a characteristic band like lymphohistiocytic infiltration occurs. A conspicuous basal cell damage demonstrated by presence of apoptosis, ballooning degeneration due to intracellular edema and formation of colloid (civatte) bodies. Rete ridge pattern is seen over tongue dorsum. Severe ulceration is seen in lesions of oral cavity.[11] Malignant transformation rate is <1%.[6]

Table 2 : Classification of epithelial precursor cells[6]

2005 WHO Classification	Squamous intraepithelial neoplasia (SIN)	Ljubljana classification squamous intraepithelial lesions (SIL)
Squamous cell hyperplasia		Squamous cell hyperplasia
Mild dysplasia	SIN1	Basal/ parabasal hyperplasia
Moderate dysplasia	SIN2	Atypical hyperplasia
Severe dysplasia	SIN3	Atypical hyperplasia
Carcinoma in situ	SIN3	Carcinoma in situ

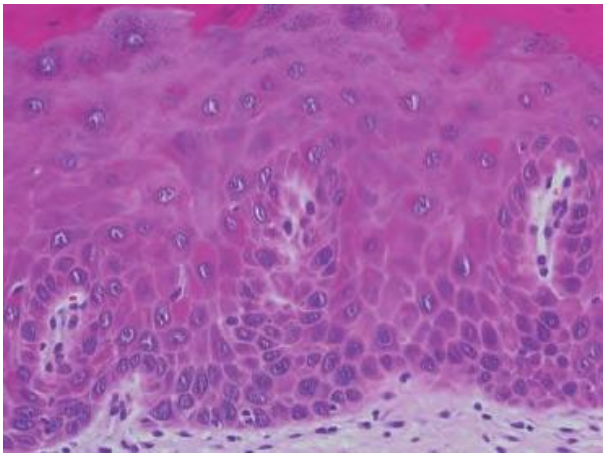


Figure 13 : MILD DYSPLASIA[9]

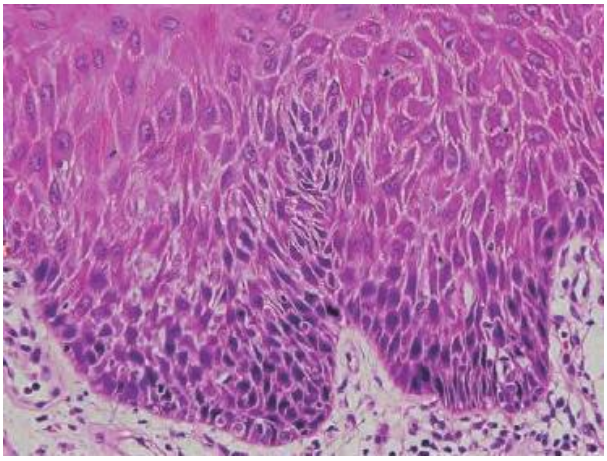


Figure 14 : MODERATE DYSPLASIA[9]

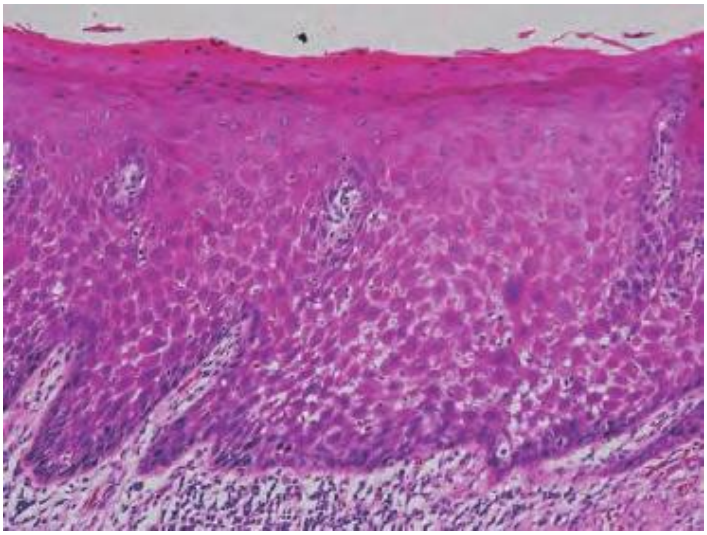


Figure 15 : SEVERE DYSPLASIA[9]

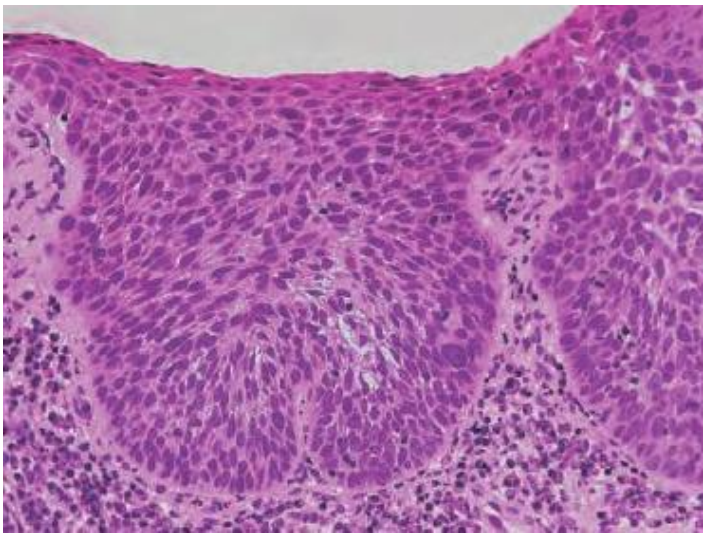


Figure 16 : CARCINOMA IN SITU[9]

Table 3 : Criteria used for diagnosing dysplasia[9]

Architecture	Cytology
Irregular epithelial stratification	Abnormal variation in nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)
Drop shaped rete ridges	Abnormal variation in cell size (anisocytosis)
Increase number of mitotic figures	Abnormal variation in cell shape (nuclear pleomorphism)
Abnormally superficial mitoses	Increased nuclear-cytoplasmic ratio
Premature keratinization in single cells (dyskeratosis)	Increased nuclear size
Keratin pearls within rete pegs	Atypical mitotic figures Increased number and size of nucleoli Hyperchromasia

Table 4 : WHO classification (1997) of precancerous lesions and conditions [9]

Precancerous lesions	Precancerous conditions
Leukoplakia	Sideropenic dysphagia
Ertroplakia	Lichen planus
Palatal keratosis associated with reverse smoking	Oral submucous fibrosis Syphilis Discoid lupus erythematosus Xeroderma pigmentosum Epidermolysis bullosa

Table 5 : Histological changes in epithelial dysplasia[9]

Histological changes in epithelial dysplasia
Loss of polarity of basal cells
The presence of more than one layer having basaloid appearance
Increased nuclear-cytoplasmic ratio
Drop-shaped rete ridges
Irregular number of mitotic figures
Mitotic figures that are abnormal in form
The presence of mitotic figures in the superficial half of the epithelium
Cellular and nuclear pleomorphism
Nuclear hyperchromatism
Enlarged nucleoli
Loss of intercellular adherence
Keratinization of single cells or cell groups in the prickle cell layer

FIELD CANCERIZATION

An inherent instability of mucosa lining entire upper aerodigestive tract combined with repeated carcinogenic insults (Eg. carcinogens present in alcohol and tobacco) leads to increased risk of developing multiple independent pre-malignant and malignant foci.[3,6,12,13]This theory was reinforced by observation of a high incidence of second primary tumors in patients with oral carcinoma (approximately 4%/ year).[6,13] The recurrence and progression of these tumors depend on malignant changes in remaining mucosa.[12] Dysplasia, carcinoma in situ are not identifiable according to typical morphological criteria and are thus frequently overlooked in routine oral cavity examination.[12]Another support to this theory is

when adjacent tissue which is normal histologically shows loss of heterozygosity and other chromosomal abnormalities.[6]

Early detection of malignancy with proper demarcation of margin of malignancy followed by radical surgery is the only way to improve cure rates.[1]

Table 6 : CLASSIFICATION OF ORAL CAVITY TUMOURS[6]

Epithelial Tumors	1. Malignant epithelial tumors a. Squamous cell carcinoma i. Verricous carcinoma ii. Basaloid squamous cell carcinoma iii. Pappilary squamous cell carcinoma iv. Spindle cell carcinoma v. Acantholytic squamous cell carcinoma vi. Adenosquamous carcinoma vii. Carcinoma cuniculatum b. Lymphoepithelial carcinoma 2. Epithelial precursor lesions 3. Benign epithelial tumors a. Papillomous i. Squamous cell papilloma ii. Condyloma acuminatum iii. Focal epithelial hyperplasia b. Granular cell tumor c. Keratocanthoma
Salivary Gland Tumors	1. Malignant a. Acinic cell carcinoma b. Mucoepidemoid carcinoma c. Adenoid cystic carcinoma d. Polymorphous low grade adenocarcinoma e. Myoepithelial carcinoma f. Carcinoma ex pleomorphic adenoma

	g. Other carcinomas 2. Benign a. Pleomorphic adenoma b. Myoepithelioma c. Basal cell adenoma d. Canalicular adenomas e. Duct papilloma
Soft Tissue Tumors	1. Kaposis sarcoma 2. Lymphangioma 3. Ectomesenchymal chondromyxoid tumor 4. Focal oral mucinosis 5. Congenital granular cell epulis
Hematolymphoid Tumours	1. Diffuse large B cell lymphoma (DLBCL) 2. Mantle lymphoma 3. Follicular lymphoma 4. Extranodal marginal zone B cell lymphoma (MALT) 5. Burkitt lymphoma 6. T cell lymphoma 7. Extramedullary plasmacytoma
Mucosa Malignant Melanoma	
Tumors of Bone	1. Giant cell lesions 2. Reparative granuloma 3. Brown tumor of hyperparathyroidism 4. Giant cell tumor of bone 5. Fibro-osseous lesions 6. Fibrous dysplasia 7. Ossifying fibroma

	8. Periapical cemental dysplasia 9. Chondrogenic neoplasms 10. Chondroma 11. Chondrosarcoma 12. Osteogenic neoplasms 13. Osteoid osteoma and osteoblastoma 14. Ameloblastoma 15. Osteogenic sarcoma 16. Other tumors 17. Histiocytosis X 18. Multiple myeloma 19. Ewing's sarcoma
Secondary Tumors	

Table 7 : TNM STAGING [AJCC][14]

Primary Tumor (T)	TX Primary tumor cannot be assessed T0 No evidence of primary tumor Tis Carcinoma in situ T1 Tumor 2 cm or less in greatest dimension T2 Tumor more than 2 cm but not more than 4 cm in greatest dimension T3 Tumor more than 4 cm in greatest dimension T4a Moderately advanced local disease* (lip) Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face, that is, chin or nose (oral cavity). Tumor invades adjacent structures only (e.g., through cortical bone [mandible or maxilla] into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face) T4b Very advanced local disease Tumor invades masticator space, pterygoid plates, or skull base and/or encases internal carotid artery
Regional Lymph Nodes (N)	NX Regional lymph nodes cannot be assessed N0 No regional lymph node metastasis N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension N2 Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension;

	or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension N2a Metastasis in single ipsilateral lymph node more than 3 cm but not more than 6 cm in greatest dimension N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension N3 Metastasis in a lymph node more than 6 cm in greatest dimension
Distant Metastasis (M)	M0 No distant metastasis M1 Distant metastasis

Table 8 : STAGING [AJCC][14]

Carcinoma in situ	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0 T1 N1 M0 T2 N1 M0 T3 N1 M0
Stage IV a	T4a N0 M0 T4a N1 M0 T1 N2 M0 T2 N2 M0 T3 N2 M0 T4a N2 M0
Stage IV b	Any T N3 M0 T4b Any N M0
Stage IV c	Any T Any N M1

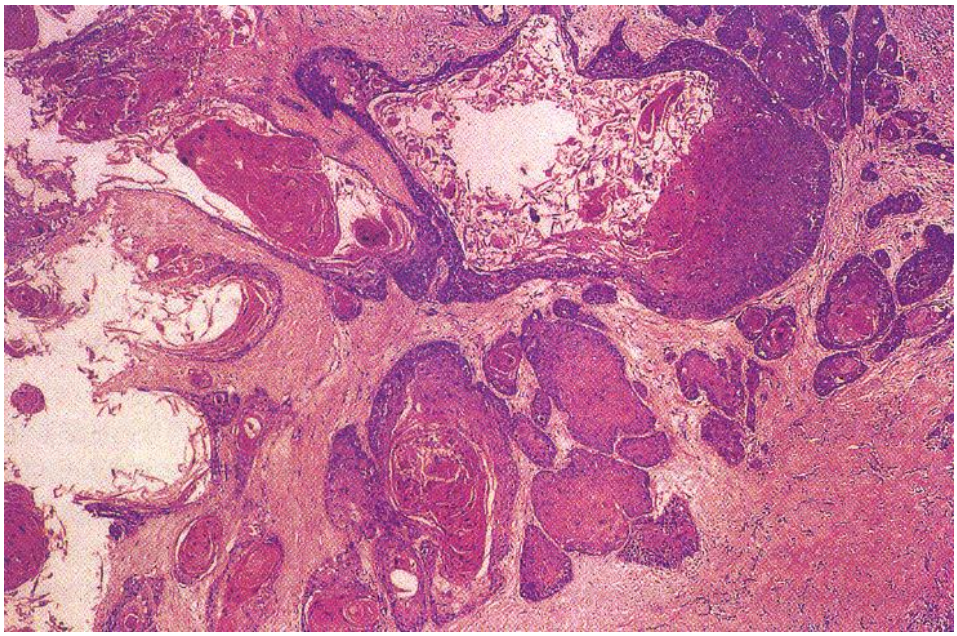


Figure 17 : HISTOPATHOLOGY OF SQUAMOUS CELL CARCINOMA[15]

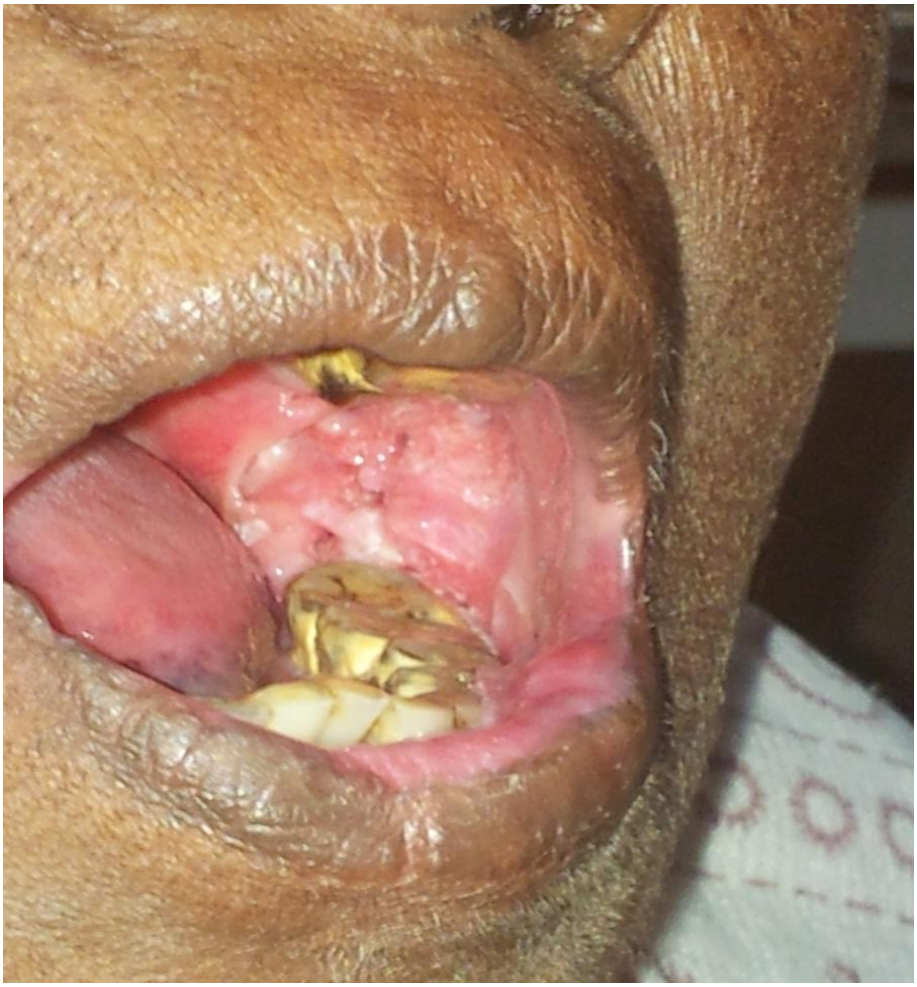


PHOTO 2 : SQUAMOUS CELL CARCINOMA

Biopsy is the standard investigation for diagnosis of oral cancer/ pre malignant lesions. False negative reports can be obtained in cases of field cancerization, associated trismus and associated mucosal benign lesion.[6]

Table 9 : Diagnostic methods for potentially malignant/ pre-malignant disorders[9]

Examination methods and adjunctive techniques	Material	Normal mucosa	Dysplasia (atypia)
Conventional oral examination	Inspection and palpation	Normal looking	Some abnormality
Vital staining: Toluidine blue Iodine	Tolonium chloride Iodine glycerol or lugol's solution	No staining Dark (parakeratotic cell)	Pale or dark royal blue No staining
Tissue reflectance light visualization: ViziLite Plus® MicroLux DL	Chemiluminescent light Battery-powered light	Light blush Light bush	Reflect and shine white Reflect and shine white
Fluorescene imaging and spectroscopy: VELscope®	Handpiece-emitted blue light	Fluorescent glow	Loss of fluorescence
Brush cytology: OralCDx®	Cytology by brush biopsy	Negative (normal)	Atypical (abnormal) Positive (dysplasia/ca)
Scalpel biopsy: Incisional Excisional	Incisional biopsy for diagnosis Diagnosis and treatment	Normal tissue	Dysplasia or cancer

Supravital Dyes

PHOTO 3 : SUPRAVITAL DYES

Supravital dyes have been in use for detection of malignancy and to assess the margin of the lesion. These also aid in the assessment of site of lesion for taking biopsy. The main supravital dyes that have been in use are – Toluidine blue, Lugol's iodine and Methylene blue.

