ROLE OF MICRONUCLEI AS A DIAGNOSTIC TOOL IN EXFOLIATIVE CYTOLOGY OF ORAL PRENEOPLASTIC AND NEOPLASTIC CONDITIONS AMONG TOBACCO CHEWERS

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

DECLARATION

I solemnly declare that the dissertation entitled "**ROLE OF MICRONUCLEI AS A DIAGNOSTIC TOOL IN EXFOLIATIVE CYTOLOGY OF ORAL PRENEOPLASTIC AND NEOPLASTIC CONDITIONS AMONG TOBACCO CHEWERS"** is a bonafide research work done by me in the Department of Pathology at Coimbatore Medical college, Coimbatore during the period from December 2012 to December 2015 under the guidance and supervision of **Dr.C.Lalitha, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical college, Coimbatore.

This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree (Branch III) in Pathology.

I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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This is to certify that the dissertation entitled **"ROLE OF MICRONUCLEI AS A DIAGNOSTIC TOOL IN EXFOLIATIVE CYTOLOGY OF ORAL PRENEOPLASTIC AND NEOPLASTIC CONDITIONS AMONG TOBACCO CHEWERS"** is a record of bonafide work done by **Dr.R.SUGANYA**, post graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the supervision and guidance of **Dr. C.Lalitha, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore and submitted in partial fulfilment of the regulations of the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the award of M.D. Degree (Branch III) in Pathology.

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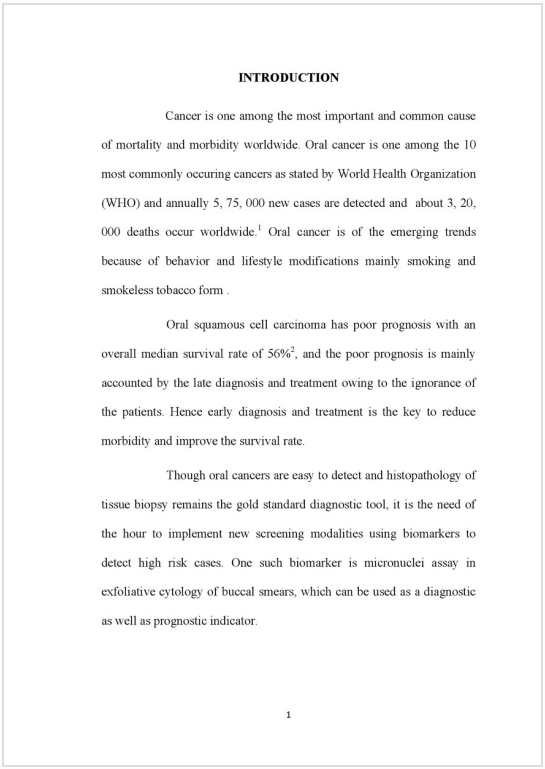


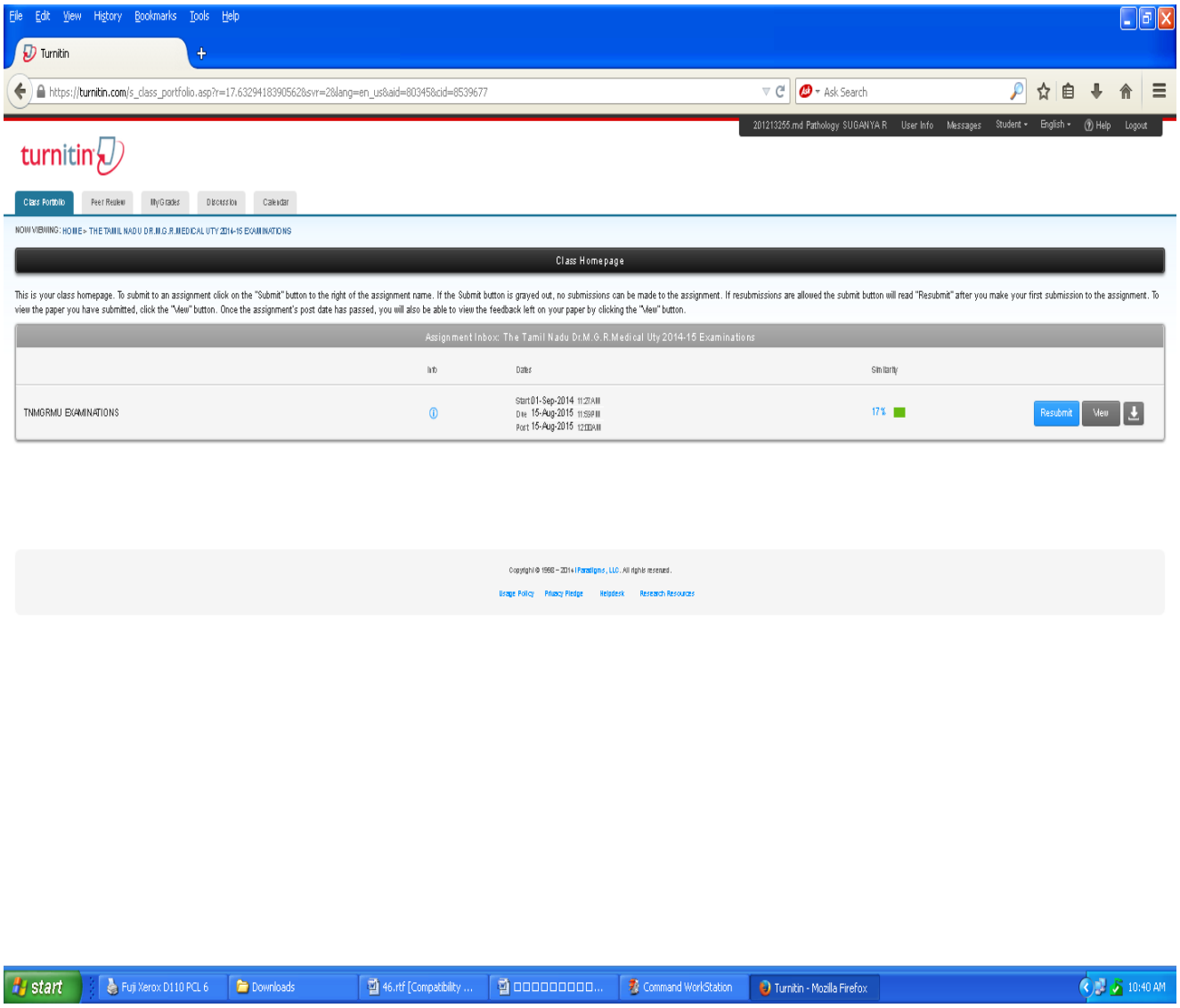
INTRODUCTION

Cancer is one among the most important and common cause of mortality and morbidity worldwide. Oral cancer is one among the 10 most commonly occuring cancers as stated by World Health Organization (WHO) and annually 5, 75, 000 new cases are detected and about 3, 20, 000 deaths occur worldwide.[1] Oral cancer is of the emerging trends because of behavior and lifestyle modifications mainly smoking and smokeless tobacco form .

Oral squamous cell carcinoma has poor prognosis with an overall median survival rate of 56%[2], and the poor prognosis is mainly accounted by the late diagnosis and treatment owing to the ignorance of the patients. Hence early diagnosis and treatment is the key to reduce morbidity and improve the survival rate.

Though oral cancers are easy to detect and histopathology of tissue biopsy remains the gold standard diagnostic tool, it is the need of the hour to implement new screening modalities using biomarkers to detect high risk cases. One such biomarker is micronuclei assay in exfoliative cytology of buccal smears, which can be used as a diagnostic as well as prognostic indicator.

1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Abstract

Background: The assessment of Micronuclei frequency in exfoliated oral epithelial cells have been shown to correlate with severity of the genotoxic damage and seem to increase steadily in order from tobacco chewers with apparently normal mucosa to premalignant and malignant lesions.

Aim: To evaluate the frequency of micronuclei (MN) in oral exfoliated cells of tobacco chewers with oral premalignant and malignant lesions and comparing them with that of healthy tobacco chewers and controls of non tobacco chewers.

Materials and Methods : The study subjects are divided into four groups consisting of tobacco chewers with apparently healthy oral mucosa, premalignant lesions and malignancy, and normal controls, each of 20 cases. The cytosmears are stained with Pap, Giemsa and Crystal violet stains. The micronuclei was identified using Tolbert's criteria.

Results: The frequency of micronuclei is found to be higher in malignant lesions as compared with premalignant lesions and healthy tobacco chewers, and controls

Conclusions: Hence, micronuclei can be used as a biomarker of genotoxic damage, which is an useful diagnostic as well as prognostic indicator.

Keywords: Micronuclei, oral exfoliative cytology, tobacco chewers, squamous cell carcinoma, premalignant lesions, Papanicolaou, Giemsa, Crystal violet stains.

INTRODUCTION

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Cancer is one among the most important and common cause of mortality and morbidity worldwide. Oral cancer is one among the 10 most commonly occuring cancers as stated by World Health Organization (WHO) and annually 5, 75, 000 new cases are detected and about 3, 20, 000 deaths occur worldwide.[1] Oral cancer is of the emerging trends because of behavior and lifestyle modifications mainly smoking and smokeless forms of tobacco.

Oral squamous cell carcinoma has poor prognosis with an overall median survival rate of 56%[2], and the poor prognosis is mainly accounted by the late diagnosis and treatment owing to the ignorance of the patients. Hence early diagnosis and treatment is the key to reduce morbidity and improve the survival rate.

Though oral cancers are easy to detect and histopathology of tissue biopsy remains the gold standard diagnostic tool, it is the need of the hour to implement new screening modalities using biomarkers to detect high risk cases. One such biomarker is micronuclei assay in exfoliative cytology of buccal smears, which can be used as a diagnostic as well as prognostic indicator.

Micronuclei are extranuclear cytoplasmic bodies seen in association with chromosomal aberrations. They are induced by many substances like alcohol, tobacco, betal nut and irradiation. Hence the micronuclei assessment in exfoliated buccal cells will turn to be a promising tool in the study of epithelial damage and to detect the pathogenesis of carcinoma in relation to chromosomal breakage or mitotic interference.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To evaluate the micronuclei frequencies in oral exfoliated cells of tobacco chewers with oral preneoplastic and neoplastic lesions and comparing with tobacco chewers of apparently healthy oral mucosa.
2. Comparison of micronuclei frequencies among nontobacco users and tobacco chewers, who are at high risk of developing oral cancer.
3. Comparison of micronuclei in oral exfoliated cells using various staining procedures like Papanicolaou, Giemsa and Crystal violet stains.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

INTRODUCTION:

Oral carcinoma globally has the age standardized incidence of 2.1% , with 2.7% in males and 1.3% in females. The mortality is 1.8% worldwide, constituting about 2.1% in males and 1.3% in females. Oral cancer is one of the top three cancers in India accounting for 30% of all cancers.[1] India is said to be the oral cancer capital of the world. In India, about 90% of the oral cancers are related to tobacco use. The World Health Organization (WHO) has calculated the rise of mortality due to tobacco related diseases in India from 1.4% in 1990 to 13.3% in 2020.[1,2] About half of all cases of oral cancer have associated leukoplakia. Other premalignant lesions and conditions are submucous fibrosis, erythroplakia, lichen planus, and chronic immunosuppression. [3]

At present, the most reliable way to control and reduce oral cancer is to merge early diagnosis with timely and appropriate treatment. As more than 90% of all oral neoplasms are squamous cell carcinomas, almost all of them are diagnosed from lesions on the mucosal surfaces. Hence oral exfoliative cytology has become a promising tool in early diagnosis of high risk individuals of oral cancer.

Oral cavity is the entrance of the gastrointestinal tract. It begins with the lips in the anterior and ends with the oropharynx.

The main functions are

i) it serves as one of the protective barrier of the body

ii) gives taste sensation

iii) provides lubrication through saliva and aids in swallowing

iv) provides immunological defense mechanism

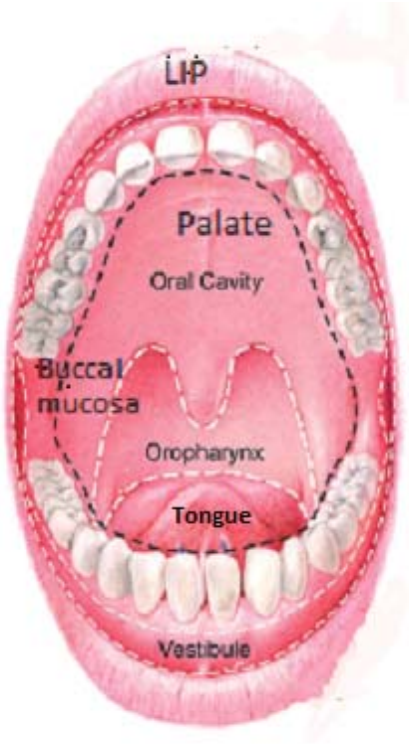
v) speech

vi) initiates digestion

ANATOMY:

The oral cavity is classified anatomically into three parts - the vestibule, oral cavity proper and the last is the oropharynx. The vestibule is the space occupied medially by teeth and laterally by the lips in the front and cheeks sideways. The oral cavity proper lies within the dental arches and is bounded by the palatoglossal arch posteriorly. Posterior to the palatoglossal arch, lies the oropharynx. The posterior one-third of the tongue, soft palate, palatine tonsils and posterior wall constitutes the oropharynx. The palatine tonsils lie between the palatoglossal arch anteriorly and palatopharyngeal arches posteriorly. The retromolar trigone is a triangular area which lies behind the last molar teeth and represents the posterior part of the vestibule of the mouth.

Based on the topography of the oral lesions, the oral cavity is divided into various regions as follows 1) Lip, 2) Buccal mucosa, 3) Tongue, 4) Hard palate, 5) Gingiva, 6) Floor of the mouth, 7) Soft palate, 8) Retromolar trigone, 9)Base of the tongue, 10) Tonsillar area and 11) Pharyngeal wall.[4]



HISTOLOGY:

Oral cavity has an overlying thicker epithelium compared to that of skin. The muscle is superficial in tongue and deep in case of buccal and labial mucosa. The minor salivary glands are mostly mucous, although serous acini are also seen throughout the oral cavity except in gingiva and tongue.

The oral mucosa is subdivided into three groups based on the lining epithelium, connective tissue structures and its functions

i) Lining mucosa

ii) Masticatory mucosa

iii) Specialized mucosa

I. **Lining mucosa** – forms the inner surface of the buccal mucosa, soft palate, floor of the mouth, lips and inferior surface of the tongue.

Buccal mucosa has nonkeratinized stratified squamous epithelium, 15 – 20 cells thick with broad tapered rete ridges, loose fibrovascular tissue in lamina propia and muscle at the base. Perinuclear halo may be seen because of glycogen.

Soft palate and floor of mouth have thin stratified squamous epithelium, 10 – 15 cells thick with poorly formed rete ridges.

Ventral tongue has serous salivary gland (anterior – glands of Blandin & Nuhn and posterior – glands of Von Ebner).

II. Masticatory mucosa – covers the gingiva and hard palate

Hard palate shows keratinized stratified squamous epithelium, thin layer of orthokeratin with thin granular layer and are 15 - 20 cells thick. It has dense lamina propria, fatty tissue with neurovascular bundles and minor salivary glands and at the base, the periosteum. There is no submucosa.

Gingiva share similar histological fingings with hard palate, but with more slender and tapered rete ridges. Rests of odontogenic epithelium (rests of Serres) with abundant clear cells are also seen.

III. Specialized mucosa (tongue):

Tongue is lined by keratinized stratified squamous epithelium with thick parakeratin layer, 20 – 30 cells thick and skeletal muscle seen superficially. Filiform papillae are keratotic spires and have surrounding bacterial colonies. They have no taste buds. Fungiform papillae (taste buds in apical surface), circumvalate and foliate papillae (lateral wall) have fibrovascular polypoid structure with taste buds. Lingual tonsils are seen in lateral tongue and posterior dorsum.[5,6]

CYTOLOGY:

Buccal cells are the first and foremost barrier for both of inhalation and ingestion route. They serve as preferred sites and target for early genotoxic events induced by carcinogenic agents. The oral epithelium is capable of continuous renewal of cells. Hence new cells formed in the basal layer by mitosis, upon time migrate to the surface and replace the shed cells. The stem cells expressing genetic damage are present in the basal layer. The cells formed will differentiate into the keratinized superficial layer and are exfoliated into the buccal cavity. The biomarkers of genomic damage like micronuclei, nuclear buds and those of cell death like apoptosis and karyolysis are identified in both the lymphocyte and buccal cells. Thus micronuclei assay is a newer novel technique in oral exfoliative cytology.[7]

Micronucleus in oral exfoliated cells is a marker of chromosomal damage caused by genotoxic agents from tobacco and tobacco-related substances, radiation and alcohol.[8] The micronucleus assay has been used to assess the genotoxic damage in oral squamous cell carcinoma and premalignant lesions.[9,10] The MN assay has been reported to correlate well with leukoplakia and the histological grading of oral squamous cell carcinoma. Incidence of micronuclei has been analyzed by various studies in oral premalignant lesions, squamous cell carcinomas and normal patients.[11-4]

HISTORICAL HIGHLIGHTS OF MICRONUCLEUS:

Micronuclei assay has been used as an indicator of genotoxic exposure since 1937, based on the radiation studies conducted by Brennecke and Mather.[15] In 1900s, Howell and Jolly found few nuclear remnants in human reticulocytes , that are Feulgen-positive and named after them as Howell-Jolly bodies. These nuclear bodies represent chromosomes separated from the mitotic spindle .[16] The term micronucleus test was first introduced in early 1970s, by **Boller** and **Schmidt** and **Heddle** who ascertained that this assay proves to be a simple method to detect the genotoxic potential of mutagens after in vivo exposure of animals using bone marrow erythrocytes. A few years later in 1976, **Countryman and Heddle** established micronucleus approach in peripheral blood lymphocytes and they recommended micronuclei assay as a biomarker in testing schemes.[17]

Stich et al is the first to develop a protocol for micronucleus assay in exfoliated buccal mucosa cells in1983.[(14-22)] It was used widely in occupational and lifestyle studies. Many studies have been published in the past 25 years using micronucleus assay in epithelial cells from oral mucosa, nasal mucosa, cervix, bronchus, bladder and oesophagus.

The Human Micronucleus **(HUMN**) project was established in 1997. This was an international collaborative program aimed to standardize micronucleus assay in peripheral blood lymphocytes and to assess the effects of protocol and scoring criteria on the values obtained. HUMN project published the results in 2001.[23-4]

MICRONUCLEUS :

M**icronucleus** is an erratic nucleus which is formed during the anaphase of mitosis or meiosis. Micronuclei are globular cytoplasmic bodies containing a portion of acentric chromosome or entire chromosome which fails to move to the opposite poles during anaphase. This results in the daughter cell that lacks a part or all of a chromosome. These chromosome fragments or entire chromosomes develop nuclear membranes and transform as micronuclei. After cytokinesis, one daughter cell ends up with one nucleus and the other ends up with one large and one small nucleus, i.e., micronucleus.

More than one micronucleus can be formed in case of more genetic damage. They are usually formed in the basal cells of epidermis. These cells are shed as exfoliated cells on maturation. Hence micronuclei assay can be used as one of the biomarkers of oral cancer, as

it is increased in oral preneoplastic and neoplastic conditions. Micronucleus can be identified by various special stains in exfoliative cytology.[19,21]

The pattern of formation of micronucleus in an individual depends on the type and amount of carcinogen exposure. The pattern produced by single and short term exposure will be different from those causing uniform and chronic exposure. The micronuclei frequency also seems to decrease with time, as this chromosomal damage can lead to apoptosis of the cell or loss of micronucleus during cell division.

The micronuclei can be demonstrated in erythrocytes, lymphocytes and exfoliated cells like oral, nasal and urothelial cells. Here, MN assay is used to evaluate the genomic damage occurred in vivo. Hence, the assay is employed in the analysis of cancers occurring in oral cavity, nasal cavity, bronchi, cervix, oesophagus, bladder and urinary tract. [25-6]

Micronucleus in a cell can be identified by many staining techniques. These include DNA specific stains like Fuelgen, acridine orange and 4',6-diaminido-2-phenylindole (DAPI) . The other non

specific stains are Giemsa, May Grunwald Giemsa, propidium iodide, Papanicolaou and crystal violet stains. Of these, DNA specific Fuelgen method is preferred by many laboratories.[27-8] Micronucleus assay is also done by using FISH (Fluorescence in situ hybridization) with a centromeric probe and micronucleus is seen as bright yellow green spots. FISH has the advantage of differentiating the mode of formation of micronuclei (clastogenic / aneuploidogenic mechanism).[29]

Micronucleus assay has been used to analyse chromosomal damage caused by various mutagenic and carcinogenic chemicals and many physical agents. These are mainly occupational hazards, lifestyle modification factors and irradiation. Some of the causative agents include all forms of tobacco, areca nut usage, alcohol, antineoplastic drugs, arsenic in drinking water, dioxin as fertilizers, pesticide mixtures, polycyclic aromatic hydrocarbons, ethylene oxide, formaldehyde, chlorants, toxic gases, lead oxide, solvents, toluene, benzene, ozone, acetone, hexane, 2-trans hexol and methl-ethyl ketone.[30-8]

Kamboj and Mahajan point out that assessment of micronuclei in buccal mucosa epithelial cells is a valuable biomarker for early detection of premalignant and malignant lesions of various sites.[39]

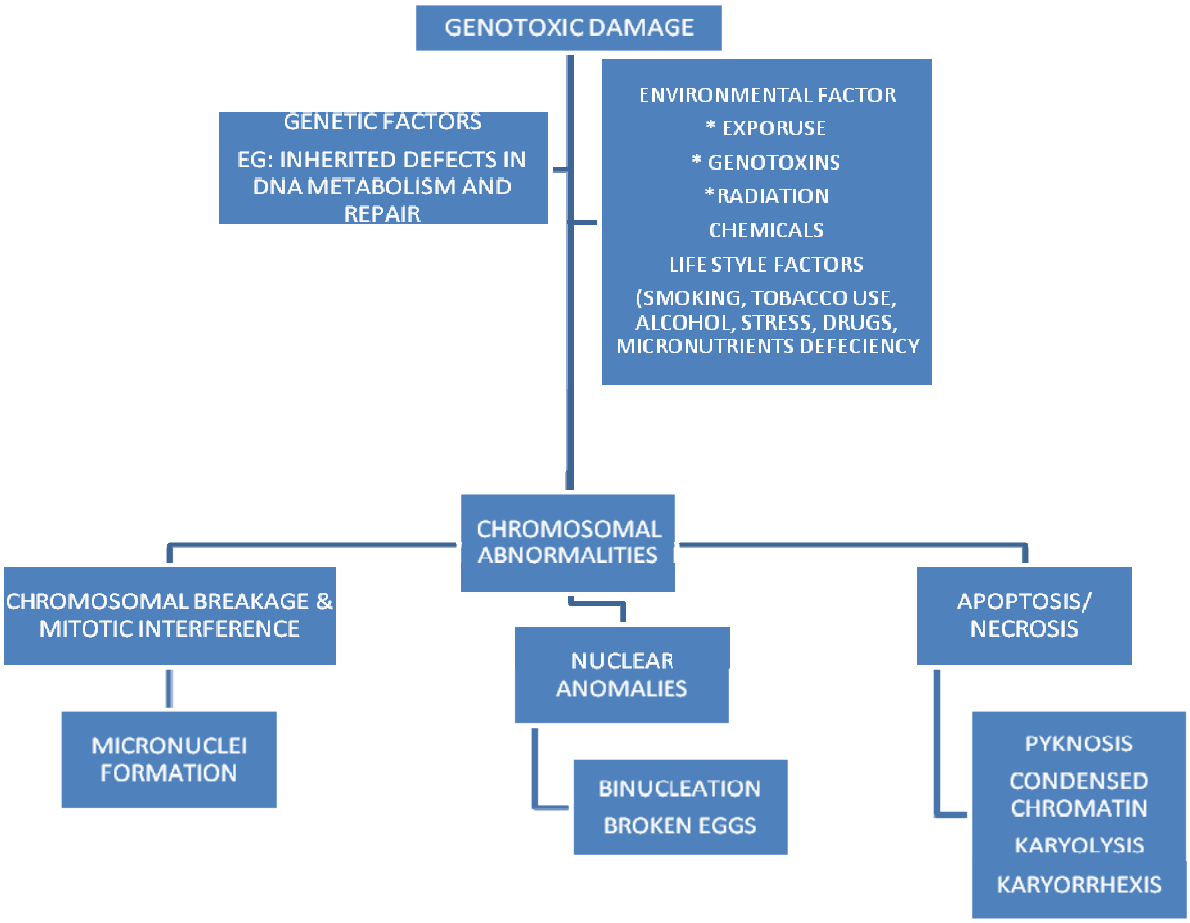
GENOTOXIC DAMAGE:

Genomic damage is the most important basic cause of degenerative and developmental diseases. It has been well established that genomic damage is caused by inherited genetic factors like defects in DNA metabolism and repair, environmental exposure to genotoxins, radiation , chemicals, micronutrients deficiency like folate and lifestyle factors (eg, smoking, alcohol, tobacco use, stress and drugs).[29,31-40]

The role of carcinogen is to induce chromosomal instabilities like deletions, translocations, gain or loss of the whole chromosomes, leading to the development of malignant cellular alterations .[40] So it has become essential to introduce reliable and relevant minimally invasive biomarkers. This will help in the implementation of diagnosis, monitoring, and treatment of diseases caused by, or associated with, genetic damage. One such valuable marker is micronuclei assay in exfoliative oral cytology.

The pathogenesis of genotoxic damage and the consequences are stated in the following diagram

PATHOGENESIS



EPIDEMIOLOGY OF ORAL CANCERS:

Oral cancer is the sixth common malignancy in the world. Annually about 5,00,000 new cases of oral cancers are diagnosed , of which three fourth of them occur in developing countries. The highest incidence is seen in France and also in Switzerland, Italy, Hungary and

Latin America. In India, about 75,000 to 80,000 cases are reported annually. In India, the age adjusted incidence rate of oral cancer is 20 per 100,000 which is very high and the overall incidence among all cancers is 30%. The oral cancer is mainly concentrated in the SEAR (South East Asian region) where the smokers and smokeless tobacco users are distributed equally, each constituting for about 250 million people. In India, tobacco chewers are about 26% and smokers are 14%. About 65% of the overall cancer cases are related to tobacco usage in India. Annually, tobacco alone contributes to 1,50,000 cancer cases, 42,00,000 heart diseases and 37,00,000 lung diseases in India. Cancer deaths in India is estimated to be around 56,00,000 /yr , of which tobacco is responsible for one third of the cases, with 2500 deaths/day contributed by tobacco related diseases. Global mortality of tobacco related diseases is said to be 22% per year.

Oral cancers have a higher incidence in men than in women. The male to female ratio is 3:1. The incidence of oral cancer in India is observed to be around 6.9% in males and 2.4% in females. This is probably because of risk factors like smoking, alcohol and chewing pan. In India, highest rates are observed in women who are heavy tobacco chewers. The mortality rate in males is 2.3% whereas in females it is 0.6%.

Oral carcinoma is commonly seen over the age of 50 years. Compared to the U.S population, the incidence is higher in India. In U.S., oral malignancies amount to only 3% of all cancers with age adjusted incidence being 10 per 100,000 as against India with 30% of all cancers and higher incidence. The difference is mainly due to increased chance of risk factors and race.

The Government of India spends about one fourth of the health expenditure ie. around Rs.300 billion annually for the treatment of tobacco associated illness.[2,41-50]

About 90% of the oral cancers are squamous cell carcinoma[51]. These tumors arise in any of the intraoral sites. Lip cancer constitutes about 20 – 40%. Intraorally, tongue is the most common site for SCC amounting to 25 – 40%, then is the soft palate complex, and less common are the buccal mucosa and floor of the mouth with 10 – 20%. But the incidence of buccal mucosal tumours seems to be higher in case of tobacco chewers.

ETIOPATHOGENESIS:

There are a number of known risk factors for oral squamous cell carcinoma. Some of the lifestyle factors are smoking, alcohol, and diet, mainly vitamin deficiencies and supplementation.[14,20,52] Most of the

studies with a significant increase in MN are related to a risk of oral cancer. They are performed in subgroups of subjects with specific lifestyle habits like chewers of betel quids (areca nut, betel leaves, slaked lime, and tobacco), snuff dippers, Khaini tobacco users (tobacco with slaked lime), reverse smokers, etc. [26,53-6] Results showed a rise in the micronuclei frequency because both smoking and chewing of tobacco mixtures cause nuclear degeneration, and in addition, the appearance of MN-like bodies in exfoliated cells are likely to be confused with micronulei. So, it is important to distinguish cell death events from genome damage in viable epithelial cells in evaluating cancer risk.