Toluidine blue use as a staining agent was first suggested in 1960[16]. Niebel and Chomet in 1964 brought about the use of toluidine blue for diagnostic purposes.[17] Toluidine blue is an acidophilic metachromatic dye of thiazine group which stains the cells due to its DNA binding property. Staining occurs as dysplastic and anaplastic cells contain more nucleic acids and also the dysplastic epithelium has loss of cohesion facilitating the penetration of the dye.[18,19,20]

Lugol's iodine/ I_2KI/ Markodine/ Strong solution/ Aqueous iodine solution BCP, first made in 1829 is a solution of elemental iodine and potassium iodide in water. Lugol's iodine is named after French physician Lugol (1786 – 1851). Principle on which iodine staining occurs – Iodine-starch reaction occurs giving a colour change when iodine reacts with glycogen in the cytoplasm. Cancer cells do not undergo this reaction due to enhanced glycolysis in cancer cells and loss of cellular differentiation.[21,22] This reaction causes the normal mucosa to stain brown or mahogany while the dysplastic tissue does not take up stain causing it to appear pale. Lugol's iodine is effective only on non-keratinized mucosa – buccal, vestibule, ventral surface and margins of tongue, floor of mouth).[23]

Methylene blue dye is preferred for large scale screening in high risk patients as they are cheap and have low toxicity. Methylene blue like toludine blue is taken up by cells with high concentration of nucleic acids and shows a deep blue shade. [24]

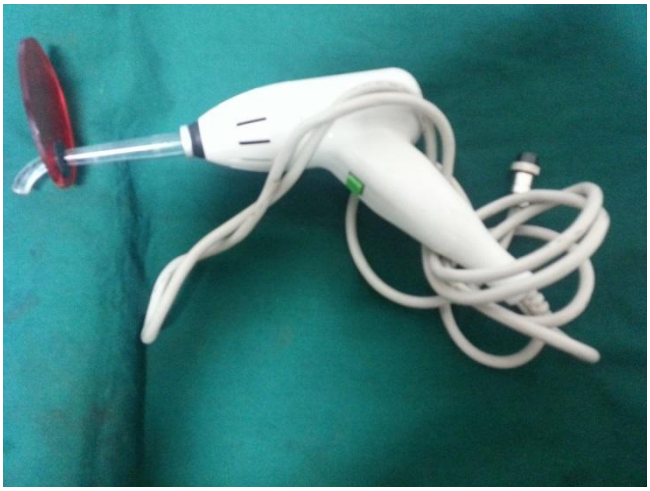
New diagnostic aids have been introduced to help in early detection of malignant lesions.[25] These technological and therapeutic advances are much needed to improve the poor outcomes associated with oral cancer due to inability to diagnose and treat the disease at an early, better

prognostic stage.[13] Oral cancer survival rates are strongly dependent on stage at diagnosis.[25] Early detection is one of the best ways to improve survival and quality of life for oral cancer patients and especially so in developing countries where there is a tendency for diagnosis at an advanced stage.[25]

5 – AMINOLEVULINIC ACID (5-ALA)

PHOTO 4: 5 – AMINOLEVULINIC ACID

PHOTO 5 : SOURCE OF LIGHT : Blue wavelength light (380-450nm) and Wood's lamp



ALA is endogenously formed in the mitochondria from Succinyl-CoA and Glycine.[26] Eight molecules of ALA give rise to Protoporphyrin IX (PpIX) and leads to heme formation.[27,28]

On exogenous administration of ALA, PpIX metabolism to heme is delayed and PpIX gets accumulated within cells. Exogenous ALA bypasses first rate limiting step and causes PpIX accumulation.[27] ALA, an early intermediate in heme biosynthesis pathway is metabolized intracellularly to PpIX which is photodynamically active.[28] Neoplastic cells selectively takes up PpIX on topical or systemic 5-ALA administration owing to altered enzymatic activity in heme biosynthetic pathway within cells.[29] There also appears to be a higher uptake of 5-ALA in malignant cell owing to reduced number of intercellular junctions and destruction of barrier functions in tumorous cells.[30] 5-ALA would help in detection of carcinoma in situ, early invasive cancer, tongue-like submucosal spread of lesions, infiltrative malignant cells diffusing into surrounding tissue layers, superficial demarcation of tumor margin which would be missed by simple inspection.[2] This is vital to ensure increased 5 year survival rates and decreased local recurrence. Topical ALA has an easy methodology and is free of side effects.[3]

ALA has low lipid solubility and bioavailability. Modifications to ALA done to improve cellular availability, increase stability in physiological pH, increase selectivity and limit side effects to extend its clinical utility. The first ALA topical application by Kennedy was for the treatment of Basal cell carcinoma in 1990.[31]

HEME BIOSYNTHETIC PATHWAY [28]

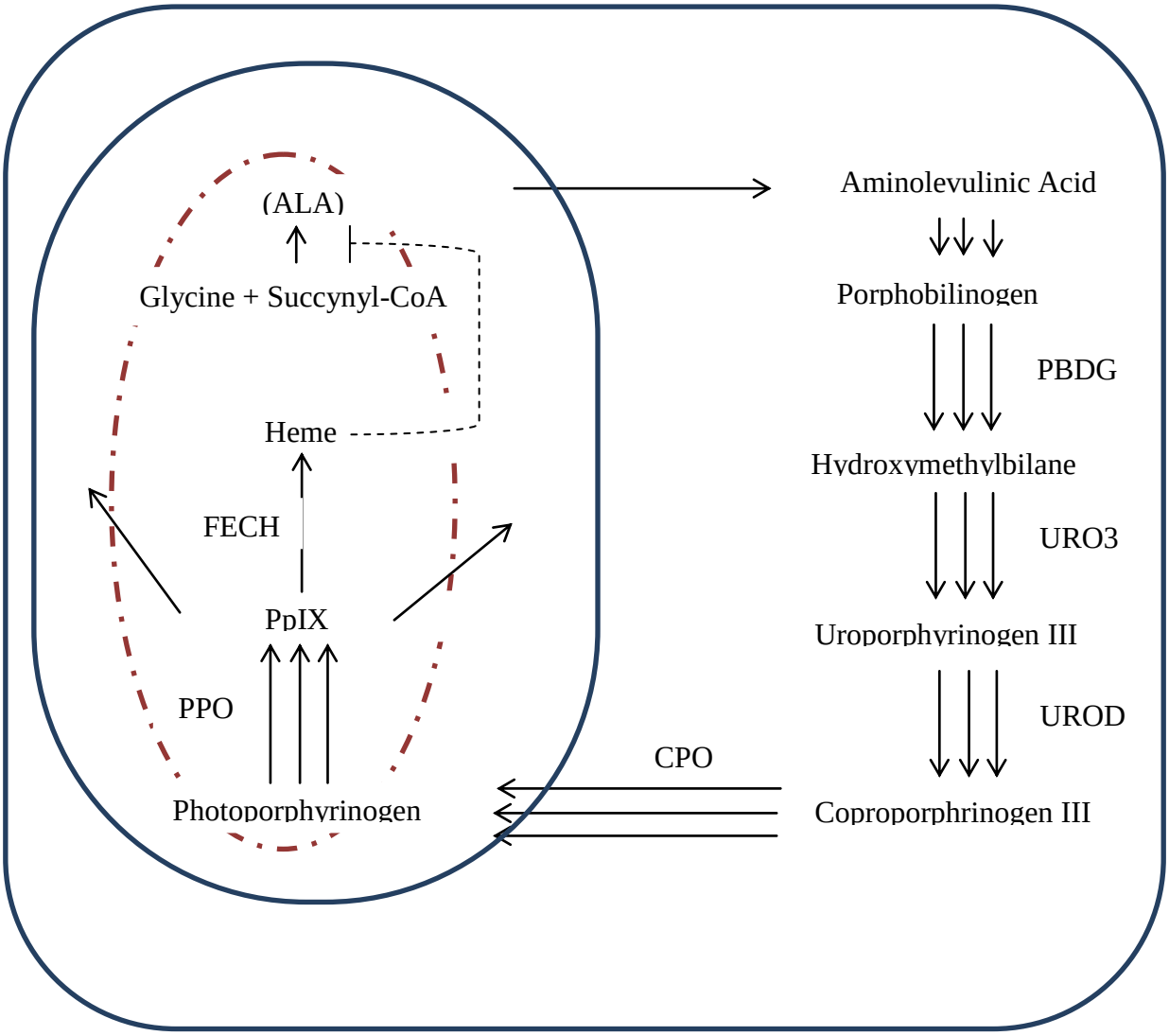


Figure 18 : HEME BIOSYNTHETIC PATHWAY

The rate limiting steps in the above pathway are : **1.** Synthesis of ALA from Succinyl CoA and Glycine, **2.** Conversion of PpIX to heam.[26]

Rate limiting enzymes: Aminolevulinic acid Synthase (ALAS1) and Ferrochelatase respectively.[27,28]

EXOGENOUS ALA

Heme regulates ALAS1 activity by negative feedback mechanism by preventing transport of the ALAS1 precursor to mitochondria by binding to heme-regulatory motif (HRM) in the mitochondrial targeting sequence of ALAS.[32] Attenuation of ALAS transcription is also caused by heme.[28]

Exogenous ALA leads to increased production of PpIX as it bypasses the natural heme regulation pathway.[33] Accumulation of PpIX within cells occurs as a result of low efficacy of ferrochelatase to convert PpIX to heme.[26]

There is preferential PpIX accumulation in tumour cells owing to decreased activity of ferrochelatase, limited iron availability, cellular iron depletion, enhanced enzyme activity – Aminolevulinate Dehydratase (ALAD), Uroporphyrinogen decarboxylase (UROD) or Porphobilinogen Deaminase (PBGD) and rapid cellular proliferation rate.[27,34]

The phase of the cell cycle, cellular availability of oxygen and the pH of extracellular fluid also influence ALA uptake by the cell. Endothelial cells that are quiescent (i.e predominantly in the G_0 phase of cell cycle at the plateau phase of growth) accumulate significantly less PpIX compared to cells that are actively proliferating.[26,27]

An active transport mechanism which is pH dependent causes ALA to be taken up into the cell. The optimal pH being 5.0. This greater uptake of substrate from the more acidic medium may have made some contribution to the increased PpIX production in neoplastic cells. Both macro and microvascular endothelial cells and vascular smooth muscle cells produce sufficient PpIX after a 4 hour incubation with ALA.[27,28]

PpIX accumulation was caused by increased synthesis of Coproporphyrin Oxidase leading to improved efficacy of ALA-PDT.[35]

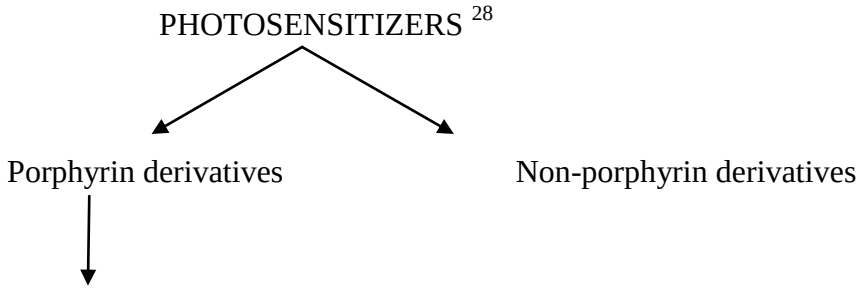
PHARMACOKINETICS

Oral ALA has lower bioavailability in comparison to intravenous route. Reduced bioavailability is owing to large biosynthetic PpIX capacity of gastrointestinal mucosal cells and hepatic first pass metabolism. On Topical ALA application, PpIX fluorescence is observed only at a depth of 0.3 – 0.6mm. Stratum corneum is the most important barrier for skin penetration.[36] ALA is hydrophilic and does not penetrate skin or cell membranes efficiently.[28,36]

ALA AND PORPHYRIN-BASED PHOTOSENSITIZERS

Characteristics of an ideal photosensitiser :[28]

- Rapid clearance
- Absorption, distribution, metabolism, excretion parameters should be favourable
- Strong selective phototoxic effect
- Generate active forms of oxygen
- High absorption coefficients allowing light to penetrate deep



- 1st generation : Hematoporphyrin derivative (HpD) and Photofrin
- 2nd generation : overcome limitations of 1st - photosensitivity
- 3rd generation : agents conjugated (for better absorption) with antibodies and other biological targeting molecules

Photosensitizers that have been approved for clinical PDT are Porfimer sodium (Photofrin), 5-aminolevulinic acid (ALA, Levulan), Methyl ester MAL (Metvix), Temoporfin/ meso-tetro-[hydroxyphenyl]-chlorin [mTMPC] (Foscan), Verteporfin (Visudyne), Talaporfin sodium (LS11) [Laserphyrin]. ALA is the only photosensitizer that can be used topically.[28]

ALA DERIVATIVES

ALA Esters – methyl aminolevulinate (MAL), hexyl aminolevulinate (HAL) are the most successful ALA derivatives with regard to PpIX uptake. Increase in skin permeability of ALA by making it lipophilic can be made by elongation of its carbon chain. Advantage of MAL and HAL over ALA is due to the faster rate at which they reach target site and intracellular space and also the faster rate of their enzymatic conversion into photoactive compounds. ALA butenyl, pentenyl and hexenyl esters cause more PpIX production than ALA and MAL.[37] Other methods to increase ALA uptake by tumour cells was by conjugation with nanoparticles(Eg. Biocompatible gold, chitosan) and also topical bioadhesive patch.[37]

A promising approach to localization of abnormal mucosa is autofluorescence imaging using differences in native autofluorescence properties between normal and neoplastic tissue to visually detect abnormal oral mucosal areas.[13] Living tissues contain fluorophores such as Nicotinamide adenine dinucleotide (NADH), Flavin adenine dinucleotide (FAD), collagen and elastin crosslinks that produce fluorescence after excitation with specific wavelengths of light.[13] Abnormal oral lesions show an increased green-to-red fluorescence intensity ratio.