TOBACCO:

The major risk factor for oral squamous cell carcinoma is all forms of tobacco. Many carcinogens have been identified in tobacco and its combustion products. The most important agents are polycyclic aromatic hydrocarbons containing benzene, tobacco-specific aromatic amines and nitrosamines. These compounds cause epithelial damage in a dose-dependent manner. Comparitively, Smokeless tobacco results in disruption of DNA repair mechanisms and harmful genetic mutations which will finally lead to malignant transformation.

Smokers have an increased risk which is about five- to ten-fold risk of developing oral cancer, as is also the case with Cigar and pipe

smokers. Smokeless tobacco has a lower risk of oral cancer than smoked tobacco. Risk depends on the composition of the particular product used and can even rise to fourfold higher than that of a non-user. The habit of chewing coarsely cut tobacco leaves (chewing tobacco) or holding finely ground tobacco leaves (snuff) in the mandibular vestibule is common among certain populations around the world, most notably in India and Southeast Asia. Either habit is referred to as smokeless tobacco use or spit tobacco use. The habit is usually started early in life, at 8 to 14 years of age.

It has been estimated that the usage of tobacco in any form among the people of 15 to 49 years of age is said to be 57% in men and 11% in women. Several health and addiction hazards may be associated with the use of spit tobacco because of the ready absorption of nicotine and other molecules through the oral **mucosa.** The theory is that tobacco-specific nitrosamines induce dysplastic changes in the epithelium and these changes are probably intensified with prolonged surface contact.[53-60]

FORMS OF SMOKELESS TOBACCO:

There are many forms of tobacco which are mostly homemade or manufactured commercially. Some of them are as follows

Tobacco products	Ingredients
1. Paan with tobacco	Betal leaf, areca nut, slaked lime, catechu, tobacco, condiments with sweetening agents.
2. Paan masala	Similar to pan, the contents are dehydrated. This is prepared commercially
3. Manipuri tobacco	Tobacco, finely cut areca nut, camphor, slaked lime and cloves. It is used in Manipuri district of Uttar Pradesh.
4.Mawa	Scrapings of areca nut, some tobacco and slaked lime. It is used in Gujarat.
5. Khaini	Combination of sun dried tobacco and slaked lime. It is used mostly in Maharastra and North India.
6. Snus	Snuff prepared in the form of tea bag like pouch, which is sucked by placing in buccal mucosa. It is marketed as 'click' (Swedish company)

Tobacco is also used in many dental care products and some advertise tobacco as a means of cleaning teeth. It indirectly causes addiction on regular usage, leading to increased ill effects of tobacco.

Few of the marketed products are Mishri, Bajjar, Gul, Gudhaku, Lal Dantamanjan, creamy snuff and tobacco water.[61]

ALCOHOL:

Alcohol consumption poses an important risk for oral cancer in "moderate to heavy" drinkers (five to eight drinks per day). Alcohol and tobacco have a *synergistic effect*, enhancing the effect of each other. Ethanol alters the permeability of the oral mucosa, by acting locally, to various substances like carcinogens and hence enhances their penetration into the tissues. Alcoholic beverages also cause decreased cell metabolism systemically, thereby leading to relative immune deficiency.[62-3]

BETEL:

Betel products, which are derived from the nut of the areca palm, prove to be potential carcinogens. They are used commonly in Southeast Asia and the Indian subcontinent. Preparations usually consist of a mixture of betel leaf, tobacco, betel nut and slaked lime (calcium hydroxide). Addition of lime potentiates carcinogenicity besides giving

an euphoric effect. Prolonged usage may lead to the development of submucous fibrosis.

Gutkha and pan masala are in more demand among all age groups. Betel quid chewing with or without tobacco is claimed to be carcinogenic in humans. Gutkha is the mixture of areca nut and tobacco with addition of catechu, cardamom, lime, spices and flavouring agents. Gutkha is found to be responsible for a number of oral diseases and has addictive effects that lead to the addiction due to the presence of areca nut and tobacco. [61,64]

VIRAL INFECTIONS:

Viruses also play a role in certain benign and malignant neoplasms of the head and neck. *Epstein-Barr Virus* (EBV) is related to nasopharyngeal carcinoma, Burkitt lymphoma, and other lymphomas. *Human herpesvirus virus 8*(HHV-8) is associated with Kaposi sarcoma in immunocompromised and HIV-infectedpatients. *Human papillomavirus* (HPV) has been well established to cause benign proliferative epithelial lesions like *squamous papilloma* and *condylomata* throughout the head and neck region and malignant tumors of the posterior oral cavity and oropharynx. The exact etiology of viral involvement is still under research.[65-7]

IMMUNOSUPPRESSION:

Immunosuppressed individuals have high risk for malignancy throughout the body, as also in oral cavity. HIV-infected patients are prone to develop Non-Hodgkin lymphoma, Kaposi sarcoma and oral Squamous cell carcinoma. Similarly, transplant patients are associated with increased risk for multiple oral malignancies .Inherited disorders like *dyskeratosis congenita*, associated with progressive bone marrow failure causing aplastic anemia and presents with skin hyperpigmentation, dystrophic nail changes and *leukoplakia* which has increased risk of malignant transformation. Yet another rare bone marrow failure syndrome *Fanconi anemia* is also associated with high risk for oral Squamous cell carcinoma.[62,68]

NUTRITION:

Nutritional factors such as β carotene, vitamin A, retinol, α tocopherol, zinc, riboflavin, selenium and Chinese tea have been proved to be protective against oral cancers. Hence vitamin and mineral deficiencies may lead to carcinogenesis, though no specific pathogenetic mechanism has been elicited. This may possibly be related to loss of antioxidant mechanism leading to the formation of free radicals. One such example is *Plummer–Vinson syndrome*, rare condition presenting in middle-aged women, with dysphagia, esophageal webs, and iron

deficiency anemia and is thought to be at increased risk for oral and esophageal carcinoma.[62,68]

SANGUINARIA:

Extract derived from *Sanguinaria canadensis*, a common bloodroot plant has been commercially used as an antibacterial agent in oral rinses and toothpaste as to reduce plaque and gingivitis. It has been said to be associated with the development of leukoplakia particularly in the maxillary vestibule. But malignant transformation in these lesions is not established.[69]

MOLECULAR GENETICS:

The common genetic alterations seen in oral squamous cell carcinoma are mutations involving p16, p53, cyclin D1, p63, PTEN , Rb , and epidermal growth factor receptor (EGFR). p16 mutations are seen in 80% of oral cancers. The critical pathways involve mainly p53 inactivation by mutation and inhibition of HPV-16 E6 protein, EGFR overexpression and activation of signal transducer and activator of transcription 3 (STAT3) and vascular endothelial growth factor receptor (VEGFR). Carcinogens in tobacco cause increased TP53 mutations. For oropharyngeal cancer, HPV is the major causative agent with more than 50% showing HPV DNA. HPV – E6 protein inactivates p53, whereas E7

protein by inactivation of Rb gene lead to overexpression of p16. Gene expression profile identifies transcriptional signatures which help in predicting the overall survival and likelihood of nodal metastasis.

Carcinogenic agents in cigarette smoke and tobacco products are mainly benzopyrene and nitrosamines, and same as are aresoline in areca nut. These products cause alteration in genes mainly in 3p, 9p and 17.[70-5]

HIGH-RISK SITES

1. BUCCAL MUCOSA:

Buccal mucosa is the most common location for oral carcinoma among tobacco users . This is probably due to the betal or tobacco quid which has been kept for quite long time in the mandibular vestibule. This further causes readily absorption of the carcinogenic agents and predisposes to cancer.

2. TONGUE:

The tongue is the next common location for oral cancer with more than half of lesions presenting on the *oral tongue*, and the rest occurring in the *tongue base*. In the oral cavity proper, lesions are most commonly seen on the lateral and ventral surfaces, and these areas remain to be the high-risk sites. Tongue base tumours seem to be more

advanced at the time of diagnosis, mostly presenting with metastasis to regional lymph nodes.

3. LIP :

The vermillion of the lip is another site for oral cancer, but the incidence is low. Most of the labial carcinomas occur on the lower lip, more common in men than women. Ultraviolet radiation exposure is the major risk factor. It may also occur in pipe smokers where the pipestem frequently contacts the lip for quite a long period of time. Usually lip cancers are diagnosed early due to its easy visibility.[62,70,72]

INVESTIGATIONS:

The investigatory modalities commonly used in diagnosis of oral cancers are

1. Tissue biopsy and histopathological examination, assisted with or without immunohistochemistry.

2. Exfoliative cytological analysis

Most of the malignancies develop in the setting of precancerous lesions. Precancerous and cancerous oral lesions can mimic a number of benign oral lesions which appear as a white or red lesion

(leukoplakia and erythroplakia). The malignant potential of these lesions is generally assessed by histopathology based on the presence and the degree of dysplasia in biopsy material. The lesions are graded as mild, moderate, and severe .Till now, tissue biopsy and subsequent histological examination remains the gold standard for the diagnosis of premalignant and malignant oral diseases. Oral tissue biopsy is an invasive procedure and involves both psychological implications for the patient and technical difficulties for the health practitioner. Extensive lesions may lead to sampling error. Moreover, there will be inter-observer variation in diagnosing dysplasia and reproducibility in morphological features of low grade dysplasia is poor.

Compared to tissue biopsy, oral cytology technique is simple, noninvasive, relatively painless, and tolerated well by patients. It can be used for diagnosis, identification of recurrence after treatment and also as a prognostic marker following therapy. Moreover, it is also used for mass screening and is reported faster within short duration. The basic requirements for a useful diagnostic technique are as follows

a) easy to use

b) minimal patient discomfort

c) adequate sampling

Ideally, a diagnostic procedure should have the following requisites

i) should not be complicated or time consuming

ii) should have high sensitivity

iii) should have the potential for automation.

The disadvantages are sampling error, inter observer variation and inability to do immunohistochemistry. The sampling error is mainly due to topography and increased size of the oral cavity causing difficulty in evaluating the whole area.[76]

The exfoliative cytology not only demonstrates increased micronuclei frequency, but also many nuclear abnormalities. The common nuclear anomalies noted are as follows

1. Increase in number of binucleated cells indicates failure of cytokinesis. The chromosomal non disjunction is increased in binucleated cells.[77]
2. Cells with nuclear buds or Broken Eggs (BEN) are indicative of elimination of nuclear material by budding. This may be probably related to DNA repair or elimination of amplified DNA.[78-9]
3. Karyolysis is nuclear dissolution. The basophilia will fade due to enzymatic digestion of the nucleus by endonucleases.

4. Pyknosis is nuclear shrinkage and has increased basophilia. This may be a mechanism of nuclear disintegration.
5. Karyorrhexis is nuclear fragmentation with loss of integrity. The nuclei have increased chromatin aggregation and speckled nuclear pattern.
6. Condensed chromatin shows intense basophilia due to aggregated chromatin and has striated nuclear pattern.

WHO classification of tumours of the oral cavity and oropharynx

Malignant epithelial tumours

Squamous cell carcinoma 8070/3

Verrucous carcinoma 8051/3

Basaloid squamous cell carcinoma 8083/3

Papillary squamous cell carcinoma 8052/3

Spindle cell carcinoma 8074/3

Acantholytic squamous cell carcinoma 8075/3

Adenosquamous carcinoma 8560/3

Carcinoma cuniculatum 8051/3

Lymphoepithelial carcinoma 8082/3

Epithelial precursor lesions

Squamous cell hyperplasia

Mild dysplasia

Moderate dysplasia

Severe dysplasia

Carcinoma in-situ

Benign epithelial tumours

Papillomas 8050/0

Squamous cell papilloma and verruca vulgaris

Condyloma acuminatum

Focal epithelial hyperplasia

Granular cell tumour 9580/0

Keratoacanthoma 8071/1

Salivary gland tumours

Salivary gland carcinomas

Acinic cell carcinoma 8550/3

Mucoepidermoid carcinoma 8430/3

Adenoid cystic carcinoma 8200/3

Polymorphous low-grade adenocarcinoma 8525/3

Basal cell adenocarcinoma 8147/3

Epithelial-myoepithelial carcinoma 8562/3

Clear cell carcinoma, not otherwise specified 8310/3

Cystadenocarcinoma 8450/3

Mucinous adenocarcinoma 8480/3

Oncocytic carcinoma 8290/3

Salivary duct carcinoma 8500/3

Myoepithelial carcinoma 8982/3

Carcinoma ex pleomorphic adenoma 8941/3

Salivary gland adenomas

Pleomorphic adenoma 8940/0

Myoepithelioma 8982/0

Basal cell adenoma 8147/0

Canalicular adenoma 8149/0

Duct papilloma 8503/0

Cystadenoma 8440/0

Soft tissue tumours

Kaposi sarcoma 9140/3

Lymphangioma 9170/0

Ectomesenchymal chondromyxoid tumour

Focal oral mucinosis

Congenital granular cell epulis

Haematolymphoid tumours

Diffuse large B-cell lymphoma (DLBCL) 9680/3

Mantle cell lymphoma 9673/3

Follicular lymphoma 9690/3

Extranodal marginal zone B-cell lymphoma of MALT type 9699/3

Burkitt lymphoma 9687/3

T-cell lymphoma (including anaplastic large cell lymphoma 9714/3

Extramedullary plasmacytoma 9734/3

Langerhans cell histiocytosis 9751/1

Extramedullary myeloid sarcoma 9930/3

Follicular dendritic cell sarcoma / tumour 9758/3

Mucosal malignant melanoma 8720/3

Secondary tumours[80]

PREMALIGNANT LESIONS:

WHO distinguishes between oral precancerous lesions and oral precancerous conditions.

A **precancerous lesion** is defined by morphologically altered tissue that is more likely to be transformed into cancer than its normal counterpart, such as leukoplakia, erythroplakia, and the palatal changes associated with reverse smoking of cigarettes.

A **precancerous condition** is a state associated with a significantly increased risk for cancer, such as syphilis, sideropenic dysphagia, and oral submucous fibrosis.

Premalignant lesions are classified by many schemes as follows[80]

LJUBIJANA Classification Squamous Intraepithelial Lesion (SIL)	**Squamous Intraepithelial Neoplasia (SIN)**	**2005 WHO Classification**
Squamous (simple) hyperplasia	Not applicable	Squamous cell hyperplasia
Basal / Parabasal cell hyperplasia	SIN 1	Mild hyperplasia
Atypical hyperplasia	SIN 2	Moderate hyperplasia
Atypical hyperplasia	SIN 3	Severe hyperplasia
Carcinoma in situ	SIN3	Carcinoma in situ

1.ACTINIC CHEILITIS (SAILOR'S LIP; SOLAR CHEILITIS)

This lesion is a form of *actinic keratosis* and occurs on the lower lip. It is directly related to prolonged exposure to sun and is common with ultraviolet B (UVB) irradiation at 290 – 320 nm. It is most common in white males and in fourth decade. The vermilion of the lip appears pale and atrophic with a glossy surface, sometimes with wrinkling and fissuring. Demarcation at the vermilion border is usually lost. Later on, as the lesion progresses, there is fissuring and ulceration, sometimes with crusting or scaling.