A detection rate of 100% with a specificity of 87.5% has been reported in literature by using fluorescence imaging system, compared with detection rate of 87.5% and specificity of 50% with standard white light dye. Stains like LI or TB have been used to improve recognition of early neoplastic lesions for a long time now. Here an increased stain uptake by abnormal mucosa is seen. Sensitivity ranges from 93.5-97.8% and specificity from 73-92.9%.[13] HpD

are also preferentially retained in abnormal (malignant) tissue and have been evaluated for localization of oral premalignant and early carcinoma lesions. Topical 5-ALA and fluorescence imaging done on patients with suspected oral neoplasia found that abnormal areas had increased red fluorescene.[13] There was an increased sensitivity and improved ability to detect peripheral extent of dysplasia compared with standard white light examination, but specificity was only 60%. On detecting under blue excitation light [380-450nm] normal mucosa appears blue, whereas lesions that retained 5-ALA emitted red fluorescence. This yielded a sensitivity of 98% and specificity of 92% for distinguishing normal from cancerous tissue and 92% and 96% for separating benign mucosa from dysplasia respectively. No photosensitivity or other complications have been reported with the procedure so far.[13]

Another study in literature shows the sensitivity and specificity of Topical 5-ALA to be 83-90% and 79-89% respectively.[38] The sensitivity of topical Photofrin was noted to be 93.75%.[39] The sensitivity and specificity on spectroscopic imaging of site stained by topical 5-ALA was 99% and 60%. The positive and negative predictive values were 77.3% and 97.5% respectively.[3]

5-ALA in the form of an intra-arterial infusion has the advantage of being used in small doses, no necessity of avoiding sun or ultraviolet light, no photosensitivity, no artefactual fluorescence and photofrin showed almost no systemic accumulation.[40]

Examination of lesions utilizing VELscope shows a sensitivity and specificity of 94.67% and 97% respectively.[41,42] However, a few studies show low sensitivity and specificity with VELscope examination.[43]

Narrow band imaging (NBI) endoscopy was first used for detection of esophageal precancerous lesions by Gono.[44] NBI assisted endoscopy has been recommended for

post-operative follow up, post radiotherapy patients. It is found to be a better diagnostic technique for identification of pharyngeal and laryngeal precancerous lesions.[45]

Thus, photodynamic diagnosis with 5-ALA represents a new alternative method for early detection of oral cancer.[12]

5-ALA can also be used for therapeutic purposes by utilizing pulsed dye laser.[46]

Detection of malignancy/ dysplasia by contact endoscopy showed a sensitivity of 91.06%, specificity of 94.06% and diagnostic accuracy of 91.75%.[47,48,49,50,51]

PHOTODYNAMIC THERAPY [PDT]

PDT is based on the ability to cause light dependent cytotoxicity in living tissues. Photosensitizers absorb light energy and transfer it to cellular substrates for cytotoxicity to take place. The subcellular localization of the photosensitizer, presence and concentration of molecular oxygen and the molecular abnormalities of target cell determine the nature of cellular damage and phenotype of cellular cytotoxicity. Also, the time interval between the administration of the photosensitizer and light delivery determines the site of damage.[27]

1st generation photosensitizers : Porphyrin structures are 1st generation photosensitizers. Samuel Schwartz in 1955 developed the HpD which was used to treat carcinomas in 1975. Porfimer sodium (Photofrin), a partially purified formulation was developed later and was considered the best agent for carcinomas and was used for carcinomas of esophagus, bronchi, skin.[27]

2nd generation photosensitizers : 5-ALA is an intermediate in the heme biosynthesis. 5-ALA is converted to protoporphyrin IX (PpIX) when exogenously administered and it accumulates intracellularly in rapidly dividing cells preferentially. It can be administered either orally, intravenous, intraperitonially or topically. PDT cytotoxicity is produced on using light source of either 630nm (red) or 400-450 nm (417nm) polychromatic blue. The

photobiological effect of subcellular distribution and tissue effect of ALA induced PpIX is similar to Porfimer sodium, but, different from exogenously administered PpIX. ALA induced PpIX concentrates in cancer cells and is used in photodiagnosis of head and neck carcinomas, peritoneal subclinical metastasis from ovarian and gartrointestinal carcinomas and malignant brain tumors. The efficiency of tumour converting ALA to PpIX maybe inversely proportional to degree of differentiation of tumor cells.[27]

Other photosensitizers are – benzoporphyrin derivative monoacid (BPD, verleporfin, visudyne) and meso-tetro-[hydroxyphenyl]-chlorin (mTMPC, Foscan). Meso-tetra-hydro phenoxy-chlorin (mTHPC) is approved by European Union for head and neck carcinoma. 2nd generation photosensitizers under investigation currently are phthalocyanine-4 (Pc4), Photochlor/ 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH), Purlytin/ tin ethyl etiopurpurin (SnET2) & Talaporfin Sodium (LS11).[27]

Light delivery and dosimetry

The light of specific wavelength is focused on areas of malignant involvement with measured dose. Damage to deeper structures is limited owing to the intrinsic physical limitation in depth of effective visible light penetration through tissue (2-10 mm for red light). [27]

Delivery systems provide uniform illumination of the desired target. Monochromatic laser light is used most often and the wavelength of the light is selected by matching absorbance peaks of photosensitizer being used with desired tissue penetration.[27]

PDT is currently carried out using a combination of the dose of the photosensitizer and the incident light fluence. The techniques being developed aim to being able to modulate light delivery in real time to achieve the desired level of PDT tissue cytotoxicity. Tissue cytotoxic effects of PDT measured by combining tissue oxygen concentration and photosensitizer with

measurements of the absorbed light dose. Limitations of dosimetric methods is spatial resolution and complexity of clinical interpretation.[27]

Oxygen effects

The efficacy of PDT is hampered by pre-treatment tumor hypoxia. Hence, the oxygen required for PDT should be taken into consideration. The rate of molecular oxygen consumption by PDT is dependent on light fluence rate (amount of light energy delivered to an area as function of time). Therefore, it is necessary to monitor the tumor blood flow and tissue oxygenation in real time and monitor light dose accordingly.[27]

Mechanisms of PDT cytotoxicity

- **Indirect:** The changes induced by PDT on tumor are vessel leakage, vasoconstriction, vascular thrombosis. Also, hypoxia plays a role in PDT owing to its requirement for molecular oxygen. PDT results in an acute vasoconstrictive response followed by thrombosis, vascular collapse and tumor hypoxia. PDT causes release of pro-inflammatory cytokines and complement fixation thus stimulating antitumor immune response. It can also result in release of immunogenic, tumor associated antigens which in turn cause lymphocytes, monocytes and granulocytes infiltration into tumors causing an antitumor immune response.[27]
- **Direct:** Here, the photosensitizer is localized at the subcellular layer leading to damage of cellular macromolecules due to PDT. As the life of singlet oxygen is at 0.03-0.18mcs which corresponds to diffusion distance of <0.2mcm or about $1/50^{th}$ of cell diameter, the cellular damage occurs very close to photosensitizer location of singlet oxygen production. Cell death phenotype is determined by specific localization of the photosensitizer i.e. at plasma membrane, lysosome, mitochondria,

golgi apparatus, endoplasmic reticulum or nuclear membrane. Eg. Porfimer sodium and BPD localize to mitochondria.[27]

There are 2 types of cell death induced by PDT – apoptotic and non-apoptotic. The apoptotic type predominates in the most PDT-sensitive cell lines at lower light or photosensitizer doses and the necrotic/non apoptotic type predominates at higher light or photosensitizer doses. It has been suggested that low dose PDT stimulates autophagy causing increased cytotoxicity in cells with deficient apoptosis. [27]

Tumor cell line and photosensitizer determines the percentage of apoptosis achieved and the mechanism (extrinsic v/s intrinsic) of apoptosis.[27]

On oral rinsing with freshly prepared 5-ALA solution, the abnormal (malignant) oral lesion shows an increased green-to-red fluorescence intensity due to PpIX accumulation in neoplastic cells.[26,27]

Clinical indications

Early stage carcinomas : Treatment of actinic keratosis, basal cell carcinoma, barrett's esophagus, microinvasive endobronchial lung cancer, head and neck carcinomas.[27]

Early stage head and neck cancer - PDT has been approved in head and neck by European Union and not by Food and Drug Administration (FDA). Of the various studies, the longest with 15 years clinical experience, where > 200 patients were treated with porfimer sodium-mediated PDT. Here, primary or recurrent T0-T2, N0 cancers were given 2mg/kg porfimer sodium 48 hours prior to delivery of 50-80 J/cm^2 630nm light. Tumor with depth <3mm was treated with light from a fiber fitted with a microlens applicator and >3mm with implantable cylindrically diffusing fibres to ensure homogenous light delivery to the tissues. For T0-T2 laryngeal lesions in 110 patients, 100% complete response seen, and on follow up of 84 months, 5 year local recurrence of 10% seen. In 112 patients with oral cancer (T0 or T1)

followed up for a mean of 80 months – 6 local failures and 2 additional regional failures were noted. Complications noted were due to cutaneous photosensitivity and local pain. Use of ALA and mTHPC (2^{nd} generation photosensitizers) has been talked about in other series.[27]

Locally advanced cancers and palliative therapy : Intraperitoneal PDT for carcinomatosis or sarcomatosis, post op PDT for pleural based spread of Non Small cell Lung carcinoma and mesothelioma, palliative care for obstructing tumors of esophagus and bronchi, hepatocellular carcinoma, prostate and bladder carcinoma and brain tumor.[27]

Molecularly targeted PDT

Studies have shown potential for increasing the efficacy of PDT by combining it with molecularly targeted therapy. Epidermal Growth Factor Receptor (EGFR) and angiogenesis are being targeted currently to improve the therapeutic index. Also, targeted photosensitizer delivery is considered to enhance efficacy of PDT. Studies with anti-EGFR antibody (OC125) covalently linked to a photosensitizer showed superior PDT efficacy in comparison to PDT using unbound photosensitizer. Nanoparticle technology can be used to target photosensitizer. Nanoparticle technology allows for targeted delivery of photosensitizer to cancer cells.[27]

Advantages of ALA over other photosensitizers :[28]

- Rapid metabolism
- High selectivity for malignant lesions
- Systemic clearance within 24 hours – prevents prolonged photosensitivity and can be used at regular intervals
- High efficacy
- Minimal side effects
- No burns/ scars

Disadvantages of ALA-PDT :[28]

- Limited depth of penetration
- Associated pain at the site of injection
- Individual variations
- Less efficient in destroying cutaneous lesions when compared to Photofrin-PDT
- Inefficacy in large or metastatic tumour management.
- Stinging, burning, pricking, smarting and itching pain during and several hours following PDT
- Unstable in alkaline environment
- Postinflammataory changes – pigmentation
- Nausea, fatigue, paraesthesia, headache

PRECLINICAL STUDIES AND CLINICAL INDICATIONS OF ALA

Photoporphyrin accumulation and photosensitivity resulting from exogenous ALA was reported in 1956 and it was first used in PDT in 1987. The first clinical trial with ALA-PDT for Superficial Basal Cell Carcinoma (BCC) was conducted in 1990.[52]

The efficacy of photodynamic diagnosis and therapy is determined by PpIX concentration and distribution, fluorescence rate, wavelength used, fluence and oxygen availability.

ALA is preferred in patients with co-morbidities and in whom surgery risk is high. ALA is better tolerated and does not affect external appearance.[28]

ADVANTAGES OVER TRADITIONAL ANTITUMOUR TREATMENTS OF ALA ARE :[28]

- Reduced long term morbidity
- Lack of intrinsic resistance mechanisms to oxygen induced cytotoxicity
- Possible treatment repetition
- No associated immunosuppression

ALA-PDT appears to be the most successful pro – drug treatment in clinical oncology.

ALA is said to have a few drawbacks, namely, hydrophilicity and low specificity which causes low cellular uptake and low bioavailability. Modifications of 5-ALA helps overcome these drawbacks.[53]

Nanoparticles is once such modification of ALA. PEG-chito (Polyethylene glycol/ chitosan)-5-ALA nanoparticles in comparison to 5-ALA, causes increased phototoxicity and higher PpIX accumulation. These cause accelerated apoptosis/necrosis of tumor cells.[53]

Nanoparticles have an excellent biodistribution, bioavailability and reduced intrinsic toxicity making them ideal vehicles to target solid tumors.[54]

PART-II

MATERIALS AND METHODS

Study to evaluate the diagnostic efficacy of 5-ALA in demarcating the actual extent of oral cancers and pre-malignant lesions was undertaken at R.L.Jalappa Hospital attached to Sri Devaraj Urs Medical College, Kolar. Biopsy was taken from visible lesion in patients with cancer and/or pre-cancerous lesions of oral cavity and another from margin of 5-ALA induced fluorescence area after an incubation period of 3 hours following rinsing with 5-ALA solution. Histopathological examination for detection of malignant or pre-malignant cells was carried out in both the specimens and usefulness of 5-ALA was determined by documenting changes (dysplasia) beyond the visible margin of the lesion.

SOURCE OF DATA :

Minimum 50 patients who came to the Out Patient Department of Otorhinolaryngology, R.L. Jalappa Hospital and Research Centre with pre malignant lesions and/or carcinoma oral cavity between November 2011 to March 2013.

INCLUSION CRITERIA

- Patients with oral squamous cell carcinoma and/or pre malignant lesions.

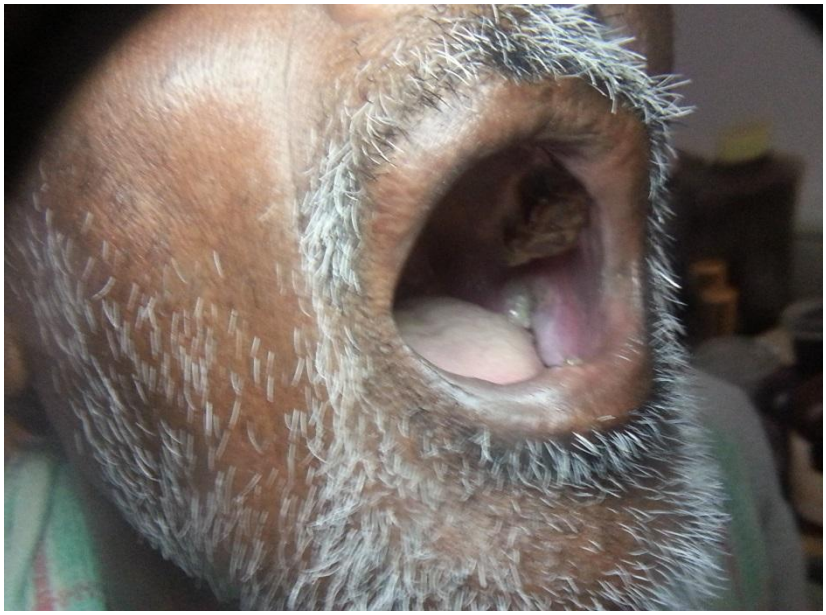
EXCLUSION CRITERIA

- Patients with recurrence.
- Post Chemotherapy/Radiotherapy patients.
- Patients with non squamous malignancies of oral cavity.

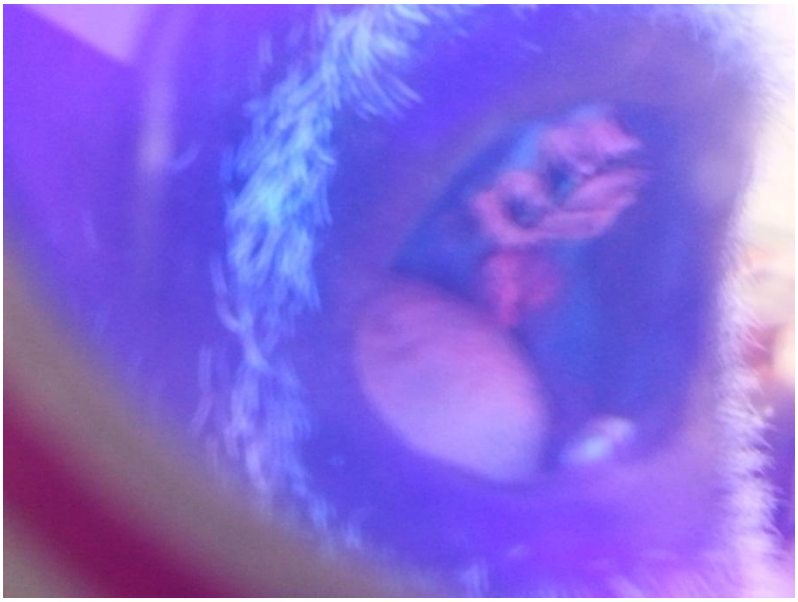
METHOD OF COLLECTING DATA :

After obtaining informed written consent of the patients, they were made to rinse the oral cavity on the side of the lesion with 5-ALA solution [200mg in 50ml of water] for 15 minutes. After incubation period of 3 hours, biopsy was taken from margin of visible lesion and another from margin of fluorescence under guidance of blue excitation light. The distance in millimeters between visible and fluorescent margin was documented and the biopsy specimens were sent for histopathological examination. The results were documented. Usefulness of 5-ALA was evaluated by calculating the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy.

PHOTO 6 : Malignancy lower alveolus – visible and fluorescent margin

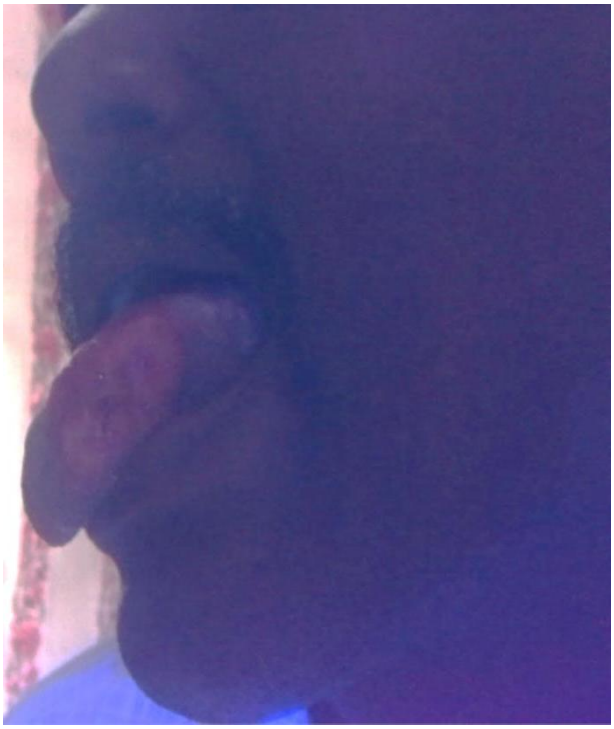


a) Clinical Examination

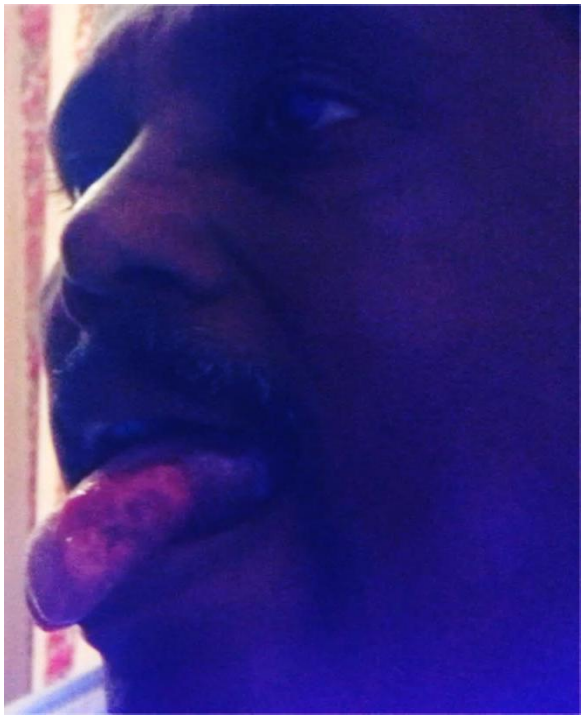


b) Examination with blue wavelength light

PHOTO 7 : Malignancy Tongue – visible and fluorescent margin



a) Clinical Examination



b) Examination with blue wavelength light

OBSERVATION AND RESULTS

Table 10 : Age Distribution (n = 50)

AGE GROUP	No. of cases
30-40	4
41-50	16
51-61	16
61-70	11
71-80	3

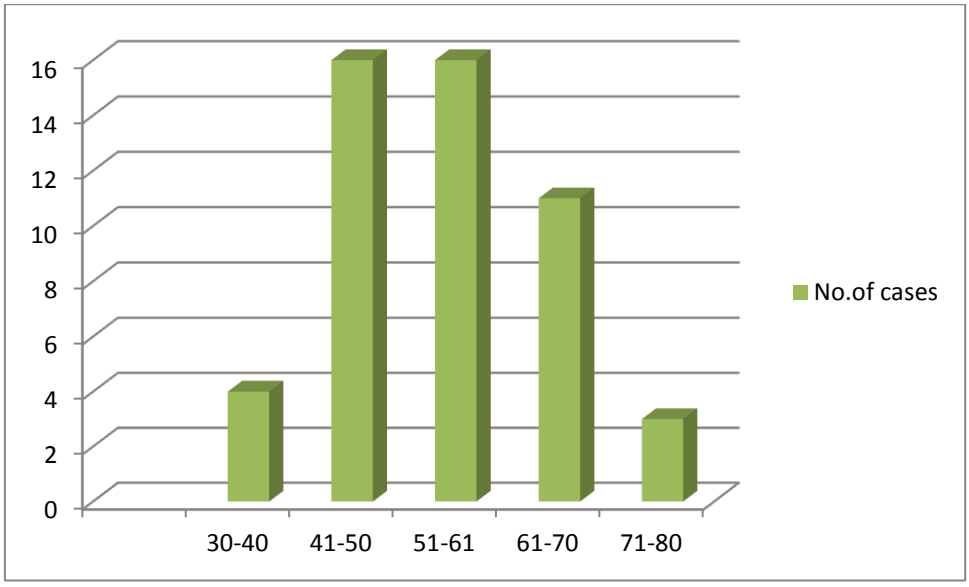


Figure 19 : The depiction of age-wise distribution shows that maximum prevalence with 16+16 patients (32 patients) was observed in patients between 4^{th} – 6^{th} decade.

Table 11 : Sex Distribution (n = 50)

	No. of Cases	Percent
Female	40	80%
Male	10	20%
Total	50	100%

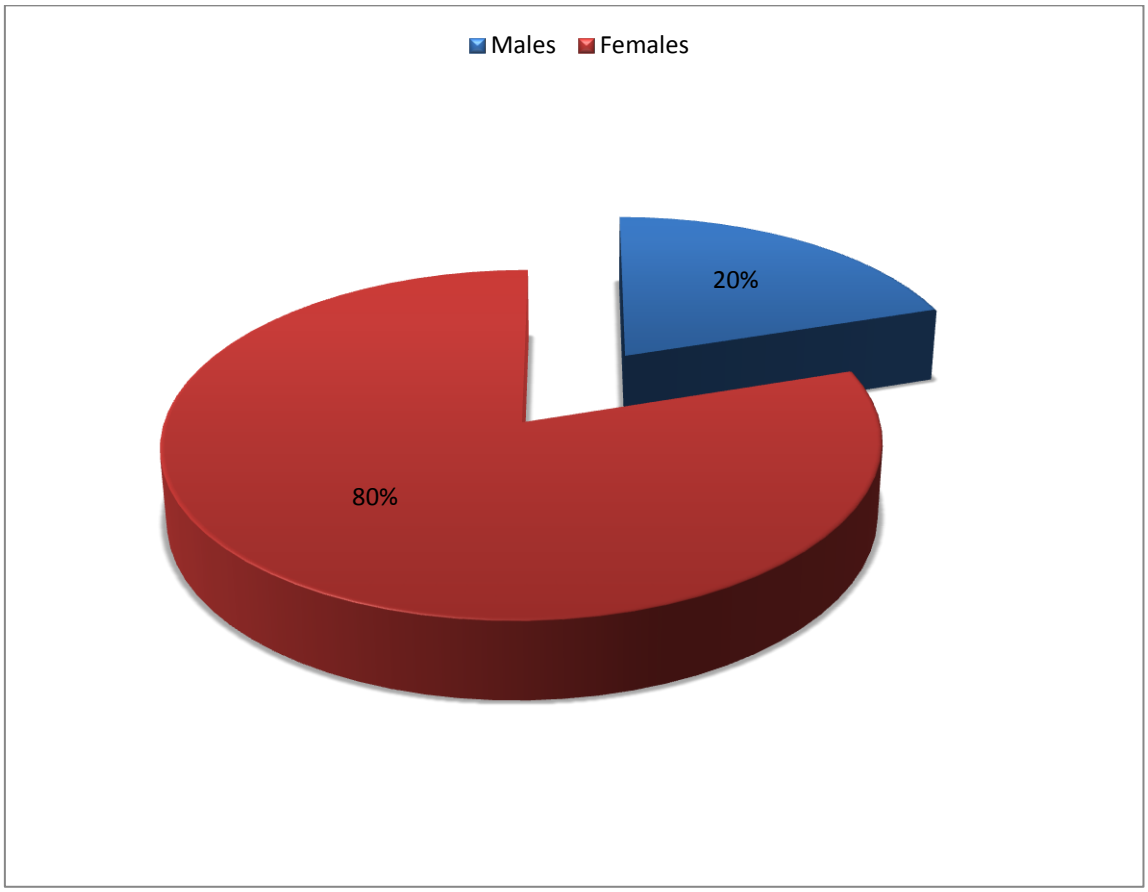


Figure 20 : The majority of patients afflicted were females with a female: male ratio of 4 : 1.

Table 12 : Diagnostic Distribution of Cases (n = 50)

Clinical Diagnosis	No. of cases
Malignancy Right Buccal Mucosa **[A]**	14
Malignancy Left Buccal Mucosa **[B]**	9
Malignancy Right Retromolar Trigone **[C]**	1
Malignancy Left Lower Alveolus **[D]**	2
Malignancy Tongue **[E]**	3
Malignancy Hard Palate **[F]**	1
Pre-malignancy (Leukoplakia) Left Buccal Mucosa **[G]**	6
Pre-malignancy (Leukoplakia) Right Buccal Mucosa **[H]**	5
Pre-malignancy (Erythroplakia) Left Buccal Mucosa **[I]**	3
Pre-malignancy (Erythroplakia) Right Buccal Mucosa **[J]**	3
Pre-malignancy (Erythroleukoplakiaplakia) Left Buccal Mucosa **[K]**	2
Pre-malignancy (Leukoplakia) Tongue **[L]**	1

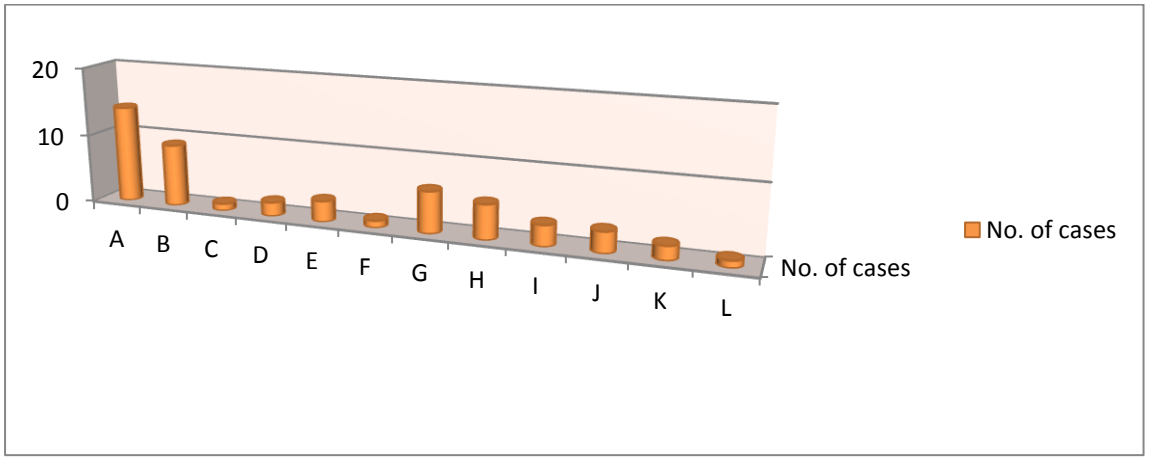


Figure 21 : There are a total of 30 cases of malignancy, of which a maximum of 23 cases are of Malignancy Buccal Mucosa. Pre-malignancy comprises 20 of the total cases.

Table 13 : Pre-malignant Lesions (n = 20)

Types of Pre-malignant Lesions	No. of Cases
Leukoplakia	12
Erythroleukoplakia	2
Erythroplakia	6

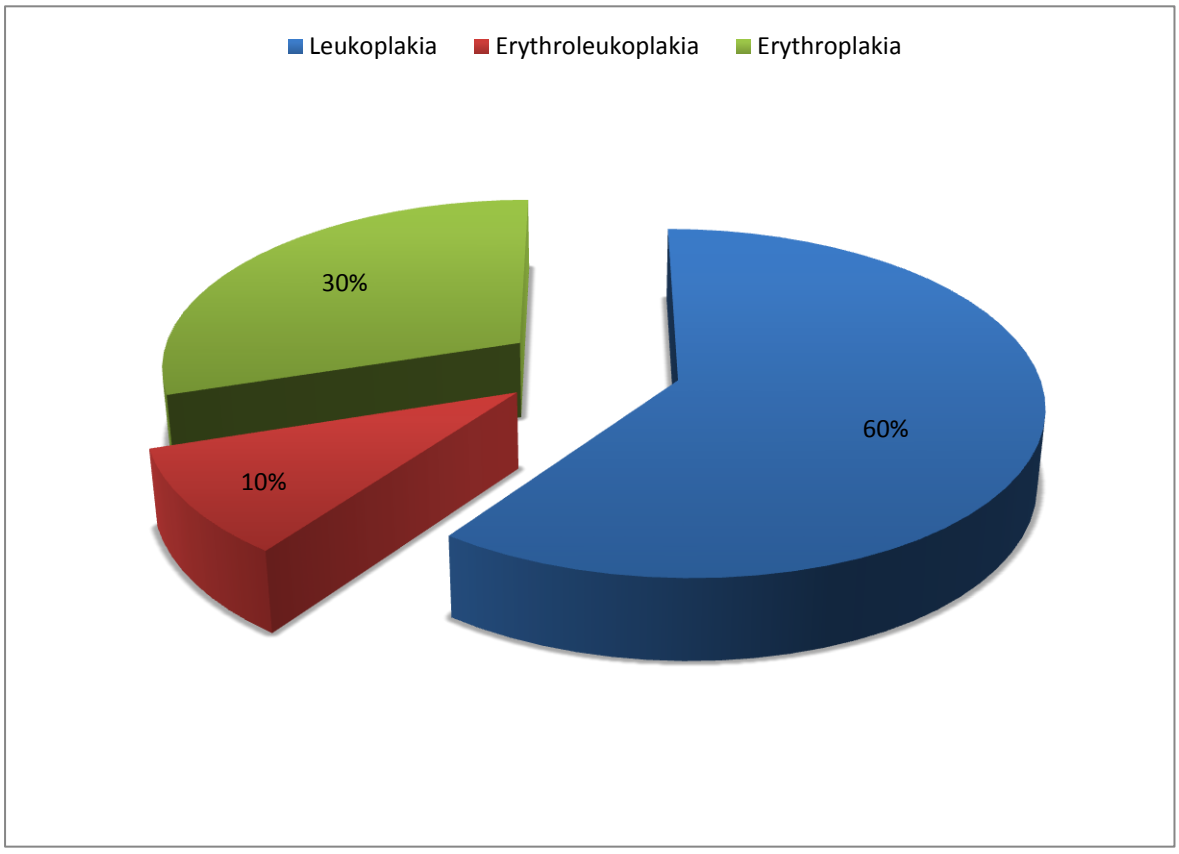
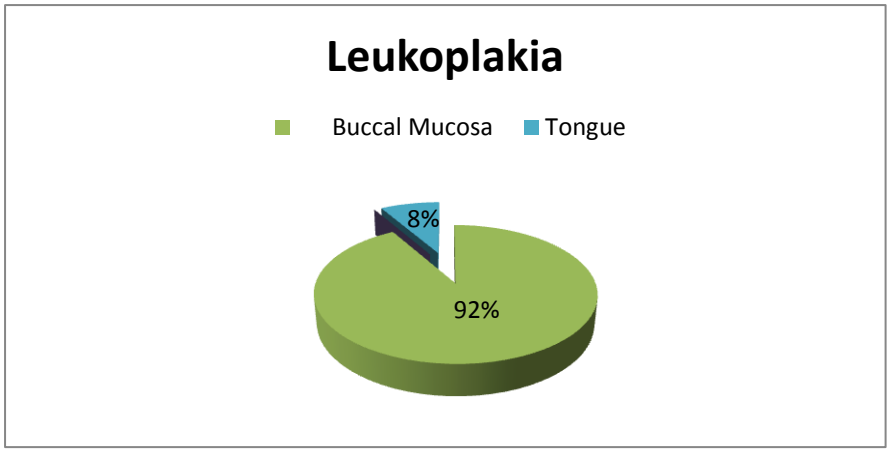


Figure 22 : Leukoplakia was the most common Pre-malignant lesion encountered in our study with 12 cases of a total of 20 cases.