Microscopic features include epithelial atrophy, hyperkeratosis and elastosis of the submucosa with lymphocytic infiltration. These changes turn to be irreversible. Risk of malignant transformation is 6 – 10%.Treatment consists of prophylactic laser ablation or vermillionectomy. These patients are also at high risk for other cancers related to sun exposure, and hence close follow up is essential.[81-2]

2.LEUKOPLAKIA:

The term leukoplakia means "white patch," in Greek. World Health Organization defines leukoplakia as a white patch, not less than 5 mm, that cannot be rubbed off easily or clinically identified as another

named entity. Leukoplakia frequently presents over the age of 40 and is common in men. But due to the change in trend with regard to smoking, leukoplakia is on increasing scene in women nowadays. The common etiopathogenesis is related to the use of tobacco in smoked or smokeless forms. Also related etiological factors are alcoholism, infection by Candida albicans , trauma and the last but one is the nutritional deficiency , particularly iron deficiency anaemia leading to sideropenic dysphagia named by the syndrome Plummer Vinson or Paterson Kelly syndrome. Mostly these lesions are benign. The common sites are ventral or lateral tongue and floor of mouth in the past decades which has now switched over to buccal mucosa and mandibular mucosa amounting for atleast half the cases. Less common to involve are the palate, lip and floor of mouth.

Clinically, it is aymptomatic with variable presentation and appearance in regard to size, shape, colour and thickness. The common presentation varies from a relative whiteness on a normal appearing non inflamed mucosa to a well defined white, leathery, thickened , fissured or warty growth. Palpation of the lesion exhibits a soft, smooth consistency to a nodular or indurated lesion. On clinical grounds, leukoplakia is further classified into four types, viz ***homogenous, non homogenous, proliferative verrucous leukoplakia and erythroplakia***. Homogenous

leukoplakia consists of more or less well demarcated uniform white plaque with or without fissuring. Non homogenous variant includes erythroleukoplakia, speckled and nodular types. These lesions have more risk of dysplasia or carcinoma. Erythroleukoplakia is a red and white lesion with fissures and usually well demarcated. This lesion needs to be differentiated from lichen planus, which show more typical reticulations.

However, around 9 to 47% of leukoplakia seems to exhibit malignant transformation with features of dysplasia or frank carcinoma and this tendency varies with the site of the lesion. About 16 – 36% of dysplastic lesions have chance of transforming into frank malignancy. Around 16% of benign lesions without dysplasia will turn to dysplasia or malignancy. Though leukoplakia in the floor of mouth is seen only in few cases, it is more prone for dysplastic changes and carcinoma. Similarly tongue, lip and soft palate also show high degree of malignant transformation. Clinically the mimics of leukoplakia include candidiasis, lichen planus, white spongy naevus and leukoedema.

Histologic changes vary from hyperkeratosis, carcinoma in situ to invasive squamous cell carcinoma. Leukoplakia shows hyperkeratosis, parakeratosis and acanthosis with hyperplastic squamous epithelium. Lichenoid changes are seen with chronic inflammatory infiltration predominantly of lymphocytes in the submucosa. Dysplasia is

noted in thick fissured leukoplakia with loss of maturation and cytologic atypia and these changes may also be seen in ducts of minor salivary glands present at that site. Most of the lesion has coexistent infection with Candida albicans. Verrucous or nodular leukoplakia show verrucous epithelial hyperplasia with bulbous rete ridges, mild to moderate dysplasia and band like lymphocytic infiltration. Erythroleukoplakia shows variable hyperkeratosis, epithelial atrophy, bulbous rete ridges with mild to severe degree of dysplasia and band like lymphocytic infiltration.[83-6]

PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

Proliferative verrucous leukoplakia (PVL) is a rare still specific type of leukoplakia, which is more prone for malignant transformation. This lesion frequently occurs in women than in men and are common in fifth to sixth decade. It usually presents as flat lesion in early stages which later turns to be thick and exophytic. The common sites differ from the conventional leukoplakia and are seen in buccal mucosa and gingiva. The etiology is unknown and relation with tobacco and human papilloma virus are yet been established. The lesion can be multifocal and persistent with high rate of recurrence. The commonest malignant change is of squamous cell carcinoma and verrucous

carcinoma. About 60 – 100% of them have chance of developing into dysplasia or carcinoma. [87]

3.ERYTHROPLAKIA:

The literal meaning of erythroplakia is 'flat red area', in Greek. Clinically erythroplakia presents as a bright red, velvety patch with well defined margins and are usually asymptomatic. Erythroplakia is less common than leukoplakia, but with higher degree of risk for malignancy. The common causative agents are tobacco, alcohol and nutritional deficiency. The common sites of predilection are tongue, floor of mouth and retromolar mucosa. Erythroplakia commonly presents in fifth to seventh decade and is equally distributed in both sexes.

The histologic features show decrease in keratin formation with increase in vascularity imparting red colour to the lesion. They also show dysplastic changes, carcinoma in situ or frank malignant changes. Risk of malignant transformation is about 40 – 50%. Surgical excision is the treatment of choice. Wide local excision with adequate margin clearance is essential since invasion into adjacent epithelium is noted. Hence follow up is essential. Differential diagnosis includes Kaposi's sarcoma, vascular malformation, contact allergic reaction, ecchymosis and psoriasis. [88]

4.TOBACCO POUCH KERATOSIS:

Tobacco pouch keratosis, also known as *snuff dipper's keratosis*, is a specific form of leukoplakia. This lesion is more frequently related to smokeless tobacco. It results from the direct effect of tobacco on the oral mucosa, with predilection for the sites of contact, being the mandibular anterior labial vestibule and the posterior buccal vestibule. The clinical presentation is gray to whitish mucosa with wrinkled appearance and associated pouch-like depression probably due to stretching of the tissue with tobacco quid. As the lesion progresses, the intensity of white colour is increased still more and it becomes leathery and nodular. Adjacent to the lesion, the surrounding gingiva becomes inflamed and retracted. This lesion tends to have relatively lower risk of malignant transformation, as it resolves on cessation of tobacco. Any persistence of ulcer or erythema after discontinuing the tobacco use must be followed up with biopsy and evaluated for malignancy. [62,89]

5.ORAL SUBMUCOUS FIBROSIS:

This lesion is common in many parts of the world with incidence of about 4 per 1000 adults in India. Because of increase in use of pan masala among young adults, around 5 million Indian youth are

getting affected. This lesion is characterized by fibroelastic transformation of the adjacent epithelial tissues resulting in mucosal stiffening and rigidity. Later it leads to fibrotic bands in the buccal mucosa and soft palate leading to difficulty in opening the mouth. The main etiologic factor is betal quid consisting predominantly of areca nut and tobacco. Additional factors include nutritional deficiency and genetic susceptibility. OSF is usually a progressive condition and is irreversible.

Histopathologic features include chronic inflammation in the subepithelial connective tissue consisting predominantly of eosinophils. As the disease progresses, there is decrease in inflammatory cells and vascularity, and increase in infiltration with abundant collagen bundles. Submucosal extension of the lesion manifests as thick band of hyalinized collagen bundles in the subepithelial tissue with replacement of fat. Moreover, similar picture is also noted in the minor salivary glands in the quid exposed area, demonstrating features of chronic inflammation and hyalinized fibrosis of the acinar structures. The overlying epithelium is atrophic. There is increased risk for malignant change accounting for 4 – 13% demonstrating epithelial dysplasia. Treatment includes local infiltration of steroids and lysis of the surgical bands. But, ultimately cessation of tobacco or betal quid usage is the only way to prevent progression to carcinoma, though this may not alter the fibrosis.[68,90-1]

7. ORAL LICHEN PLANUS:

Lichen Planus is a mucocutaneous disorder of idiopathic etiology. It commonly occurs in adults with no gender predilection. This lesion is probably immunologically mediated. The common manifestation is bilateral white patch. Many variants exist including reticular, erosive, papular, plaque and erythematosus forms. Reticular form is more common presenting with the characteristic Wickham's striae which are nothing but white keratotic lines in a lacy pattern. It is frequently seen in buccal mucosa.

The plaque variant mimics leukoplakia presenting in the buccal mucosa and dorsum of tongue. The erosive or ulcerative form manifests as fibrinous plaque with central ulceration. The erythematous variant is an atrophic form with reddish patch and fine white striae. Microscopic features consists of hyperkeratosis, vacuolization in the basal layer with keratinocytes and saw tooth rete ridges . Lymphophagocytic reaction mainly band like lymphocytic infiltration is seen at the junction between epithelium and connective tissue. The characteristic are the Civette bodies, which are eosinophilic ovoid bodies in the basal layer. Also, many Langerhans cells are seen in the epidermis. The risk of malignant transformation is minimal with 0.4 – 2.5% , mostly with erosive form of lichen planus.[70,85]

DYSPLASIA:

Dysplasia refers to abnormal epithelium and disordered growth. Dysplasia is graded into three histologic types and is designated as mild, moderate, and severe dysplasia. The microscopic features of dysplasia are as follows

I. ARCHITECTURAL DISTURBANCE:

(1) Irregular stratification

(2) Basal cell crowding with loss of polarity

(3) Drop-shaped epithelial ridges

(4) Premature keratinization in single cells

(5) Reduced intercellular adhesion

(6) Keratin pearls within rete ridges

(7) Increased mitotic figures

(8) Abnormal superficial mitosis

II. CYTOLOGIC ATYPIA

1. Nuclear pleomorphism and hyperchromatism
2. Anisonucleosis
3. Increased nuclear size
4. Anisocytosis and cellular pleomorphism

5. Increased nucleus to cytoplasmic ratio
6. Abnormal mitotic figures
7. Increased number and size of nucleoli

Mild dysplasia

Mild dysplasia is defined as the architectural disturbance limited to the lower third of the epithelium with associated cytological atypia. It is characterized by basal zone hyperplasia with mild increase in thickness. Cellular crowding is seen only in the lower one third of the epithelium and mitosis is absent. Nuclei show mild degree of pleomorphism.

Moderate dysplasia

Moderate dysplasia relates to architectural disturbance extending into the middle third of the epithelium. Anyhow, it is upgraded in view of increased cytologic atypia. There is moderate increase in thickness with basal zone hyperplasia. Cellular crowding involves the lower two thirds of the epithelium with no loss of polarity. Nuclear grooves and lobulations may be seen with moderate degree of cellular and nuclear pleomorphism. Mitosis is appreciated in the lower one third.

Severe dysplasia

Severe dysplasia involves architectural irregularity and increased cytologic atypia in more than two thirds of the epithelium. Markedly increased thickness with basal zone expansion is noted. There is loss of polarity with mitosis in the lower two thirds of the epithelium. Cells demonstrate high nuclear cytoplasmic ratio, nuclear folding, coarse chromatin and prominent nucleoli with increased pleomorphism.

CARCINOMA IN SITU:

Carcinoma in situ is defined as architectural irregularity and increased cytologic atypia involving full thickness of the epithelium, but invasion is absent. There is epithelial disarray with cellular crowding, loss of polarity and full thickness atypia. Marked degree of cellular and nuclear pleomorphism is noted and mitotic figures are seen throughout the epithelium. No flattening of surface layer is seen. The basement membrane is intact, but may have thinning when seen with immunostains. [80,92]

SQUAMOUS CELL CARCINOMA:

Squamous cell carcinoma is the most common malignancy of the oral cavity. Almost 90% of the oral tumours come under this

category. In India, tumours tend to arise in the vicinity of leukoplakia, whereas in western countries, red lesion or normal epithelium is much more common. SCC is characterized by lobules of squamous cells with cytologic pleomorphism and abundant keratinization. The malignant epithelial cells proliferate and invade the stroma singly or as islands or cords. There is increase in nuclear cytolopasmic ratio with increased apoptotic bodies. Mitosis is increased with many abnormal mitotic figures. Variable amounts of desmoplasia and inflammatory infiltration with lymphocytes and eosinophils are seen. Perineural and vascular invasion is noted. The adjacent epithelium shows dysplastic changes or features of carcinoma in situ.

Squamous cell carcinoma is graded based on the degree of differentiation of the epithelium, nuclear pleomorphism and mitotic activity. SCC is usually graded into three categories viz., well differentiated, moderately differentiated and poorly differentiated grade.

Well differentiated SCC:

In this type, the tumour resembles normal squamous epithelium and consists of large differentiated keratinocyte like squamous cells with the periphery of the tumour having small basal type cells. Keratinisation is present throughout. The characteristic is the presence of intercellular bridges. Only few mitoses are noted.

Moderately differentiated SCC:

In this type of SCC, there is increase in nuclear pleomorphism and keratinization is reduced. Mitosis is increased with many abnormal mitotic figures.

Poorly differentiated SCC:

Here the basal type cells predominate. Mitoses are numerous with increased abnormal mitotic figures. The intercellular bridges are barely recognized and keratinization is usually minimal.[68,70,72,80,92]

VARIANTS OF SQUAMOUS CELL CARCINOMA

There are many variants of squamous cell carcinoma. It is important to distinguish them as they differ in their prognosis. Only few tumours present entirely of their classical features. Most of them occur in combination with conventional SCC presenting as mixed tumours. However, it is better to mention the histological variation, which would be possible for the clinician to delineate the tumours with aggressive behavior or poor prognosis.

VERRUCOUS CARCINOMA:

About 5% of the oral carcinomas belong to this category. Initially this term was first used to describe a large exophytic , warty, acanthotic lesion with mild cytologic atypia. Friedell and Rosenthal in

1941 was the first to demonstrate verrucous carcinoma of oral cavity, which was later well established by Ackerman in 1948 as a non metastazing, but locally invasive SCC. This is commonly seen in men over the age of 70 years. Verrucous carcinoma has predilection for buccal mucosa, alveolar ridge and mandibular sulcus with occurrence in other sites like gingiva, tongue, soft palate and tonsillar fossa. This tumour has strong association with tobacco in both smokers and smokeless tobacco users. The clinical manifestation is that of an exophytic growth with warty or papillary surface.