Table 14 : Diagnostic distribution of Pre-malignant Lesions

Types of Pre-malignant Lesions	Buccal Mucosa	Tongue
Leukoplakia	11	1
Erythroleukoplakia	2	
Erythroplakia	6	



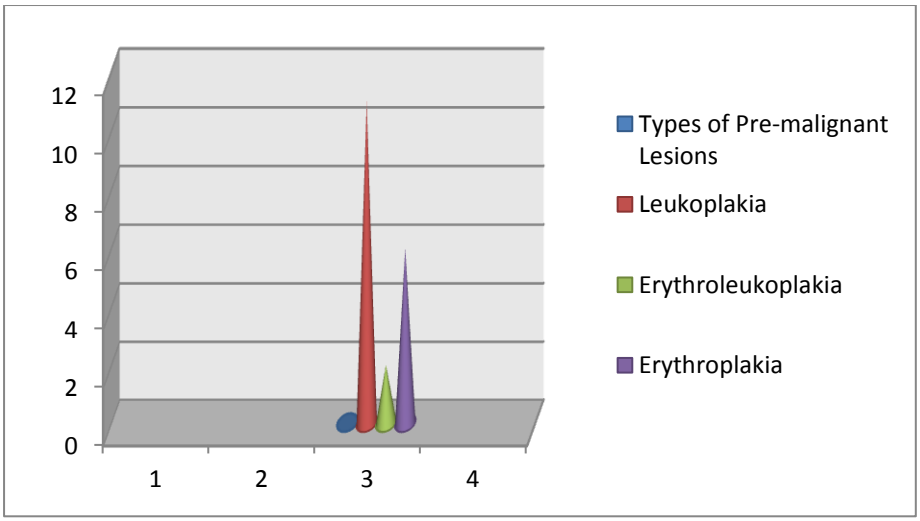


Figure 23 and 24 : Of the 20 pre-malignant lesions a maximum of 19 cases were involving Buccal Mucosa and 1 case was of Tongue. 11 cases were of Leukoplakia of Buccal Mucosa, 6 cases of Erythroplakia of buccal mucosa and 2 cases of Erythroleukoplakia.

STAGING

Table 15 : Malignancy Buccal Mucosa – Staging (n = 23)

	T2	T3	T4a
N0	1	2	2
N1	3	6	6
N2a		1	1
N2b		1	

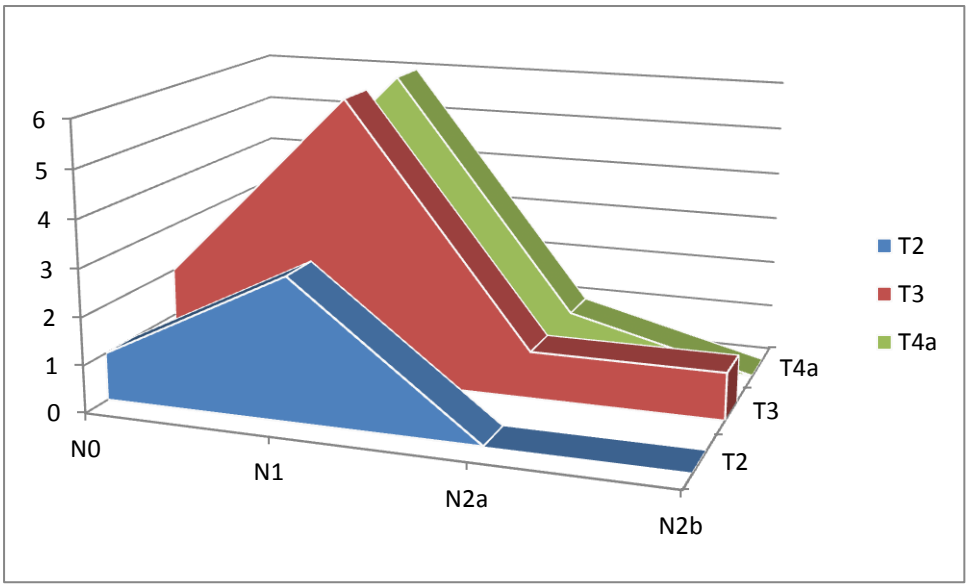


Figure 25 : It is depicted in this chart that patients in and around Kolar present at an advanced stage of malignancy. 19 of 23 patients of malignancy buccal mucosa presented with a T3 or T4a lesion.

Table 16 : Malignancy Tongue – Staging (n = 3)

	T2	T3	T4a
N0	1		
N1		1	1

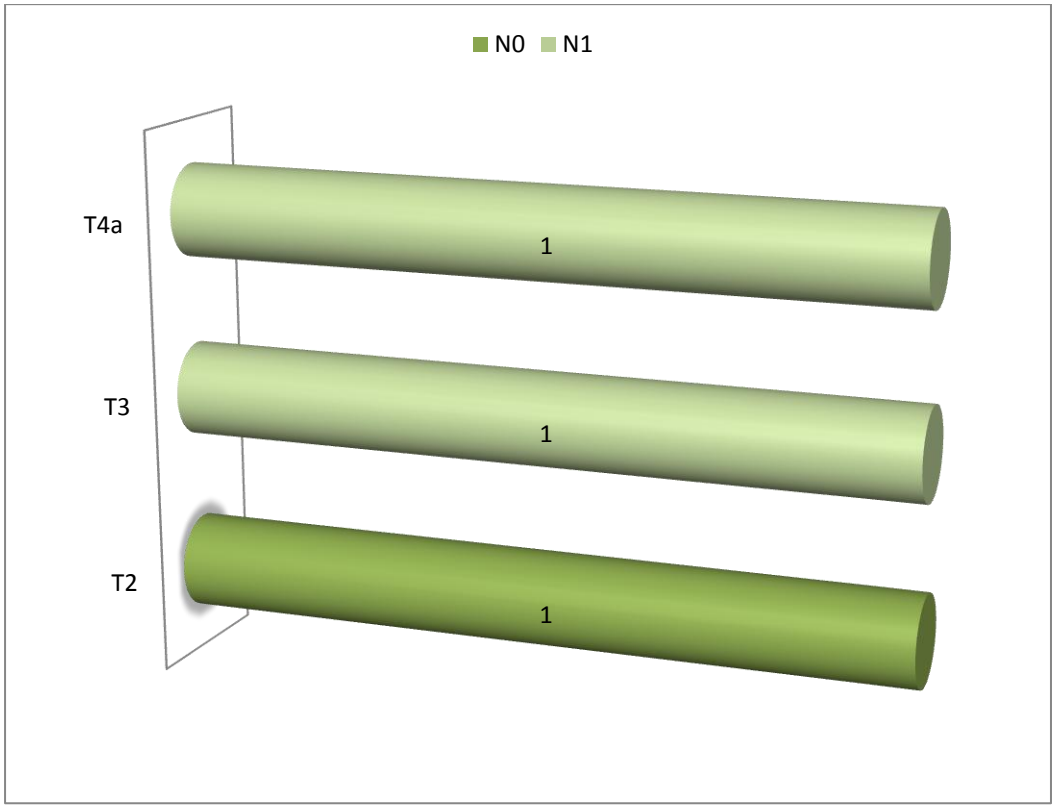


Figure 26 : 2 of the 3 tongue malignancy cases presented with a T3 and T4a lesion respectively.

Table 17 : Malignancy Lower Alveolus (n = 2)

	T2	T3
N0	1	
N1		1

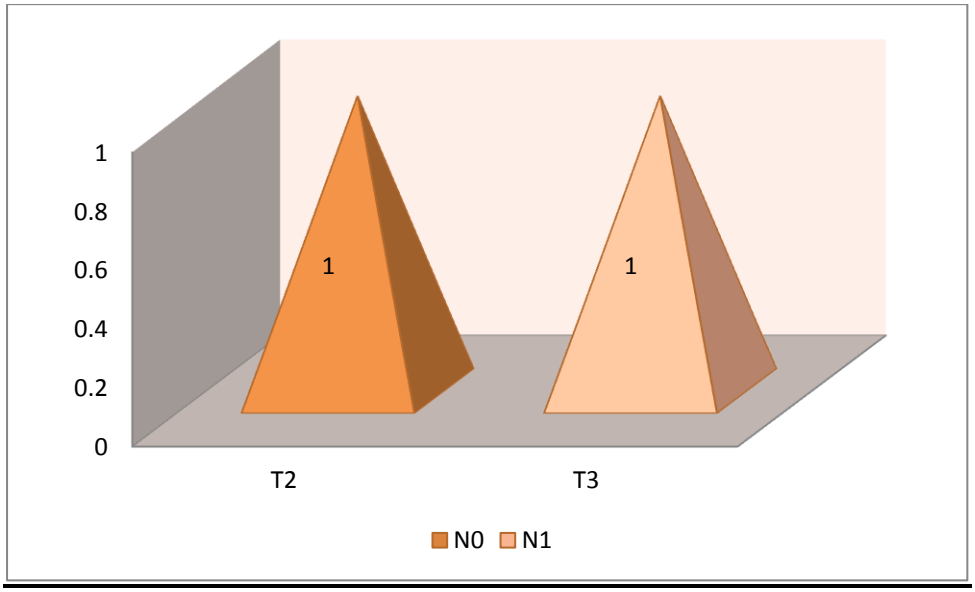


Figure 27 : Cases of malignancy lower alveolus presented with a T2N0 and T3N1 lesion respectively.

Malignancy Hard Palate - T2 N0 M0

Malignancy Retromolar Trigone - T2 N1 MO

Table 18 : Depiction of Histopathology Reports of Biopsy from Visible Margin (n = 50)

Results of Biopsy from Visible Margin	**No. of cases**
Well differentiated SCC **[A]**	14
Well to Moderately differentiated SCC **[B]**	9
Moderately differentiated SCC **[C]**	3
Moderately to Poorly differentiated SCC **[D]**	1
Verrucous Carcinoma **[E]**	1
Severe Dysplasia **[F]**	5
Moderate to Severe Dysplasia **[G]**	1
Moderate Dysplasia **[H]**	5
Mild to Moderate Dysplasia **[I]**	4
Mild Dysplasia **[J]**	4
No evidence of Dysplasia **[K]**	3

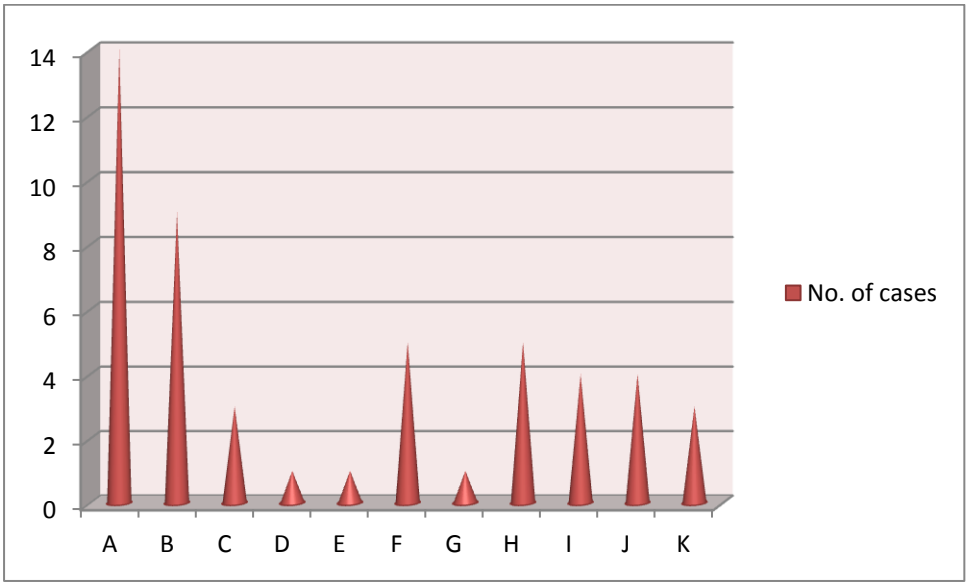


Figure 28 : The 2 cases showing no evidence of dysplasia from biopsy taken from visible margin were of Pre-malignancy (Leukoplakia) Left Buccal Mucosa.

Table 19 : Depiction of Histopathology Reports of Biopsy from Fluorescence Margin

(n = 50)

Results of Biopsy from Fluorescence Margin	**No. of cases**	**Fluorescence/ Visible Margin**
Well differentiated SCC **[A]**	8	8/14
Well to Moderately differentiated SCC **[B]**	6	6/9
Moderately differentiated SCC **[C]**	2	2/3
Poorly differentiated SCC **[D]**	1	1/1
Verrucous Carcinoma **[E]**	1	1/1
Severe Dysplasia **[F]**	7	7/5
Moderate to Severe Dysplasia **[G]**	2	2/1
Moderate Dysplasia **[H]**	6	6/5
Mild to Moderate Dysplasia **[I]**	3	3/4
Mild Dysplasia **[J]**	9	9/4
No evidence of Dysplasia **[K]**	2	2/3
No extension **[L]**	3	-

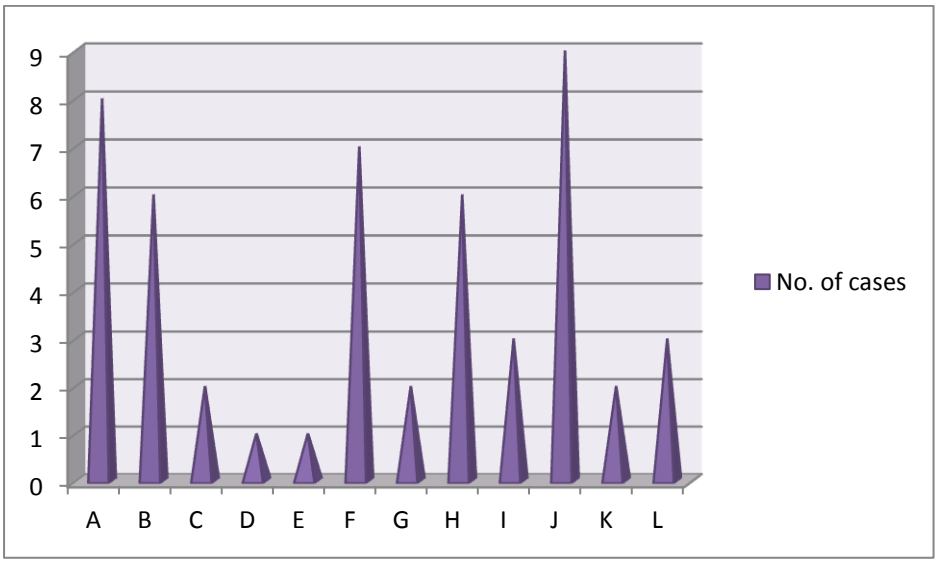


Figure 29 : It is to be noted here that, 3 cases showed no extension of margin from visible margin on fluorescence diagnostic staining. All the 3 cases were of Pre-malignancy (Leukoplakia) Left Buccal Mucosa. The 2 cases showing no evidence of dysplasia were of Pre-malignancy (Leukoplakia) Left and Right Buccal Mucosa respectively.

In the tumours that were well differentiated the fluorescence margin being positive were relatively less. Whereas the tumours that were less differentiated the fluorescence margin being positive increased showing that these were more aggressive and had microscopic spread of tumour beyond visible margin.

Table 20 : Sites of Pre-malignant Lesions which were positive on fluorescent margin (n = 17)

Site	No. of Cases	Avg. Extension (in mm.)
Buccal Mucosa	16	3.35mm (2-5mm)
Tongue	1	3mm

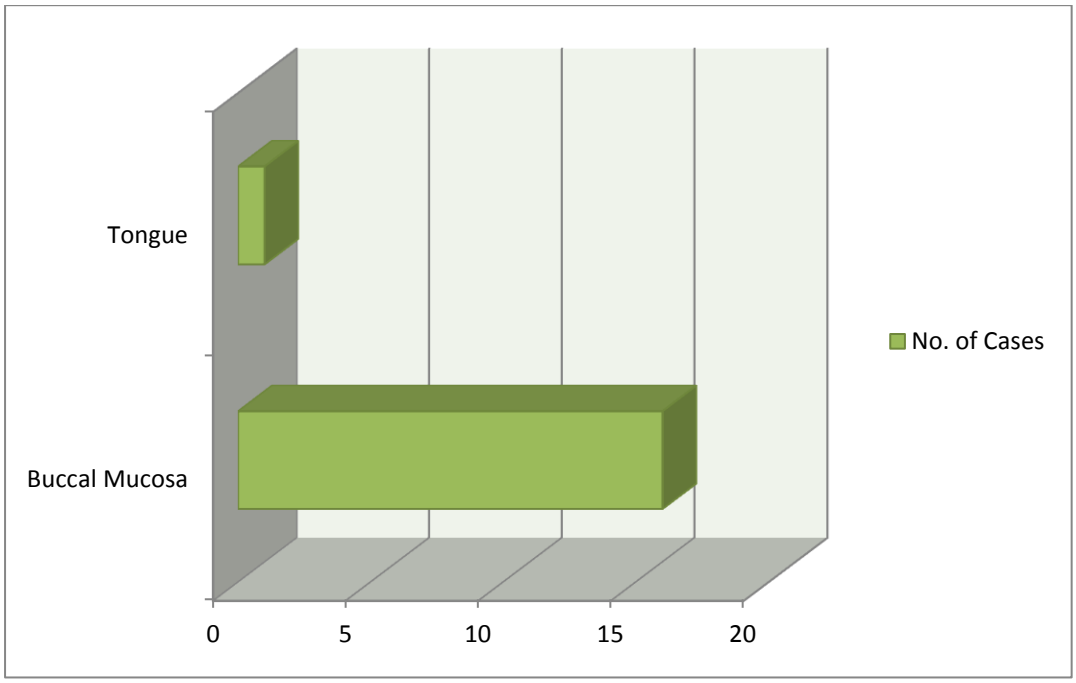


Figure 30 : 17 cases of pre-malignant lesions showed extension beyond visible margin on fluorescence. Of which 16 were involving buccal mucosa and 1 involving tongue. The average extension (in mm.) of fluorescence from visible margin in buccal mucosa lesions is 3.35mm. (2-5mm.) and in tongue is 3mm.

Table 21 : Demonstration of cases showing fluorescence beyond visible margin with the distance (in mm.) range [n = 47]

Distance in mm.	No. of cases
< 3mm	8
3mm - 5mm	31
6mm - 8mm	6
9mm - 12mm	1
> 12mm	1

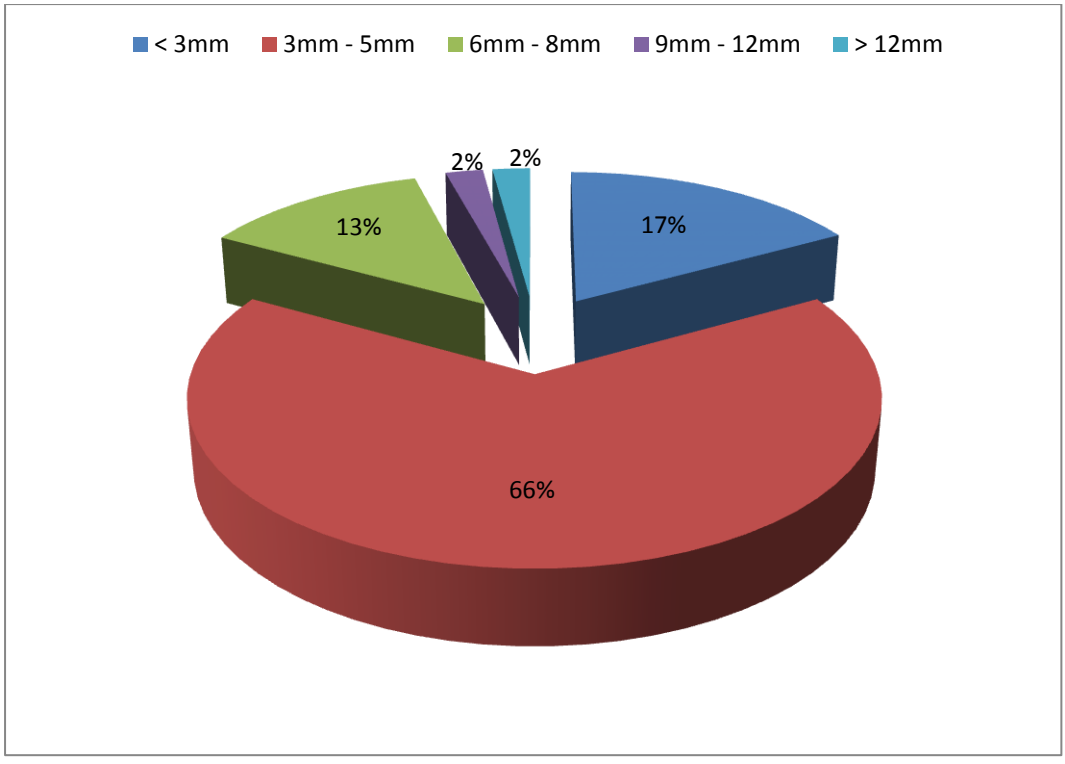


Figure 31 : Majority of the cases - 31, showed a 3-5mm extension of fluorescence margin beyond visible margin.

Table 22 : Subsites showing >5mm extension from visible margin (n = 8)

Subsites	No. of cases	Average distance of extension (by fluorescence) in mm.
Malignancy Buccal Mucosa	5	6.6mm [6-7mm]
Malignancy Tongue	3	11mm [8-13mm]

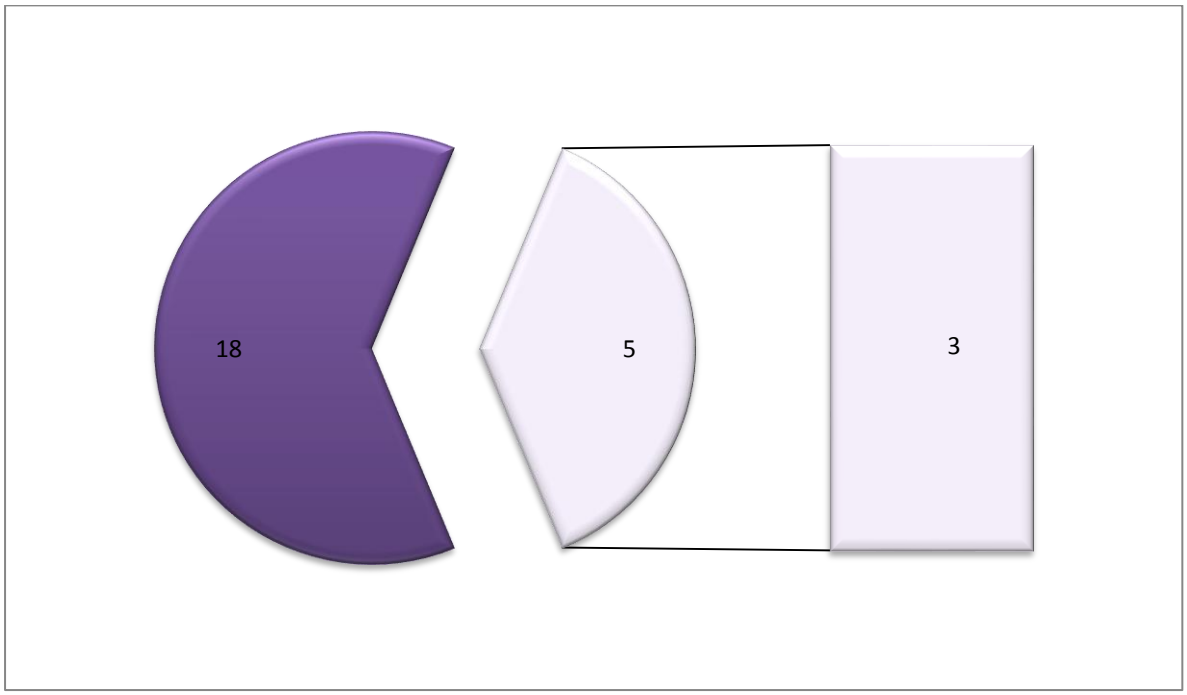


Figure 32 : This pie chart demonstrates the subsites of oral cavity showing extension of >5mm beyond visible margin on fluorescence under blue wavelength light.

5 cases of a total of 23 cases of Malignancy Buccal Mucosa and 3 cases of a total of 3 cases (100%) of Malignancy Tongue showed extension of >5mm., thus depicting the significance of fluorescence staining – especially in cases of Tongue Malignancy.

Table 23 : Number of patients showing Field Cancerization (n = 3)

	Site	No. of Positive Lesions
Leukoplakia	Buccal Mucosa	2
Erythroplakia	Buccal Mucosa	1

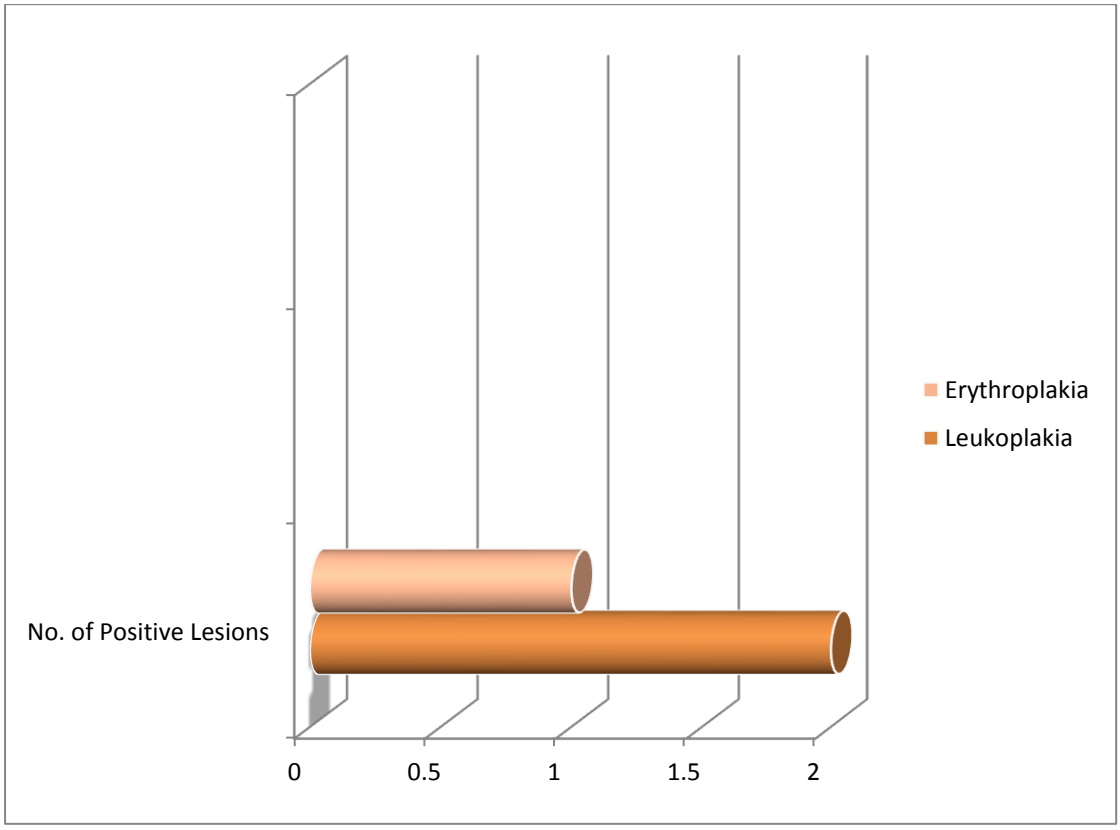


Figure 33 : Of the biopsies taken from patients having field cancerization under guidance of blue wavelength light 2 leukoplakia and 1 erythroplakia cases showed dysplasia in the 2 cases of leukoplakia and severe dysplasia in case of erythroplakia.

DISCUSSION

This study was done at R.L.Jalappa Hospital, Kolar. A total number of 50 patients were included in our study. Majority of patients in our study – 80% were females. Increased incidence was seen in the 4^{th}-6^{th} decade of life. Buccal mucosa was the site of predilection. This can be attributed to poor socioeconomic status in Kolar and addiction to chewable tobacco (cudd) among ladies. In contrast, the men of this region are addicted to smoking beedis or cigarettes.

In our study, 23 of the 30 malignancy patients were of squamous carcinoma buccal mucosa. Pre-malignant lesions constituted 20 cases, of which leukoplakia buccal mucosa constituted 11 cases and 1 case of leukoplakia tongue. 6 cases were of erythroplakia buccal mucosa and 2 of erythroleukoplakia buccal mucosa. Carcinoma oral cavity is the most common malignancy in Kolar district.

Malignancy Buccal Mucosa was observed to be the most afflicted subsite (23/30 malignancy patients). Patients in this region usually present at an advanced stage – T3N0: 2 patients, T3N1: 6 patients, T3N2a and T3N2b: 1 patient each; T4aN0: 2 patients, T4aN1: 6 patients, T4aN2a: 1 patient. The TNM staging of malignancy tongue consisting of 3 patients was T2N0, T3N1and T4aN1. This late presentation is due to poverty and ignorance of the people of this region.

In our study, 47 out of 50 patients were found to have extension (fluorescence) beyond visible margin when visualized with the aid of blue wavelength light. Majority of the cases – 31 (66%), showed extension between 3-5mm. beyond visible margin. All cases of

malignancy (30 patients) showed extension (fluorescence) beyond visible margin. Histopathology of biopsy taken from fluorescent margin showed frank malignancy in 18 patients and dysplasia in 12 patients.

16 cases of pre-malignancy buccal mucosa and 1 case of pre-malignancy tongue demonstrated fluorescence beyond visible margin.

Histopathology report of 2 Pre-malignancy (Leukoplakia) Left Buccal Mucosa showed no evidence of dysplasia on biopsy from visible margin. This is because of poor oral hygiene, field cancerization and ulcerated mucosa which makes it difficult to select the biopsy site.

In our series, 3 patients had no fluorescence beyond visible margin and in 2 patients the biopsy taken from fluorescent margin did not show any dysplasia. All these 5 patients were clinically diagnosed as leukoplakia buccal mucosa.

27 cases (15 - Pre-malignancy and 12 – Malignancy) demonstrated dysplasia on histopathological examination of biopsy from fluorescent margin (extention beyond visible margin).

8 patients in our study had >5mm extension (fluorescence) beyond visible margin.The subsites showing >5mm extension of fluorescence constituted 5 cases of malignancy buccal mucosa and 3 cases of malignancy tongue. It is to be noted here that all 3 cases of malignancy tongue that were taken in this study showed an extension of >5mm from visible margin on fluorescence staining.

It was observed that poorly differentiated tumours showed more extension on fluorescence, demonstrating that these tumours are more aggressive owing to microscopic extension beyond visible margin on clinical examination.

Field cancerization is seen in buccal mucosa malignancies most commonly. In our study, in biopsies taken from areas of field cancerization in 3 cases, 2 cases of leukoplakia showed dysplasia and 1 case of erythroplakia showed severe dysplasia. In these 3 patients, the appropriate site for biopsy could be selected only after staining with 5-ALA.