The histologic features are the epithelium showing marked acanthosis, marked parakeratosis and hyperkeratosis. There is papillary exophytic and endophytic proliferation of well differentiated squamous epithelium. The bulbous frond like rete ridges push downward into the submucosa with parakeratin plugging. Only minimal cytologic atypia is noted. Lamina propria consists of chronic inflammatory cell infiltration with lymphocytes and subbasal clefting. The prognosis is comparatively better. [92-98]

BASALOID SQUAMOUS CELL CARCINOMA:

This is a rare variant of squamous cell carcinoma. Basaloid Squamous cell carcinoma was first described by Wain et al in 1986. The common sites involved intraorally are tonsils and base of tongue. BSCC

has a high male to female sex ratio, predominant in smokers and alcoholics and are common over the age of 60 years. BSCC in oral cavity seems to be associated with HPV 16. It is a highly aggressive tumour , mostly presenting in Stage III or IV with regional metastasis.

Microscopically, BSCC is classically arranged in nests, lobules and cribriform pattern with basaloid cells having squamous differentiation in the nests. The cells are small, with scant cytoplasm, indistinct cell borders and dark nuclei exhibiting pleomorphism. Mitotic activity is increased. Admixed with them are larger cells having abundant cytoplasm, vesicular nuclei with small nucleoli. Nuclear palisading may be seen in the periphery of the nests. The characteristic is the presence of necrosis with both single cell and comedonecrosis. Some of them may have pseudoglandular pattern filled with hyaline or mucoid material which is PAS or Alcian blue positive. The stroma may be hyalinized or myxoid . BSCC is frequently seen in combination with conventional SCC and squamous CIS. BSCC has to be differentiated from basaloid lesions like salivary duct carcinoma, solid variant of adenoid cystic carcinoma and peripheral ameloblastoma. BSCC commonly metastasize to the lungs. The three year survival rate is only 35%.[94-7,99]

PAPILLARY SQUAMOUS CELL CARCINOMA:

Papillary carcinoma is commonly seen in larynx and hypopharynx, but is uncommon in the oral cavity. This presents as an exophytic, cauliflower like mass with broad base or as finger like papillary projections with slender fibrovascular core. Microscopically it consists of non keratinizing epithelial proliferation in papillary exophytic or endophytic pattern with considerable cytological atypia, paraorthokeratosis and often microabscess at the tips of bulbous rete ridges. Hyperkeratosis is minimal and stromal invasion is not well defined.

Depending on the maturation of the overlying epithelium, two patterns of proliferations are identified. One type is undifferentiated variant with close resemblance to small cell CIS, and consists of non keratinizing basal and parabasal like cells with dysplastic features. The proliferation is seen in the entire thickness of the epithelium. This variant is frequently seen in tonsils and oropharynx. The next variant comprises of varying degrees of keratinization with dysplastic changes in the epithelium. PSCC should be differentiated from verrucous carcinoma, exophytic conventional SCC and squamous papilloma. The prognosis is only 44% and most of them die within 2 years. [95-7,100]

SPINDLE CELL (SARCOMATOID) CARCINOMA:

Spindle cell squamous carcinoma is commonly seen in the head and neck region, but is rare in the intraoral site, presenting in the tongue, lower lip, gingiva and alveolar ridge. Spindle Cell Carcinoma is common among males in their sixties. The tumour presents as red lesions, non healing ulcer associated with pain or as exophytic bosselated mass . The etiological factors include tobacco use, previous radiation, poor oral hygiene and alcohol abuse. It is a spindle cell tumour and consists predominantly of sheets of spindled pleomorphic cells. This tumour may resemble malignant fibrous histiocytoma and hence needs to be differentiated from other soft tissue sarcomas. However, the spindle cell component is admixed with conventional squamous cell carcinomatous areas. The metastatic deposits of the sarcomatoid carcinoma show pure carcinomatous or mixed components in lymph node and distant sites. [95-7,101]

ADENOID / ACANTHOLYTIC SQUAMOUS CELL CARCINOMA

In this variant, the tumour show pseudoglandular or alveolar architecture. This tumour is more common in men. The usual site involved is the lip, but also seen in tongue and gingiva. The main etiological factor is irradiation. The tumour is arranged in biphasic pattern

with proliferation of malignant epithelial cells associated with acantholysis and formation of pseudoglandular structures. Here, there is loss of intercellular adhesion within the tumor cell nests creating a glandular pattern. The tumour is more aggressive with poor prognosis. [95-7,102]

ADENOSQUAMOUS CARCINOMA:

This is a rare tumour which exhibits both squamous and glandular differentiation. The glandular differentiation is thought to have arisen from minor salivary glands. The involvement of the salivary gland ducts in these tumors support the hypothesis, but is still controversial. The currently favoured explanation is the derivation from surface epithelium. The main etiological factor is proposed to be tobacco and alcohol use, but is not confirmed. The sites involved are floor of mouth, tongue, tonsil, palate and larynx. This tumour is common in males in their sixties.

The tumour is biphasic with proliferation of malignant squamous and basaloid epithelial cells admixed with duct like structures having mucous cells. This neoplasm has to be differentiated from mucoepidermoid carcinoma and conventional SCC invading the normal salivary gland. These tumors have an aggressive course with poor prognosis. There is increased tendency for local recurrence (45%) and

early nodal metastasis (65%). The five year survival rate is 13% and at 10 years it is only 4.5%. The presenting feature is erythroplakia with ulcer or submucosal nodule.

Microscopically adenosquamous carcinoma is characterized by three distinct components a) squamous cell carcinoma b) adenocarcinoma c) admixture of glandular mucous cells with squamoid differentiation which resemble mucoepidermoid carcinoma. Many densely keratinized glassy cells are seen. Adjacent areas show foci of dysplasia or carcinoma in situ. There is widespread and extensive permeation into adjacent soft tissues. Perineural invasion is also noted. [95-7,103]

SMALL CELL CARCINOMA:

Small cell carcinoma has histologic features akin to lung carcinoma. This is an aggressive tumour. The tumour consists purely of small cells or has an admixture of squamous component. Few of them have Merkel cell carcinoma features.[94-7,104]

LYMPHOEPITHELIOMA LIKE CARCINOMA:

This is a rare tumour of oral cavity. The appearance is similar to that of the tumour found elsewhere in head and neck like nasopharynx and tonsil.[80,95,105]

CLEAR CELL CARCINOMA:

This is a rare variant of squamous cell carcinoma.[95,106]

NUT (MIDLINE) CARCINOMA:

This is a newly recognized type involving midline structures, mainly in the head and neck region. This is frequently seen in children and young adults, but affects all age groups. It is characterized by rearrangement of the *NUT* gene on chromosome 15q14. The tumour consists of dual population of cells composed of islands of undifferentiated cells and islands of keratinization. There is a sharp distinction between these two regions. The diagnosis is confirmed immunohistochemically by nuclear expression of NUT in undifferentiated cells. NUT carcinoma has an aggressive course, but a very good response to chemotherapy. [80,95]

CUNICULATE CARCINOMA:

This is a rare variant seen commonly on plantar aspect of foot and skin lesions. In oral cavity, this lesion is seen in gingiva and alveolar ridge and most of them have intraosseous extension. It has an indolent course. Histologically, the tumour is characterized by proliferation of stratified squamous epithelial cells in trabecular or ribbon

like pattern with complex arborizing architecture and variable cytologic atypia. Many convoluted irregular cysts or crypts filled with keratin which may burrow into bone are noted. Obvious cytological malignant features are not seen. This tumour has to be differentiated from verrucous carcinoma. They usually do not metastasize. [80,107]

IMMUNOHISTOCHEMISTRY:

Premalignant lesions:

Keratosis without dysplasia : show keratin 19, epidermal growth factor and Ki-67 expression limited to basal layer.

Keratosis with dysplasia : show keratin 19, epidermal growth factor, $p16^{INK4A}$ and Ki-67 expression extending to suprabasal cells.

Ki-67 is the most important marker helpful in identification and grading of dysplasia in premalignant lesions. Few cases of dysplasia show p53 overexpression.

Squamous cell carcinoma:

Cytokeratin : CK5/6, CK8, and CK19 positive, but are CK20 negative

- Overexpression of *TP53* oncogene is seen in 30% to 50% cases.

Basaloid squamous cell carcinoma show immunoreactivity for high molecular weight keratin (detected with the 34ßE12 antibody)

Adenosquamous carcinoma show positivity for CEA, CAM 5.2 and CK7.[80,95-7]

CANCER STAGING:

Squamous cell carcinoma is graded according to TNM staging of tumours by AJCC. The tumour staging is of important prognostic significance and aids in treating the patient.

2002 AJCC Staging Guidelines for Tumors of the Oral Cavity

PRIMARY TUMOR (T)

Primary tumor cannot be assessed (TX)

No evidence of primary tumor (T0)

Carcinoma in situ (Tis)

Tumor ≤ 2 cm in greatest dimension (T1)

Tumor > 2 cm but not > 4 cm in greatest dimension (T2)

Tumor > 4 cm in greatest dimension (T3)

Tumor invades adjacent structures (i.e., through cortical bone, into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face) (T4a)

Tumor invades masticator space, pterygoid plates, or skull base and/or encases internal carotid artery (T4b)

(Lip) Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face, i.e., chin or nose (T4)

REGIONAL LYMPH NODES (N)

Regional lymph nodes cannot be assessed (NX)

No regional lymph node metastasis (N0)

Metastasis in a single ipsilateral lymph node, ≤ 3 cm in greatest dimension (N1)

Metastasis in a single ipsilateral lymph node, > 3 cm but not > 6 cm in greatest dimension (N2a)

Metastasis in multiple ipsilateral lymph nodes, none > 6 cm in greatest dimension (N2b)

Metastasis in bilateral or contralateral lymph nodes, none > 6 cm in greatest dimension (N2c)

Metastasis in a lymph node > 6 cm in greatest dimension (N3)

DISTANT METASTASIS (M)

Distant metastasis cannot be assessed (MX)

No distant metastasis (M0)

Distant metastases (M1)

STAGE GROUPING

The overall pathologic AJCC stage is

Tis/N0/M0 (Stage 0)

T1/N0/M0 (Stage I)

T2/N0/M0 (Stage II)

T3/N0/M0 (Stage III)

T1/N1/M0 (Stage III)

T2/N1/M0 (Stage III)

T3/N1/M0 (Stage III)

T4a/N0/M0 (Stage IVA)

T4a/N1/M0 (Stage IVA)

T1/N2/M0 (Stage IVA)

T2/N2/M0 (Stage IVA)

T3/N2/M0 (Stage IVA)

T4a/N2/M0 (Stage IVA)

T4b/Any N/M0 (Stage IVB)

Any T/N3/M0 (Stage IVB)

Any T/Any N/M1(Stage IVC)[80,108]

PROGNOSIS:

The most important prognostic indicators of oral cancers are as follows

1. Location: The overall five year survival rates vary depending upon the site of tumour. Lip has a good prognosis with 90% survival rate for tumors of lower lip. Next is the anterior tongue with 60% survival rate and floor of the mouth, posterior tongue, tonsil, hard palate and gingiva having 40%. The poor prognostic tumors are those in the soft palate with only 20- 30% survival rate.

2. Tumour stage: This is the most important prognostic index. The five year survival rates without recurrence for various stages are as follows : stage I - 91%, stage II - 77.2%, stage III - 61.2%, stage IV A - 32.4% , stage IV B - 25.3% and for stage IV C -3.6%

3. Grade: This is a separate prognostic marker. The grading of deep invasive margins in a tumour proves to be more useful than that of entire tumour.

4. Tumour size: It has nothing to do with prognosis except for small neoplasms.

5. Depth of invasion: This play a role in grading at selected sites. Tumour thickness correlates well with recurrence, lymphnode metastasis and survival rate.

Tumour < 3 mm has 8% subclinical metastasis, no recurrence and good survival rate. Tumour > 9 mm has 53% metastasis with recurrence 24% and a 5 year survival rate of 66%.

6. Tissue eosinophilia: Dense infiltration of eosinophils in the tumour is a better prognostic factor.

7. Desmoplasia: In squamous cell carcinoma of lip, presence of abundant desmoplastic reaction is considered as worse prognosis with increased chance of metastasis.

8. Lymph node metastasis: This is an important factor in staging and hence of worst prognosis. The prognosis still more decrease with extracapsular spread.

9. DNA ploidy: DNA ploidy has much correlation with microscopic grading. Most of the oral cancers are polyploid or aneuploid. The nondiploid tumors have worse prognosis compared with that of diploid tumours.

10. HPV status: This is considered as an independent and most significant prognostic implicator. The tumors associated with HPV expression have better prognosis.

11. P21 expression: P21 overexpression is considered as worst prognostic factor in squamous cell carcinoma of tongue.

12. P16: p16 expression is found to be a good prognostic indicator. The 5 year survival rate is 80% for p16 positive cases and 40 – 50% for negative cases.

13. H antigen: Loss of expression of H blood group antigen is found to have more chance of invasion and distant metastasis.

14. TROP2: TROP2 is human trophoblast cell surface antigen, whose overexpression decreases the survival rate.

15. 3q26.3 locus: This genetic locus amplification causes progression of tumour and decreased survival rate.[80,94-7]

TREATMENT:

The main stay of treatment for oral carcinoma is surgery and radiation therapy, which are used either alone or in combination. Surgery and irradiation hold good in early stage lesions. More advanced cases are treated with a combination of radiotherapy and chemotherapy. Tumours without TP53 expression respond well to radiation due to the high proliferation index compared to those with TP53 expression and low Ki-67 index.[62,95-6]

MATERIALS AND METHODS

MATERIALS AND METHODS

- This is a case control study carried over a period of 1 year from July 2013 to July 2014.
- The study was conducted on patients of Coimbatore Medical College.
- The age of the patients range from 20 to 65 years.
- They are randomly selected from Dental , ENT and Surgical Oncology OPD
- The study sample consists of 80 subjects and is divided into four groups as follows:

Group 1: Control group consists of 20 healthy subjects, who are non tobacco users, with clinically normal oral mucosa .

Group 2: Consists of 20 healthy subjects, who are tobacco chewers, with clinically normal oral mucosa.

Group 3: Consists of 20 subjects, who are tobacco chewers, with oral premalignant lesions.

Group 4: Consists of 20 subjects, who are tobacco chewers, with oral malignant lesions.

Exclusion criteria:

1. Smokers
2. Alcoholics
3. Patients undergoing radiotherapy in head and neck region.
4. Patients previously diagnosed and on treatment for oral premalignant and malignant lesions.
5. Patients with cancer other than in oral cavity.