Supravital dyes – toluidine blue, lugol's iodine, methylene blue, aminolevulinic acid and other porphyrins are utilized to determine the prescence of malignancy and to obtain definite margins and the extension beyond the gross margin.[23] Vital staining serves as a means of surveillance of high risk cases and also for those diagnosed with neoplasm of other parts of the aero digestive tract.[16]

Toluidine blue has been shown to have better sensitivity and specificity as compared to other supravital dyes. Dysplastic cells take up toluidine blue owing to their DNA binding property.[17] Studies done show sensitivity varying between 91-92% and 100% specificity. A false negative of 2% was noted. The retention of dye by filiform papillae duct to a high rate of protein synthesis was observed.[16,55] In contrast to the above studies, few studies in literature showed a sensitivity ranging 65.5-79.5% and specificity of 73.3%, with a false negative rate of 20.5%. The positive and negative predictive values were found to be 35.2% and 90.6% respectively. Diagnostic accuracy of 65% was observed.[56,20]

In 1989 Rosenberg and Cretin performed a meta-analysis wherein the sensitivity was 97.7 +/- 4.65% and specificity was 90.8 +/-9.34%.[57] False positive results occur due to inflammatory and ulcerative lesions which retain the dye.[57] In a multi-institutional study done in Europe, the average Positive Predictive Value (PPV) varied between 50.4% to 86%. The Negative Predictive Value (NPV) was averaged to be 75.2% with the study in Universities across

U.S.A showing 100% NPV.[20,58,59,60.] Toludine blue was said to have a high percentage of false positive and false negative by a study done at University of Detroit.[61]

A study regarding the efficacy of Toluidine blue and Brush biopsy (BB) compared to wedge biopsy in oral lesion, done at our institute included a total of 172 patients. Sensitivity and specificity of evaluation of TB and BB was found to be 93% and 95% respectively for malignant lesions and 88% and 90% in pre-malignant lesions. PPV was found to be 96%. As quoted in other studies, false positive results were noted in inflammatory lesions. TB is an ideal screening tool which can minimize false negatives.[62]

Lugol's iodine was the earliest dye to be used for staining tissues for detection of dysplastic/ malignant cells. This dye does not cause staining of malignant/ dysplastic cells due to the enhanced glycolysis occurring in these cells. The main drawback of this dye is that its usefulness is confined to non-keratinized mucosa.[23] The pioneer study in 1990 by Shiozaki et al, wherein 178 patients were screened by lugol's iodine staining, identified 13 cases with oral cancer and dysplasia.[23]

A study comparing lugol's iodine and toludine blue done at University of British Columbia, Vancouver which included 59 cases showed sensitivity and specificity of toludine blue to be 92.5% and 63.2% respectively and that of lugol's iodine to be 87.5% and 84.2% respectively. When both the stains were used on each patient the sensitivity and specificity was 85% and 89.5%. This study gives a conclusion that lugol's iodine has less sensitivity and greater specificity.[63] The study done at Shinshi University School of Medicine emphasized the importance of margins by demonstrating delineation of margins by lugol's iodine. The margins were identified by iodine colour lining. This study suggested involving 5mm of normal tissue in lesion excision beyond lesion on the iodine positive area.[64]

Action of methylene blue is similar to toluidine blue and is preferred for large scale screening.[24] Study done at Institute of Dental Sciences and Hospital, Lucknow showed a sensitivity of 91.4%, specificity of 66.6%, positive predictive value of 97.7%, negative predictive value of 33%. The overall diagnostic accuracy of methylene blue stain was 90%.[24]

A study done at Chi – Mei Medical Center, Tainan, Taiwan shows a sensitivity of 90%.[65]

5-ALA uptake is facilitated mainly by decreased ferrochelatase action, enchanced enzymatic activity and reduced intercellular junctions. Topical ALA has the advantage of absence of side effects and of having a high selectivity for malignant cells. 5-ALA shows high specificity in comparison to hematoporphyrin derivatives.[2] Topical 5-ALA is an upcoming new procedure for early detection of malignant lesions and can be used on outpatient basis as well as fluorescence guided laser resection of oral cancer. The only drawback being the high costs associated in procuring 5-ALA.[3] As the use of 5-ALA in oral malignancies has been recently adopted, very few studies on this topic are available in literature.

Real margins of malignant tumour are identified through fluorescence diagnostics (FD).[40] Photofrin said to be the most popular sensitizer, is administered exogenously to enhance tumour demarcation by emission of a red fluorescence under wavelength of around 400nm.[40]

In our study we have used only topical 5-ALA stained red fluorescence for demarcating extent of malignant and pre-malignant lesions of oral cavity under blue light.

Topical photofrin used in a study conducted at Chang Gung Memorial Hospital, Taiwan, to identify early oral cancer showed a sensitivity ranging between 92.45%- 93.75%.[39]

Besides diagnostic application, 5-ALA can also be used as a form of photodynamic therapy for therapeutic purposes. 5-ALA application in a study conducted at University of Arkansas for Medical Sciences, Little Rock and Regensburg University, Germany, was used for photodynamic treatment of oral leukoplakia by utilizing a pulsed dye laser. Of 17 patients, 1(6%) showed no response whereas 7(41%) showed significant response and 9(53%) showed partial response. It was observed that Buccal mucosa lesions showed significant response.[46]

Contact endoscopy has been done in laryngeal and pharyngeal malignancies using 5-ALA. But in contrast to our study involving oral cancers, these are different malignancies with different biological behavior and the endoscopy is using narrow band imaging and filters in the microscope.[12]

Study conducted at Medical University Hannover, Germany, included 42 patients who were examined for pharynx, hypopharynx and laryngeal lesions. This study showed a sensitivity and specificity of 90% and 93.8% and a diagnostic accuracy of 88%.[47]
Other studies in Eastern Europe show a sensitivity and specificity of 80-90%.[48,49]

In a study done at the Chinese University of Hong Kong, 64 patients with previous irradiation for nasopharyngeal carcinoma were examined. This study showed a 100% sensitivity and specificity for prediction of persistant and recurrent disease and a diagnostic accuracy of 92.1%.[50]

Study conducted at University Hospitals, Giessen, Germany, observed a sensitivity and specificity of 94.7% and 95.5% respectively in 83 patients with laryngeal lesions. The diagnostic accuracy of this study was 94%.[51]

Contact endoscopy in a non-invasive, in vivo microscopic examination done by placing a rigid telescope on mucosa that has been stained by a dye. This produces real time images of the cellular architecture with magnification, thereby eliminating need for biopsy – which is the gold standard for diagnosis.[12]

Similar sensitivity and specificity was observed at a study held at National Cancer Center and Singapore General Hospital, Singapore.[66]

In a collaborated study involving Taiwan and Japan, white light and narrow band imaging were compared in detection of cancerous lesions. The diagnostic accuracy of white light and NBI was 100% and 97% for oral lesions, 69% and 100% for oropharyngeal lesions, 39% and 100% for hypopharyngeal lesions respectively. Detection of CIS was found to be superior in NBI ($p<0.001$).[45]

In a study conducted at University of British Columbia, Canada, detection of surgical tumor margins for oral cancer by fluorescence visualization utilizing VELscope was demonstrated in 20 patients. 19 patients observed a loss of autofluorescence 25mm beyond the visible margin of tumor. Caricnoma or dysplasia was observed in 89% of biopsies taken from these areas.[67,41]

On using VELscope, neoplastic cells appear dark brown to black due to decreased autofluorescence and healthy mucosa emits pale green autofluorescence. The sensitivity and specificity for differentiation of normal mucosa and severe dysplasia/CIS/invasive carcinoma in 50 biopsies was noted to be 98% and 100% respectively.[42] Another study at the same institute demonstrated sensitivity and specificity for oral neoplasia detection by utilizing VELscope to be 97% and 94% respectively.[41] In contrast, a study to assess accuracy of

VELscope in detection of 256 oral lesions done at Moti Lal Nehru Medical College, India, showed sensitivity and specificity to be 50% and 38.9% respectively.[43]

Newer techniques like contact endoscopy and endoscopic high frequency ultrasound have been used.[68] Study done at Biophotonics Laboratory, Trivandrum, India, the above techniques have be used for identifying oral cavity lesions and show sensitivity of 91.3% and specificity of 100%.[68] Study at University of Brescia, Italy, utilizing NBI for detection of oral cavity lesions demonstrated a sensitivity and specificity of 96% and 100% respectively. Positive and negative predictive value was found to be 100% and 93%.[69]

In a study done at Vilnius University, 84 patients with morphologically verified malignant tumour were subjected to Fluorescence Detection (FD) following intravenous or intra-arterial administration of photosensitizer (Photofrin). This study, conducted in 1990 was the pioneer of intra-arterial (i/a) hematoporphyrin derivative mediated photodynamic therapy (i/a PDT) in head and neck cancers. Visualization under 405+/- 5nm wavelength of light after 1-4hours showed a pink fluorescence. Also, spectroscopic examination of malignant and healthy tissues was performed. It was seen that all cases demonstrated fluorescence. Of the 20 cases who underwent intravenous administration of Photofrin, 6 were noted to be false positive. Glow artefacts produced a non-specific lilac fluorescence. The sensitivity of this study was 100%.[40]

A study done at University of Munich, Germany, utilizing topical ALA solution in the form of gargling, showed a sensitivity of 99%, specificity of 60%, a positive predictive value of 77.3% and a negative predictive value of 97.5%.[3] Spectroscopic imaging of the lesion was

done post staining for fluorescence and on examination, results were classified with regard to fluorescence as strong positive, weak positive and negative. All strong positive cases considered as specific were positive for malignancy/ dysplasia. Weak positives constituted 30-40% of the total cases and were found to be negative for malignancy on histopathology.[3] No major adverse reactions like photosensitivity or systemic absorption were seen in this study. Detection of flat lesions and margin demarcations were unsatisfactory as 66% of evident tumors did not show endogenous fluorescence and 33% showed a non homogenous and partial staining.[3]

In our study, no spectroscopy was done. As a result, there were no weak positives. Hence, there is a discrepancy with regard to the specificity. Therefore the sensitivity and specificity in our study was 95.74% and 100% respectively because biopsy from fluorescent margin (naked eye under blue light) would have been taken only from strongly fluorescent margin.
Study done at Eastman Dental Institute for Oral Healthcare Sciences, London, consisted of 71 patients with clinically suspicious lesions. These patients performed an oral rinse with ALA solution. The sensitivity and specificity for identifying dysplasia or carcinoma in situ was 83-90% and 79-89% respectively.[38]

In a study done at University of Munich, Germany, topical ALA was used as a rinsing solution. The lesion was examined under 375-440 nm (blue) wavelength of light for fluorescence and biopsy was performed and evaluated for malignancy. Comparable results were observed in white light diagnosis and Combined Fluorescence Diagnosis (CFD) for all biopsy specimens taken. PPIX fluorescence fared best and autofluorescence scored worst for tongue lesions.[2] Sensitivity of white light imaging was 99.2%, autofluorescence photodetection was 87.8% while CFD and PPIX was 100%. Specificity of autofluorescence

photodetection was 56.4% and was superior to white light (42.9%), PPIX and CFD. CFD outperforms autofluorescence photodetection, PPIX and white light inspection in the detection and delineation of tumor margins. Tumors at rim of the tongue showed best results through PPIX diagnosis while tumors at the floor of the mouth and oropharynx showed better results with autofluorescence diagnosis.[2] Hematoporphyrin derivative (HpD) was not of much help for early carcinoma as it showed poor selectivity and can pose harmful effects on blood agglutination. This study demonstrates that white light was found to be better than other methods for detection and demarcation of tumors.[2] Autofluorescence was best for small sized nonkeratinizing tumors at floor of mouth or oropharynx while PpIX was best for large lesions at rim of the tongue. The best method was said to be a combination of autofluorescence photodetection and topical 5-ALA induced PpIX fluorescence for detection of precancerous lesions, tumor borders and areas of field cancerization.[2]

Similar to the above study, our study emphasized the efficacy of 5-ALA induced PpIX fluorescence in detection and delineation of tongue malignancies. We observed that all malignancy tongue cases showed >5mm extension beyond visible margin on fluorescence.

In our study, using topical 5-ALA, sensitivity and specificity was found to be 95.74% and 100% respectively. Positive predictive value was 100% and negative predictive value was 60%. Diagnostic accuracy was found to be 96%. The data was analysed using OpenEpi Software Version 2. As noted in a study in literature, there appears a better detection and delineation of tongue cancers by PpIX-induced fluorescence in comparision to other sub-sites of oral cavity in our study too.[2]

The sensitivity of our study was comparable to the study done at Germany. Specificity of our study was higher as we have taken only visible fluorescence and not spectroscopy so the weak positive fluorescence would not have shown.

To infer, staining with 5-ALA shows autofluorescence and as seen in our study the lesion may be extending beyond visible margin. This shows that tumours extend beyond visible margin as do pre-malignant lesions and a wide excision is mandatory. The average extension in Buccal Mucosa lesions was 4.35mm (2-7mm) and in tongue was 11mm (8-13mm). The pre-malignant lesions show an average extension of 3.35mm (2-5mm).

The same autofluorescence using 5-ALA may be used in future to ensure margins of resection during surgery. This may bring down the number of local recurrence in oral malignancies.

CONCLUSION

- There is high prevalence of oral squamous cell carcinoma in Kolar region.
- Most of the patients are females and present with locally advanced disease (T3/ T4a).
- Staining with 5-ALA produces red fluorescence when seen with blue light (380-450nm).
- This ALA induced fluorescence shows the true extent of the lesion in oral malignancies and pre-malignant lesions.
- Staining with 5-ALA dye is superior to other supravital dyes like toluidine blue, lugol's iodine and methylene blue in demarcating oral malignant and pre-malignant lesions.
- In our study, topical ALA staining and visualization under blue light showed extension of fluorescence beyond the visible margin in 94% of patients.
- The sensitivity and specificity in our study for this fluorescence (extension of lesion beyond visible margin was 95.74% and 100%. The positive and negative predictive value was 100 % and 60 % respectively. Diagnostic accuracy was 96%.
- The average extension of tumour beyond visible margin was 3.95 mm in buccal mucosa and 9 mm in tongue lesions.
- In field cancerization, staining with 5-ALA can help to select the appropriate site for biopsy.
- 5-ALA staining demarcates the malignant and pre-malignant lesions efficiently and can therefore be used while resecting lesions to ensure complete clearance in future.
- 5-ALA staining holds promise in photodynamic therapy and laser excision of lesion in future.
- Modifications of ALA maybe used to deliver nanoparticles to the tumour in future.

- Other techniques like narrow band imaging also hold promise in showing full extent of lesion during resection in future.

SUMMARY

This study was done between November 2011 to March 2013 in R.L. Jalappa Hospital and Research Centre, Kolar. 50 patients with oral cancers and pre-malignant lesions were evaluated by taking biopsies from the visible margin and fluorescent margin of the tumour after staining with 5-ALA under blue light.

Majority of patients were females and most were from the 4^{th}-6^{th} decade of life. Buccal mucosa was found to be the most common site affected.

Maximum cases were of malignancy buccal mucosa (23 of 30 malignancy patients).

20 patients with pre-malignant lesions were included in the study, of which leukoplakia constituted the maximum number of cases (12 patients). Site-wise distribution shows buccal mucosa involvement in 19 patients and 1 patient with leukoplakia tongue.

Patients with oral malignancy in this region presented at an advanced stage of disease (22 of 30 patients).

The staining was done by making patient rinse oral cavity with freshly prepared 5-ALA solution for 15 minutes following which biopsy was taken after an incubation period of 3 hours.

The two biopsies were subjected to histopathological examination to find out whether there was microscopic extension of tumour or pre-malignant lesions beyond visible margin.

Histopathological reports of biopsy of lesions from visible margin showed a maximum of well differentiated SCC in 14 patients. 3 patients showed no evidence of dysplasia/ malignancy.

Histopathological reports of biopsy of lesions from fluorescence margin showed 2 cases with no evidence of dysplasia/ malignancy, 3 cases with no extension from visible margin on fluorescence and 8 cases showing features of well differentiated SCC.

A total of 47 patients showed extension of fluorescent margin further to the visible margin. The range of extension varies between 2-13mm. 31 patients were observed to have an extension (fluorescence) beyond visible margin varying between 3mm – 5mm. 5 cases of malignancy buccal mucosa and all 3 cases of malignancy tongue showed extension (of fluorescence) >5mm beyond visible margin.

17 pre-malignancy (16 – buccal mucosa and 1 – tongue) cases showed extension on fluorescence beyond visible margin with average distance of fluorescence beyond visible margin to be 3.35mm and 3mm respectively.

Cases of malignancy tongue showed more extension under fluorescence (average of 9mm) compared to other subsites.

Field cancerization is an important aspect to be noted while inspecting the oral cavity with blue wavelength light due to high incidence of second primaries in oral cavity malignancies. Here, in our study, of the 3 patients showing field cancerization, the 2 cases of leukoplakia demonstrated dysplasia and 1 case of erythroplakia showed severe dysplasia.

Topical 5-ALA is non-invasive and has an easy methodology. Fluorescence on visualization under blue wavelength light was noted due to accumulation of Protoporphyrin IX in malignant cells. This aids in delineation of lesion margins accurately ensuring complete clearance of tumour intra-operatively. Our patients did not suffer from any side effects.

Our study showed a diagnostic accuracy of 96%. Sensitivity and specificity of the study was 95.74% and 100% respectively. The positive and negative predictive values were found to be 100% and 60% respectively.

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ANNEXURES

EVALUATING USEFULNESS OF 5-AMINOLEVULINIC ACID INDUCED FLUORESCENCE TO GUIDE BIOPSY OF ORAL CANCERS AND PRE MALIGNANT LESIONS

PROFORMA

I. PERSONAL DETAIL

Name : Age : Sex : M F

Address : Date : Occupation :

Telephone : Hospital no:

E-mail ID :

II. PRESENTING COMPLAINT

CHIEF COMPLAINTS	YES/NO	SINCE
Presence of ulcer/mass in oral cavity		
Presence of burning sensation in oral cavity upon taking spicy food		
Restricted mouth opening		
Presence of mass/swelling in neck		

Lesion Site : Buccal mucosa/ Tongue/ Gingivobuccal sulcus/ Retromolar Trigone/

Hard palate/Floor of Mouth/Alveolus

Side : Right/ Left

III. HISTORY OF PRESENT ILLNESS

Onset : Progression :

Aggravating factors :

Relieving factors :

H/O trauma : Y/ N

H/O difficulty in swallowing : Y/ N

H/O difficulty in breathing : Y/ N

H/O change in voice : Y/ N

H/O weight loss : Y/ N

IV. PAST HISTORY

History of Diabetes mellitus, Hypertension, Tuberculosis, Bronchial asthma, Drug allergy

History of previous surgery : Y/ N

Treatment History (if any) : Surgery/ Radiotherapy/ Chemotherapy

V. FAMILY HISTORY

VI. PERSONAL HISTORY

Loss of appetite: Y/ N

Disturbed sleep: Y/ N

Bowel and bladder disturbances: Y/ N

Habits –

- Tobacco chewing :

  Type – Betel nut/ Pan masala/ Gutka

  Duration - Frequency –

  Side – Right/ Left/ Both Leaves overnight – Y/ N

  Tobacco – Y/ N Lime – Y/ N

  Stopped since –

  (if stopped)

- Smoking :

  Type – Filtered Cigarette/ Unfiltered Cigarette/ Beedi/ Hookha/ Pipe

  Duration - Pack years -

  Reverse smoking :Y/ N Stopped since –

  (if stopped)

- Alcohol :

  Duration - Type -

  Amount/ day - Stopped since –

  (if stopped)

EXAMINATION

VII. GENERAL PHYSICAL EXAMINATION

Built: Poor/ Medium/ Well-built Nutritional status: Poor/ Satisfactory

Temperature: Pulse: BP: RR:

Pallor: Y/ N Icterus: Y/ N Cyanosis Y/ N

Clubbing: Y/ N Lymphadenopathy: Y/ N Oedema: Y/ N

VIII. E.N.T EXAMINATION

- **Oral Cavity :**

Mouth opening : Adequate/ Trismus Grade of Trismus (if any) :

Oro – dental Hygiene : Poor/ Satisfactory Nicotine stains : Y/ N

Site : Lip/ Buccal mucosa/ Tongue/ Floor of Mouth/ Retromolar Trigone/

Gingivo-buccal Sulcus/Alveolus/ Hard palate

Side : Right/ Left/ Both

Type of Lesion: Leukoplakia/ Erythroplakia/ Erythroleukoplakia/ Lichen planus/

Oral Submucous Fibrosis/ Verrucous/ Ulceroproliferative/

Ulcerative

Dimension:

Extent – Superior :

Inferior :

Medial :

Lateral :

Edge:

Tender : Y/ N

Skin involvement : Y/ N

Bleeds on touch : Y/ N

Extension of fluorescenced margin beyond visible margin, if any (in mm.) :

- **Indirect Laryngoscopy :**

- **Neck :**

Level/ s involved : Number :

Size :

Mobile/ Fixed Consistency : Hard/ Firm

- **Nose :**

- **Ear :**

IX . SYSTEMIC EXAMINATION

Cardio vascular system :

Respiratory system :

Abdomen :

Central nervous system :

X . CLINICAL DIAGNOSIS

XI. INVESTIGATIONS

Hb: BT: CT: HIV: Y/ N HbsAg: Y/ N

XII. BIOPSY REPORT

Non–Fluorescenced (Visible) Margin:

Dysplasia/Leukoplakia/Erythroplakia/Erythroleukoplia/ Lichen planus/ Well differentiated Squamous cell Carcinoma/Moderately differentiated Squamous cell Carcinoma/Poorly differentiated Squamous cell Carcinoma

Fluorescenced Margin :

Dysplasia/Leukoplakia/Erythroplakia/rythroleukoplakia Lichen planus/ Well differentiated Squamous cell Carcinoma/Moderately differentiated Squamous cell Carcinoma/Poorly differentiated Squamous cell Carcinoma

XIII. TREATMENT

Surgery :

Radiotherapy :

Chemotherapy :

Default :

XIV . Signature of the Guide

CONSENT FOR BIOPSY

I Mr/Mrs _____ have been explained in a language I understand, that I will be included in this diagnostic study which includes taking 2 biopsies from the oral cavity lesion that I have presented with. The study requires me to rinse my oral cavity with a freshly prepared solution of 5 – Aminolevulinic Acid for 15 minutes. The biopsies will be taken after an incubation period of 3 hours with the aid of a blue wavelength light (380-450nm). The 2 biopsy specimens will be sent for histopathological reporting to identify and document the presence or absence of malignancy/ dysplasia.

I understand this is a relatively new procedure which has given good results, according to previous studies performed and that this procedure helps in delineating the tumour margin accurately as it stains even the microscopic subclinical malignant cells.

I have been made to understand that I will not incur any added expenditure other than investigations required prior to performing biopsy and the cost for the histopathological examination of the 2 biopsy specimens.

I have also been made to understand that I will be advised treatment in accordance with the histopathological report of the biopsy and that I can withdraw myself from the study at any period of time and that there will be no compromise in my treatment in case I withdraw myself from the study.

I have been informed that the topical application of this chemical solution does not have any side effects. However, in case any adverse reactions occur to me during the period of study, immediate appropriate treatment will be given by the attending doctor.

I have been informed regarding confidentiality that will be strictly maintained regarding my personal details throughout study and if details regarding procedure needs to be published, I will be informed in advance prior to the publication.

I have understood the same and willingly give valid consent and agree to be a part of this study.

Patient’s signature/ thumb impression:

Witness signature :

DATE: TIME : PLACE :

KEY TO MASTERCHART

F	⇨	Female
M	⇨	Male
BM	⇨	Buccal Mucosa
RMT	⇨	Retromolar Trigone
T	⇨	Tumour size and extent
N	⇨	Regional nodal metastasis – clinically
M	⇨	Distant metastasis
SCC	⇨	Squamous Cell Carcinoma
Cis	⇨	Carcinoma in situ
*	⇨	Pre-malignant lesions

MASTER CHART

SL. NO.	NAME	AGE	SEX	HOSPITAL NO.	DIAGNOSIS	STAGING	RESULTS OF BIOPSY FROM VISIBLE MARGIN	RESULTS OF BIOPSY FROM FLUORESCENT MARGIN	EXTENSION (IF ANY) OF FLUORESCENT MARGIN
1	Kanthamma	50	F	824120	Malignancy Right BM	T4aN1M0	Well differentiated SCC	Well differentiated SCC	4mm
2	Muniyappa	43	M	823830	Malignancy Right BM	T3N1M0	Severe Dysplasia (Cis)	Severe dysplasia (Cis)	3mm
3	Nagamma	45	F	823424	Malignancy Right RMT	T2N1M0	Mild dysplasia	Mild dysplasia	2mm
4	Chinamma	46	F	823758	Pre-malignancy (Erythroplakia) Left BM	*	Well differentiated SCC	Severe dysplasis (Cis)	3mm
5	Yuvaraj	44	M	774560	Pre-malignancy (Leukoplakia) Tongue	*	Mild to Moderate Dysplasia	Moderate to Severe Dysplasia	3mm
6	Rajamma	50	F	816017	Pre-malignancy (Leukoplakia) Left BM	*	Mild to Moderate Dysplasia	No evidence of Dysplasia	2mm
7	Nagamma	45	F	823424	Pre-malignancy (Leukoplakia) Left BM	*	Severe Dysplasia - Leukoplakia	Severe Dysplasia	3mm
8	Muniyamma	50	F	832719	Pre-malignancy (Leukoplakia) Left BM	*	No evidence of Dysplasia	No extension	-

9	Sakamma	60	F	835412	Malignancy Right BM	T4aN1M0	Well to Moderately differentiated SCC	Well to Moderately differentiated SCC	5mm
10	Muniyamma	70	F	879508	Pre-malignancy (Leukoplakia) Right BM	*	Mild to Moderate Dysplasia	No evidence of Dysplasia	2mm
11	Nagamma	50	F	887563	Pre-malignancy (Erythroplakia) Left BM	*	Moderate Dysplasia - Erythroplakia	Moderate Dysplasia - Erythroplakia	3mm
12	Noor Jan	55	F	891891	Malignancy Left Lower Alveolus	T3N1M0	Well differentiated SCC	Mild to Moderate Dysplasia	5mm
13	Shanthamma	55	F	881973	Malignancy Right BM	T2N0M0	Well differentiated SCC	Mild dysplasia	5mm
14	Raghu Reddy	49	M	841826	Malignancy Tongue	T3N1M0	Well differentiated SCC	Well differentiated SCC	13mm
15	Venkatamma	63	F	925386	Malignancy Right BM	T3N1M0	Well to Moderately differentiated SCC	Well to Moderately differentiated SCC	7mm
16	Nagamma	50	F	887563	Pre-malignancy (Leukoplakia) Right BM	*	Moderate Dysplasia - Leukoplakia	Moderate Dysplasia - Leukoplakia	2mm
17	Bhagyamma	78	F	924138	Malignancy Left BM	T2N1M0	Well differentiated SCC	Mild Dysplasia	4mm
18	Bhoolakshmamma	40	F	903939	Pre-malignancy (Erythroleukoplakia) Left BM	*	Severe Dysplasia - Erythroleukoplakia	Mild Dysplasia	3mm
19	Gulab Jan	40	F	914241	Malignancy Right BM	T4aN2aM0	Moderately differentiated SCC	Moderately differentiated SCC	7mm
20	Muniyappa	60	M	893587	Malignancy Right BM	T3N1M0	Moderately differentiated SCC	Severe Dysplasia	2mm

21	Subamma	50	F	913858	Malignancy Right BM	T2N1M0	Well to Moderately differentiated SCC	Mild Dysplasia	4mm
22	Muniyamma	60	F	916384	Malignancy Right BM	T2N1M0	Well to Moderately differentiated SCC	Mild to Moderate Dysplasia	5mm
23	Mangamma	59	F	932348	Malignancy Left BM	T4aN1M0	Well differentiated SCC	Well differentiated SCC	3mm
24	Lakshmidevamma	54	F	866291	Malignancy Left BM	T3N2aM0	Well differentiated SCC	Well differentiated SCC	6mm
25	Zaheera Bee	65	F	837856	Malignancy Left BM	T4aN0M0	Moderately differentiated SCC	Moderately differentiated SCC	4mm
26	Krishnamma	67	F	844226	Malignancy Hard Palate	T2N0M0	Moderate to Poorly differentiated SCC	Poorly differentiated SCC	2mm
27	Ramaiah	33	M	846536	Pre-malignancy (Erythroplakia) Right BM	*	Mild Dysplasia	Mild dysplasia	2mm
28	Muniyappa	60	M	837803	Malignancy Right BM	T3N2bM0	Well differentiated SCC	Well differentiated SCC	7mm
29	Deviramma	58	F	928106	Malignancy Left BM	T4aN0M0	Moderate Dysplasia	Moderate Dysplasia	3mm
30	Manjamma	40	F	932312	Malignancy Right BM	T4aN1M0	Well differentiated SCC	Well to Moderately differentiated SCC	6mm
31	Sampuranamma	65	F	913821	Pre-malignancy (Leukoplakia) Right BM	*	Mild to Moderate Dysplasia	Mild to Moderate Dysplasia	5mm
32	Nanjundappa	75	M	903600	Malignancy Left Lower Alveolus	T2N0M0	Well differentiated SCC	Severe Dysplasia	3mm
33	Narsamma	55	F	899930	Pre-malignancy (Leukoplakia) Right BM	*	Mild Dysplasia	Mild dysplasia	4mm

34	Narasamma	55	F	895011	Malignancy Left BM	T4aN1M0	Verrucous Carcinoma	Verrucous Carcinoma	2mm
35	Thayamma	55	F	898247	Malignancy Right BM	T3N0M0	Well differentiated SCC	Moderate Dysplasia	4mm
36	Rathnamma	55	F	896613	Pre-malignancy (Leukoplakia) Left BM	*	No evidence of Dysplasia	No extension	-
37	Jayamma	55	F	871208	Malignancy Left BM	T3N0M0	Well differentiated SCC	Well to Moderately differentiated SCC	3mm
38	Byramma	65	F	872140	Malignancy Left BM	T4aN1M0	Well differentiated SCC	Well differentiated SCC	3mm
39	Varadamma	60	F	871123	Malignancy Right BM	T3N1M0	Well to Moderately differentiated SCC	Well differentiated SCC	5mm
40	Hemavathi	46	F	873258	Pre-malignancy (Leukoplakia) Left BM	*	No evidence of Dysplasia	No Extension	-
41	Venkatamma	65	F	873089	Pre-malignancy (Erythroplakia) Left BM	*	Moderate Dysplasia	Moderate Dysplasia	5mm
42	Venkatamma	65	F	873089	Pre-malignancy (Erythroplakia) Right BM	*	Severe Dysplasia	Moderate Dysplasia	3mm
43	Jayamma	45	F	915751	Malignancy Left BM	T3N1M0	Well to Moderately differentiated SCC	Well to Moderately differentiated SCC	5mm
44	Rathnamma	50	F	914120	Pre-malignancy (Leukoplakia) Left BM	*	Severe Dysplasia	Severe Dysplasia	4mm
45	Venkateshappa	65	M	912628	Malignancy Right BM	T3N1M0	Well to Moderately differentiated SCC	Severe Dysplasia	3mm

46	Chikkaramappa	56	M	922087	Malignancy Tongue	T2N0M0	Well to Moderately differentiated SCC	Well to Moderately differentiated SCC	12mm
47	Rajamma	75	F	920669	Pre-malignancy (Leukoplakia) Right BM	*	Moderate to Severe Dysplasia	Moderate to Severe Dysplasia	4mm
48	Munirathnamma	70	F	864648	Pre-malignancy (Erythroleukoplakia) Left BM	*	Mild dysplasia	Mild dysplasia	4mm
49	Subbaraju	47	M	914702	Malignancy Tongue	T4aN1M0	Well to Moderately differentiated SCC	Well differentiated SCC	8mm
50	Munirathnamma	70	F	864648	Pre-malignancy (Erythroplakia) Right BM	*	Moderate Dysplasia	Mild Dysplasia	5mm
* ⇨Pre-malignant lesions									