Sample Collection:

Oral exfoliative cytology is used for mass screening. The sensitivity is about 94% and specificity of 100%. Exfoliative cytology of buccal cells is collected by various methods like wooden tongue depressor, toothpicks, metal spatula, toothbrushes and cytobrush.

Before sample collection, written and informed consent is obtained from the patient. The patient is enquired about the lifestyle habits including smoking, alcohol and tobacco habits, duration of the habits, frequency of usage, any complaints, investigations taken and finally about treatment and medication details.

Immediately before cell collection, the participants are instructed to rinse their mouth twice with tap water. Subsequently, the cells are scraped with wooden spatulas from each cheek, smeared on precleaned slides and smears are fixed with 95% isopropyl alcohol.

Staining Procedures:

A variety of stains have been employed in assessment of micronuclei. Some of them are Fuelgen, acridine orange, DAPI (4',6 – diaminido-2-phenylindole), Papanicolaou, May Grunwald Giemsa, Giemsa, Crystal violet and propidium iodide stains. In this study, the smears are separately stained with Papanicolaou, Giemsa and Crystal violet stains.

REAGENTS REQUIRED:

1. OG 6
2. EA-36
3. Harri's Haematoxylin

} Papanicolaou stain

4. Giemsa solution
5. Crystal violet powder

The procedures involved in the different staining techniques are as follows

PAPANICOLAOU'S STAINING METHOD:

FIXATION

95% Isopropyl Alcohol - 30 min

PROCEDURE

1. Place in 80% Alcohol – 1 min
2. Dip in 70% Alcohol – 1 min
3. Dip in 50% Alcohol – 1 min
4. Wash in Tap Water - 10 min
5. Immerse in Harri's Haematoxylin solution – 5 min
6. Rinse in tap water gently and briefly
7. Quick differentiation with 1% Acid alcohol.
8. Wash in Tap water (blueing) -10 min
9. Dip in 70% Alcohol - 5 min
10. Dip in 90% Alcohol - 5 min
11. Place in OG II Solution - 2 min (monochromate solution)
12. Dip in 95% Alcohol - 1 min
13. Dip in 95% Alcohol - 1 min
14. Dip in 95% Alcohol - 1 min

15. Place in EA 50 solution - 4 min (polychromate solution)

16. Dip in 95% Alcohol - 1 min

17. Dip in 95% Alcohol - 1 min

18. Dip in 95% Alcohol - 1 min

19. Place in Xylene : Alcohol (50:50) mixture – 5 min

20. Clear in Xylene - 10 min

21. Mount in DPX

Papanicolaou staining method is named after George Papanicolaou and is commonly used in cytopathology. The pap stain gives good visualization of the exfoliated epithelial cells. It is a polychrome stain which demonstrates variations in cellular morphology including cellular maturity and metabolic activity.

The advantages are differential cytoplasmic counterstaining and transparency with well stained nuclear chromatin. The nuclei are stained blue, cytoplasm of keratinized squamous cells are orange or pink and those of non keratinized cells are stained blue or green.[109-11]

GIEMSA STAINING TECHNIQUE

FIXATION:

Isopropyl Alcohol - 30 minutes.

Preparation of Working Solution:

Giemsa Stock solution - 1 ml

Distilled water - 9 ml (1: 9 ratio)

PROCEDURE:

1. Wash in distilled water - 15 dips
2. Place in Giemsa working solution for 2 hours
3. A quick dip in 1% acetic acid
4. Slides are blotted dry with filter paper
5. Differentiate with 100% ethyl alcohol until there is only a slight bluish tint to alcohol
6. Clear in xylene 10 dips – two changes.
7. Mount with DPX.

Giemsa is a Romanowsky polychromatic stain. The cells are stained purple in colour. It is one among the commonly used cytological stains.[109-10]

CRYSTAL VIOLET STAINING METHOD

FIXATION:

Fix in Isopropyl Alcohol - 30 minutes.

Preparation of working solution: (1% crystal violet solution)

Crystal violet powder - 1 gm

Distilled water - 100 ml

PROCEDURE:

1. Stain with 1% crystal violet working solution for 1 minute
2. Blot with filter paper
3. Clear in two changes of xylene
4. Mount with DPX medium.

Mitotic cells are stained magenta and stand out distinctly against a light blue background of resting cells.

Crystal violet is a basic dye and has more affinity for the highly acidic chromatin of mitotic cells. It is used to demonstrate high mitotic counts. The major advantage is quick staining method and easy identification of mitotic figures at a lower magnification compared to H/E-stained section (Ankle et al., 2007).[112]

IDENTIFICATION OF MICRONUCLEUS:

Many factors influence the assessment of micronuclei which are as follows

1. Timing and implements used in collection of cells

2. Fixation and staining methods

3. Selection and number of cells counted

4. Scoring criteria adopted

5. Associated nuclear anomalies

6. Presence of other cellular structures like bacteria and keratohyaline globules.

SCORING CRITERIA:

The criteria for selecting cells with micronuclei as provided by **Tolbert et al** is as follows:

1. Intact cytoplasm and relatively flat cell position
2. Little or no overlap with adjacent cells
3. Little or no debris
4. Nucleus normal and intact, nuclear perimeter smooth and distinct.

The recommended criteria for the identification of micronucleus is

1. Rounded smooth perimeter
2. Less than one third the diameter of the associated nucleus
3. Staining intensity similar to that of the nucleus
4. Texture similar to that of nucleus
5. Same focal plane as nucleus
6. Absence of overlap with, or bridge to, the nucleus.[25]

In this study, cellular evaluation is performed using optic microscope with 100X magnification. 500 cells per smear are counted in zigzag method. The presence of micronucleus in all subjects and the number of cells showing micronuclei are calculated. The mean number of micronuclei in nuclei can also be determined using the following formula

$$\frac{\text{The total number of micronuclei in each cell}}{\text{The number of cells with micronucleus}} \quad X \ 100$$

Statistical analysis:

Data obtained was coded and entered into Microsoft excel spread sheet (Annexure II). The data was analysed using ratio and percentage. Continuous data was expressed as mean and median. Correlation between the micronuclei frequency in the four study population was calculated using Annova test.

OBSERVATION AND

RESULTS

OBSERVATIONS AND RESULTS

The present study was a case control study conducted in the Department of Pathology, Coimbatore Medical College Hospital. A total of 80 cases of buccal smear were taken over the period from July 2013 to July 2014 and cytological examination was done.

Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

The micronuclei frequency in oral exfoliative cytology is evaluated in precancerous and cancerous oral conditions in tobacco chewers and are compared with that of apparently healthy tobacco chewers and normal persons.

TABLE 3. INCIDENCE IN DIFFERENT AGE GROUPS.

AGE GROUP (Age in Years)	MALIGNANCY	PREMALIGNANT LESIONS	HEALTHY TOBACCO CHEWERS	CONTROLS
<40	10%	20%	40%	15%
41-50	20%	35%	30%	25%
51-60	20%	25%	10%	20%
61-70	35%	15%	10%	25%
>70	15%	5%	10%	15%

In the present study, the incidence of malignant cases seems to be higher over the age of 50 years. Comparitively, healthy tobacco chewers and premalignant cases are higher in less than 50 years of age.

CHART 1: INCIDENCE IN DIFFERENT AGE GROUPS

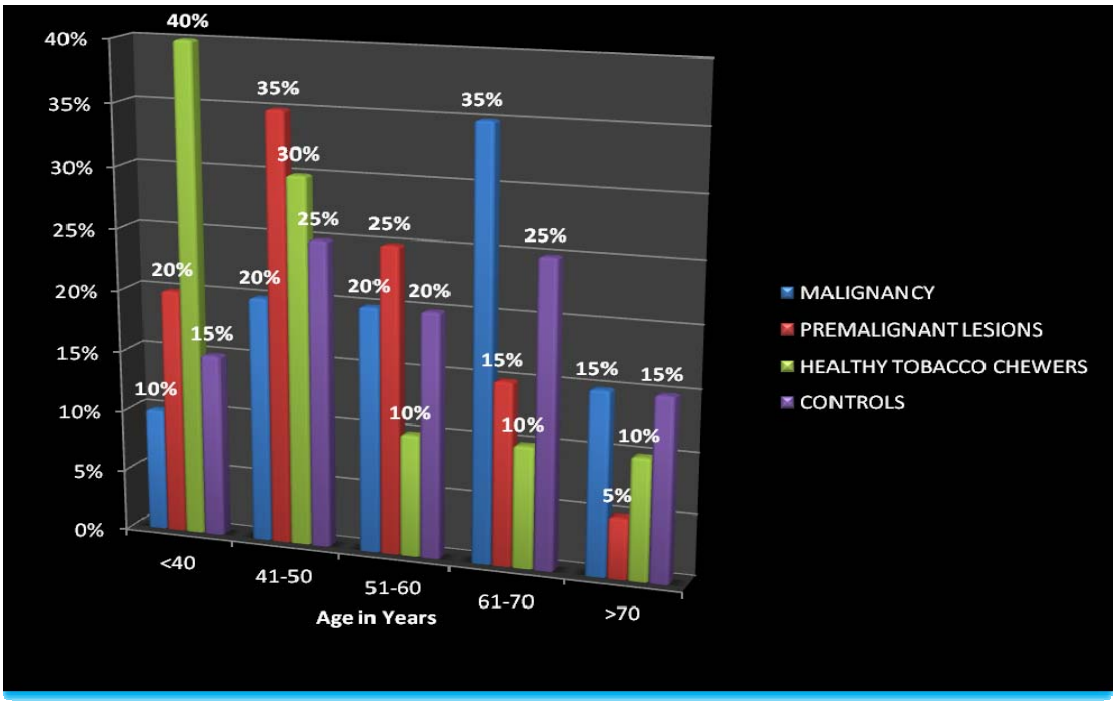


TABLE 4. DIFFERENCE IN GENDER DISTRIBUTION

	MALE	**%**	**FEMALE**	**%**
MALIGNANCY	7	35%	13	65%
PREMALIGNANT LESIONS	8	40%	12	60%
HEALTHY TOBACCO CHEWERS	7	35%	13	65%
CONTROLS	8	40%	12	60%

In the current study, the study population show female preponderance. This is probably a selection bias as the three study groups are selected on exclusion of smoking, alcoholism and other cancers. The male to female ratio is observed to be 1:2.

CHART 2. DIFFERENCE IN GENDER DISTRIBUTION

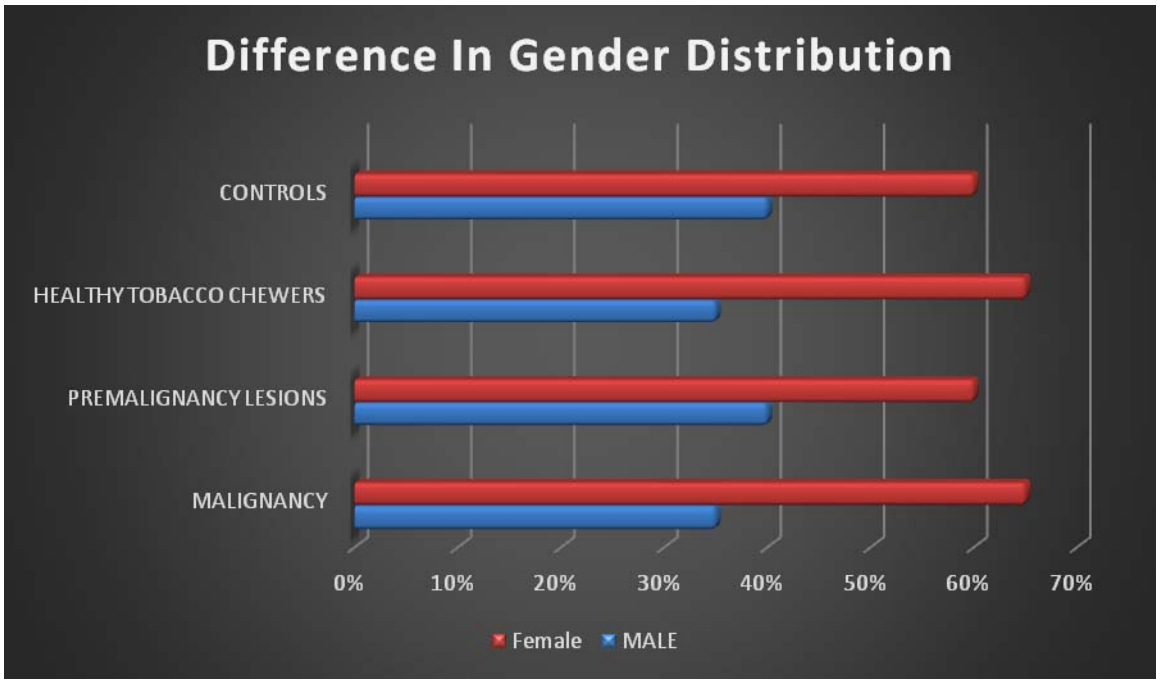


CHART 3. AGE AND SEX DISTRIBUTION IN VARIOUS STUDY GROUPS

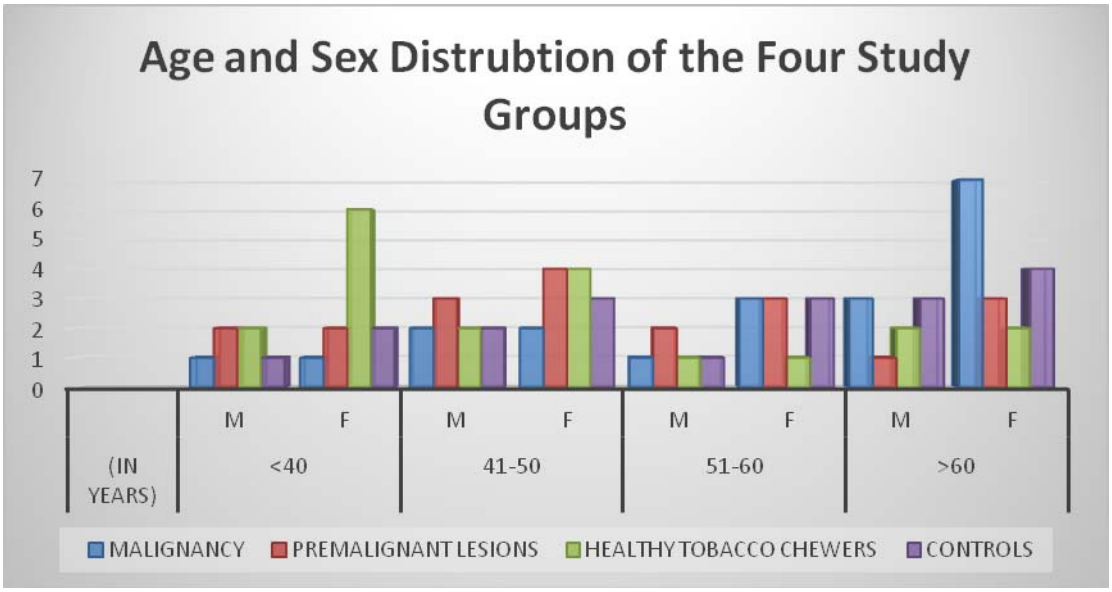


TABLE 5. INCIDENCE IN VARIOUS SITES OF THE LESIONS

SITE	MALIGNANCY	Percentage	PREMALIGNANT LESIONS	Percentage
BUCCAL MUCOSA	10	50%	12	60%
TONGUE	6	30%	8	40%
LIP	1	5%		
FLOOR OF MOUTH	1	5%		
HARD PALATE	1	5%		
SOFT PALATE	1	5%		

In the present study, about half of the malignant and premalignant lesions are seen in the buccal mucosa, the commenest site where the quid is placed. Next comes the tongue carcinoma with 30% occurrence. Other sites like lip, floor of mouth, hard and soft palate each share a single case.

CHART 4. DIFFERENCE IN INCIDENCE OF VARIOUS SITES

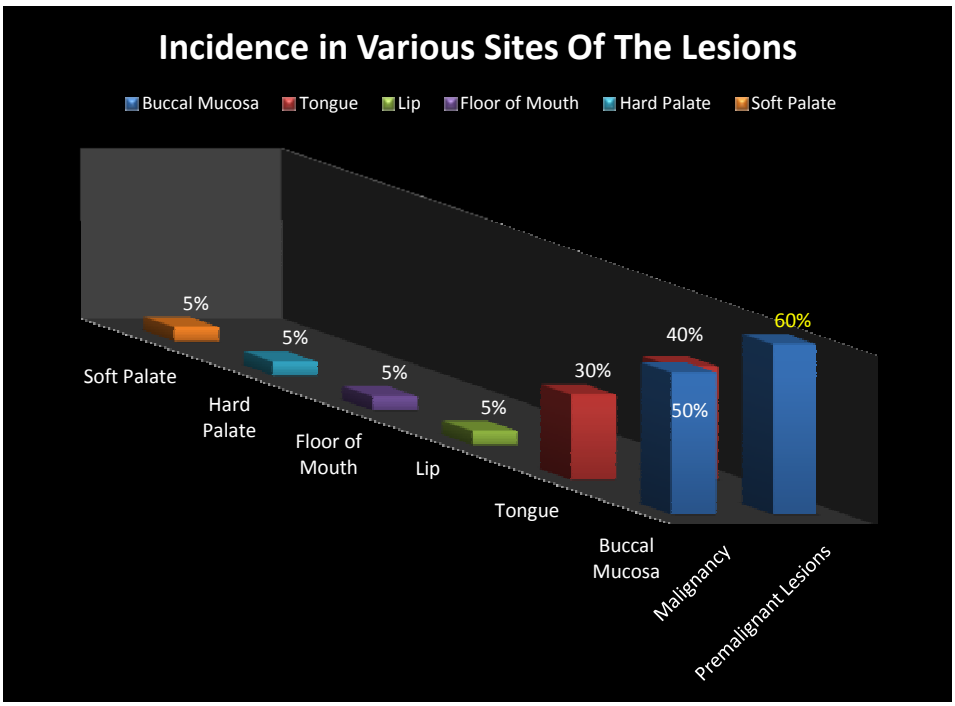


CHART 5. FREQUENCY OF MN /500 CELLS IN NORMAL CONTROLS

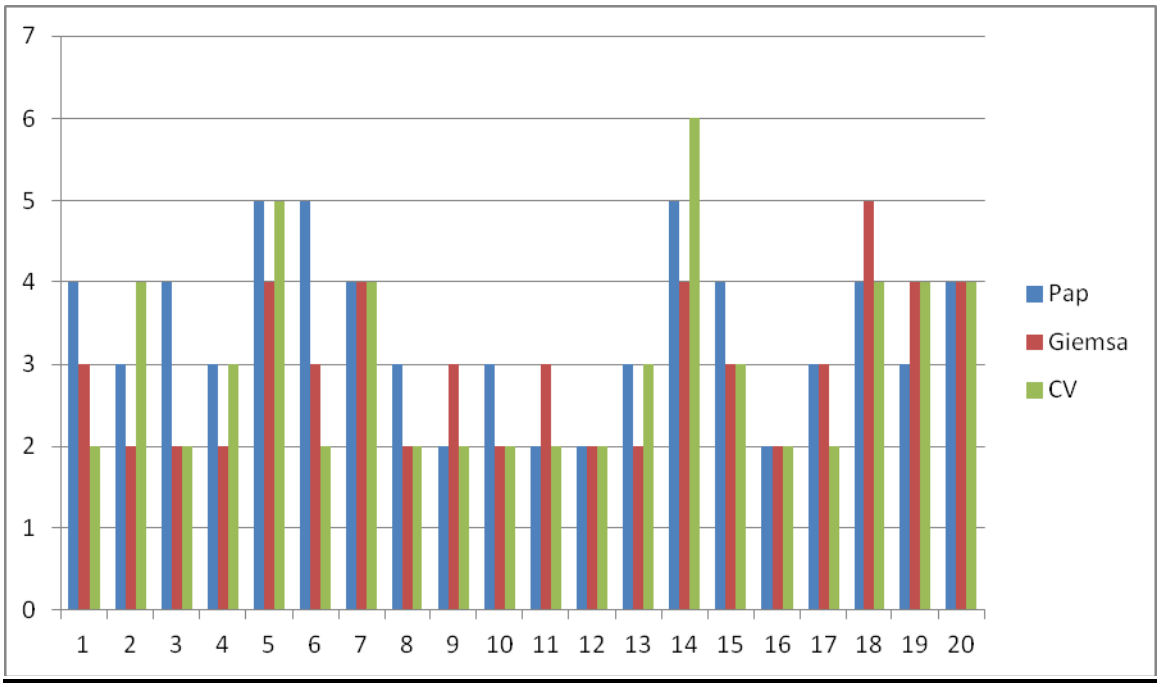


Table 6 : One-Sample Statistics for Normal Controls (n = 20)

	N	Mean	Std. Deviation	Std. Error Mean
PAP	20	3.40	.995	.222
GIEMSA	20	2.95	.945	.211
CRYSTAL VIOLET	20	3.00	1.214	.271

The mean number of micronuclei observed in the controls and the difference in the three staining methods are shown in the above table.

CHART 6. FREQUENCY OF MN /500 CELLS IN HEALTHY TOBACCO CHEWERS

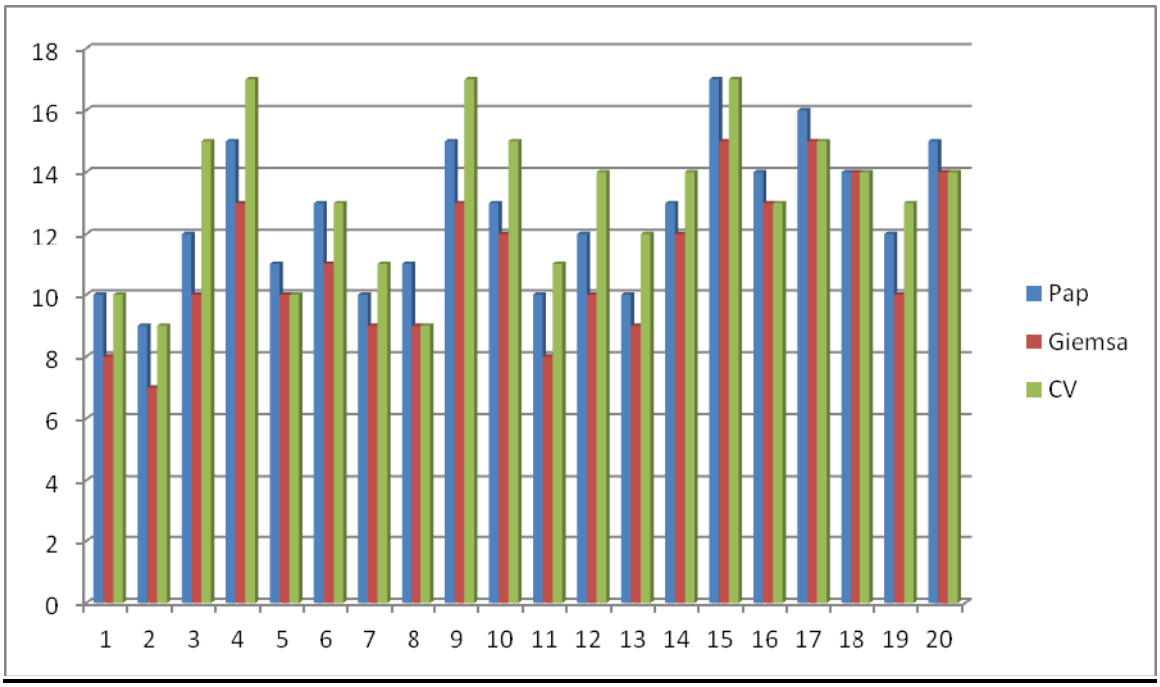


Table 7 : One-Sample Statistics for Healthy Tobacco Chewers (n=20)

	N	Mean	Std. Deviation	Std. Error Mean
PAP	20	12.60	2.280	.510
GIEMSA	20	11.10	2.447	.547
CRYSTAL VIOLET	20	13.15	2.540	.568

The mean number of micronuclei observed in the tobacco chewers with normal oral mucosa and the difference in the three staining methods are shown in the above table.

CHART 7. FREQUENCY OF MN /500 CELLS IN PREMALIGNANT LESIONS

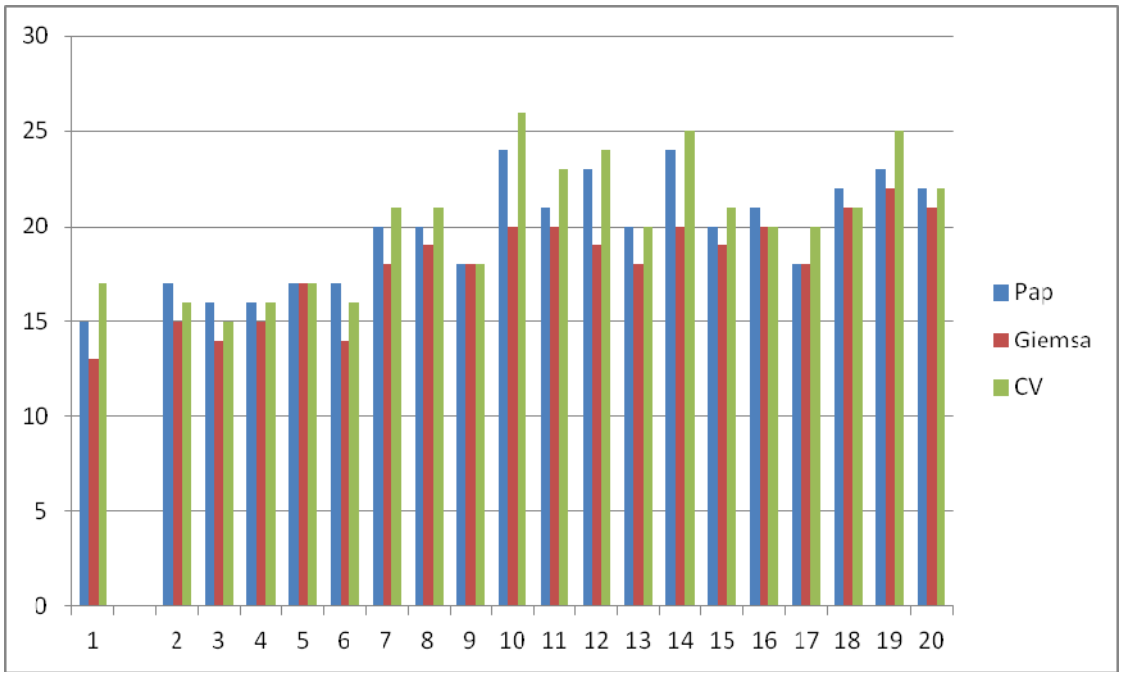


Table 8 : One-Sample Statistics for Premalignant Lesions (n = 20)

	N	Mean	Std. Deviation	Std. Error Mean
PAP	20	20.20	2.881	.644
GIEMSA	20	19.75	3.831	.856
CRYSTAL VIOLET	20	26.60	3.350	.749

The mean number of micronuclei observed in tobacco chewers with premalignant lesions and the difference in the three staining methods are presented in the above table.

CHART 8. FREQUENCY OF MN /500 CELLS IN MALIGNANT LESIONS

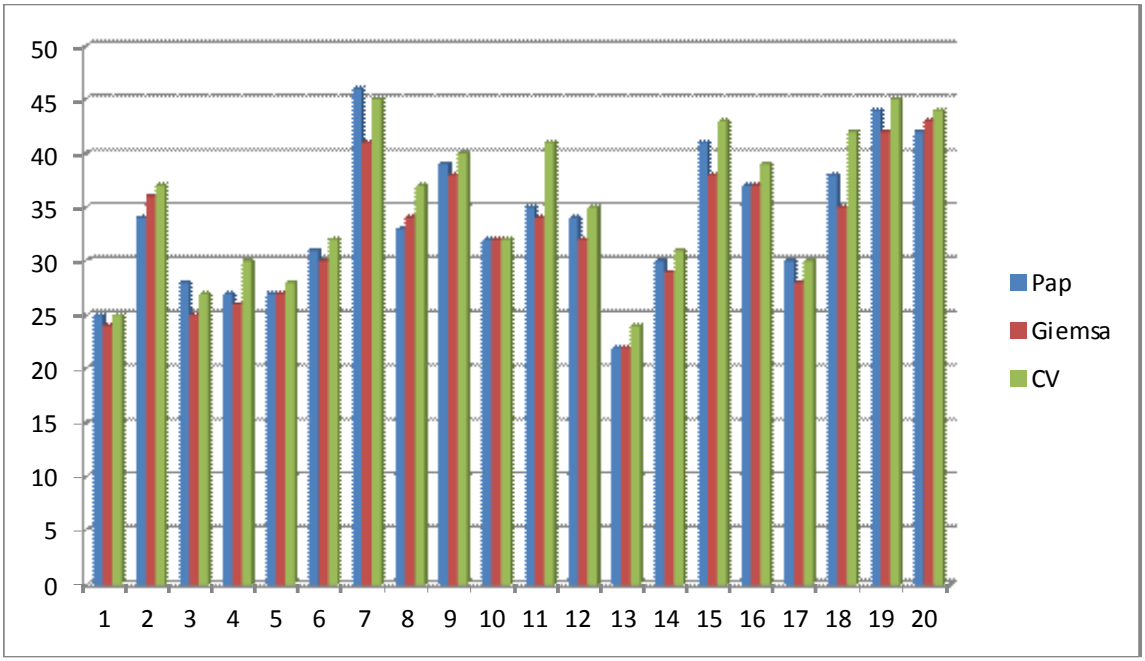


Table 9 :One-Sample Statistics for Malignant Lesions (n = 20)

	N	Mean	Std. Deviation	Std. Error Mean
PAP	20	33.75	6.536	1.462
GIEMSA	20	32.65	6.184	1.383
CRYSTAL VIOLET	20	35.35	6.862	1.534

The mean number of micronuclei observed in tobacco chewers with carcinoma and the difference in the three staining methods are presented in the above table.

TABLE 10 .Comparison of Mn/500cells in Various Study Groups Using Different Staining Techniques

STUDY GROUP	PAP	GIEMSA	CRYSTAL VIOLET
Controls	3.40	2.95	3.00
Healthy tobacco chewers	12.60	11.10	13.15
Premalignant	20.20	19.75	26.60
Malignancy	33.75	32.65	35.35

In the current study, the micronuclei frequency is studied in four different populations of malignancy, premalignancy, healthy tobacco chewers and controls without tobacco habit. The micronuclei frequency is assessed by various staining procedures like papanicolaou, Giemsa and crystal violet stains and their staining quality are compared. The MN frequency is found to be slightly higher with Pap stain compared to Giemsa, and still more higher with crystal violet stain. It is observed that there is no significant difference seen in evaluating micronuclei in the three staining techniques, as only mild variation in the values noted with $p > 0.05$.

CHART 9. Comparison of MN/500cells in Various Study Groups Using Different Staining Techniques

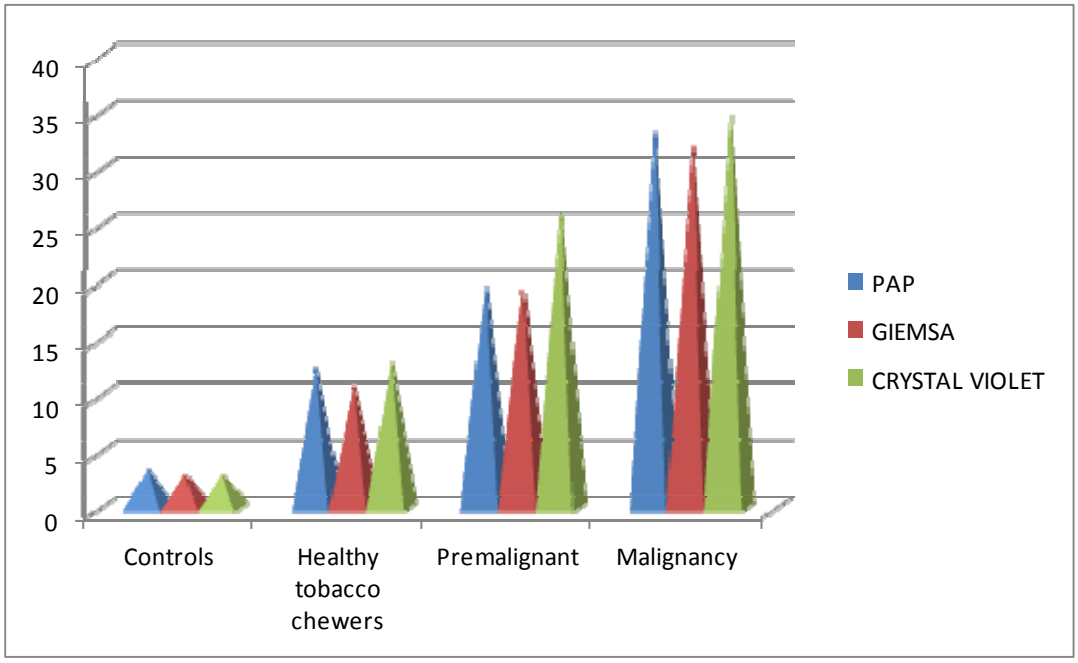


TABLE 11.Comparison of Micronuclei in Tobacco Chewers with Normal Oral Mucosa, Premalignant Lesions and Malignant Lesions.

Descriptives

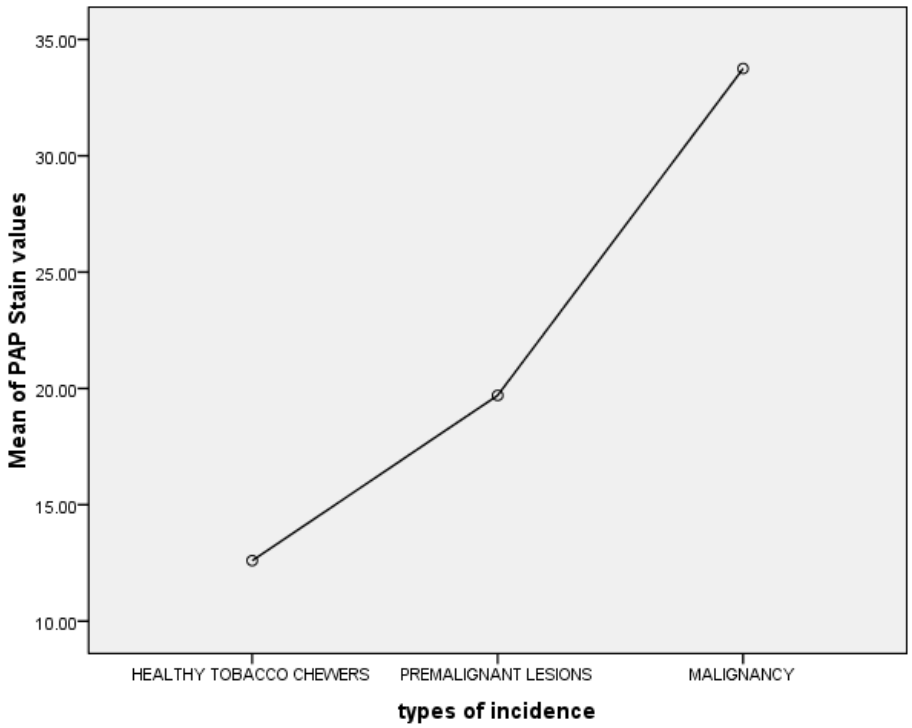
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
HEALTHY TOBACCO CHEWERS	20	12.6000	2.28035	.50990	11.5328	13.6672	9.00	17.00
PREMALIGNANT LESIONS	20	20.2000	2.81163	.62870	18.3841	21.0159	15.00	24.00
MALIGNANCY	20	33.7500	6.53634	1.46157	30.6909	36.8091	22.00	46.00
Total	60	22.0167	9.82472	1.26837	19.4787	24.5547	9.00	46.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4634.233	2	2317.117	124.512	<.001*
Within Groups	1060.750	57	18.610		
Total	5694.983	59			

As there is no significant difference in the mean micronuclei in the three staining techniques, comparison of MN frequency among the study groups is done with Pap stain, which is commonly used in most laboratories.

CHART 10. Comparison of Micronuclei in Tobacco Chewers with Normal Oral Mucosa, Premalignant Lesions and Malignant Lesions.



INTERPRETATION ANALYSIS:

From table; we found H1-1: Types of incidence has significant difference to the micronuclei counting and decision factors; however, mean of micronuclei have significant difference to types of incidence as F=124.512, P=<.001*. Therefore, Hypothesis (H1) is supported; thus H1 is accepted. From Mean plot we interpret that there is maximum number of micronuclei counting in Malignancy and immediately followed by Premalignant lesions and healthy tobacco chewers.

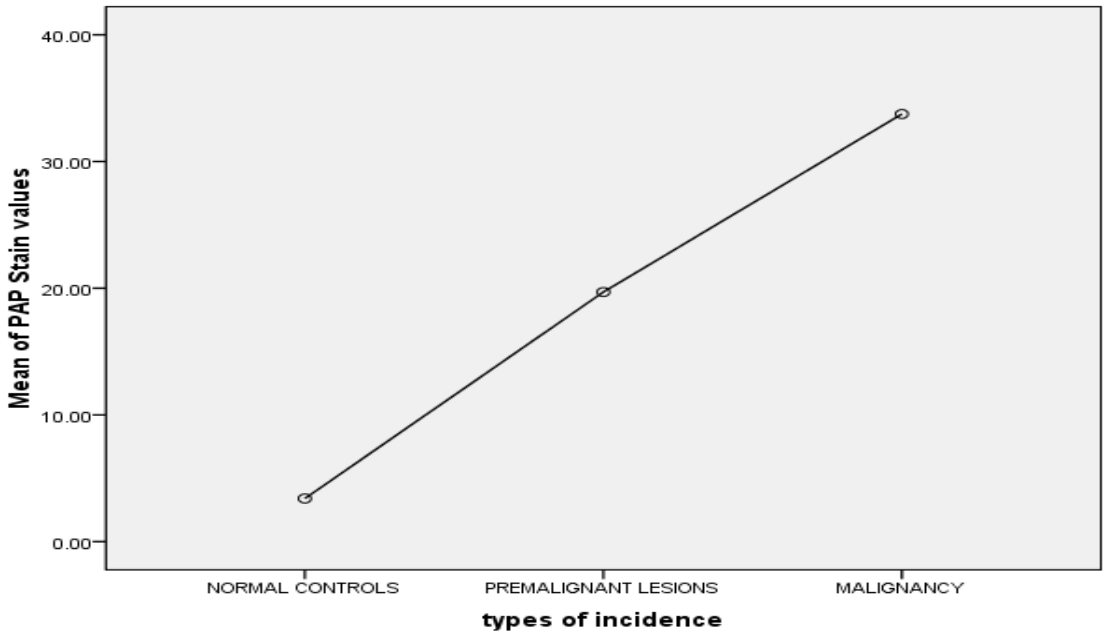
TABLE 12. Comparison of Micronuclei in Malignant and Premalignant lesions with controls

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NORMAL CONTROLS	20	3.4000	.99472	.22243	2.9345	3.8655	2.00	5.00
PREMALIGNANT LESIONS	20	20.2000	2.81163	.62870	18.3841	21.0159	15.00	24.00
MALIGNANCY	20	33.7500	6.53634	1.46157	30.6909	36.8091	22.00	46.00
Total	60	18.9500	13.15414	1.69819	15.5519	22.3481	2.00	46.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9228.100	2	4614.050	268.163	<.001*
Within Groups	980.750	57	17.206		
Total	10208.850	59			

CHART 11.Comparison of Micronuclei in Malignant and Premalignant lesions with controls



INTERPRETATION ANALYSIS:

From table; we found H1-1: Types of incidence has significant difference to the micronuclei counting factors; however, mean of micronuclei have significant difference to types of incidence as F=268.163, P=<.001*. Therefore, Hypothesis (H1) is supported; thus H1 is accepted. From Mean plot we interpret that there is maximum no of micronuclei counting Malignancy and immediately followed by Premalignant lesions and Normal Controls.

TABLE 13.Comparison of Micronuclei in controls and healthy tobacco chewers

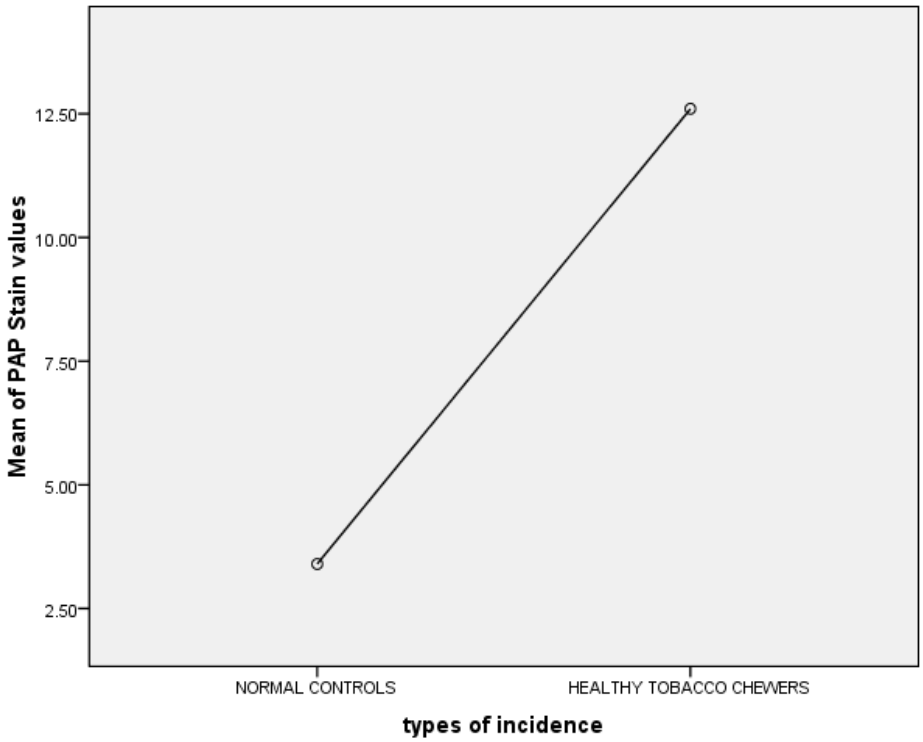
Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NORMAL CONTROLS	20	3.4000	.99472	.22243	2.9345	3.8655	2.00	5.00
HEALTHY TOBACCO CHEWERS	20	12.6000	2.28035	.50990	11.5328	13.6672	9.00	17.00
Total	40	8.0000	4.97171	.78610	6.4100	9.5900	2.00	17.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	846.400	1	846.400	273.497	<.001*
Within Groups	117.600	38	3.095		
Total	964.000	39			

CHART 12.Comparison of Micronuclei in controls and healthy tobacco chewers



INTERPRETATION ANALYSIS:

From table; we found H1-1: Types of incidence has significant difference to the micronuclei counting and decision factors; however, mean of micronuclei have significant difference to types of incidence as F=273.497, P=<.001*. Therefore, Hypothesis (H1) is supported; thus H1 is accepted. From Mean plot we interpret that there is maximum number of micronuclei counting in healthy tobacco chewers as compared with normal controls which is very less.

TABLE 14: Multiple comparison of micronuclei in four study groups

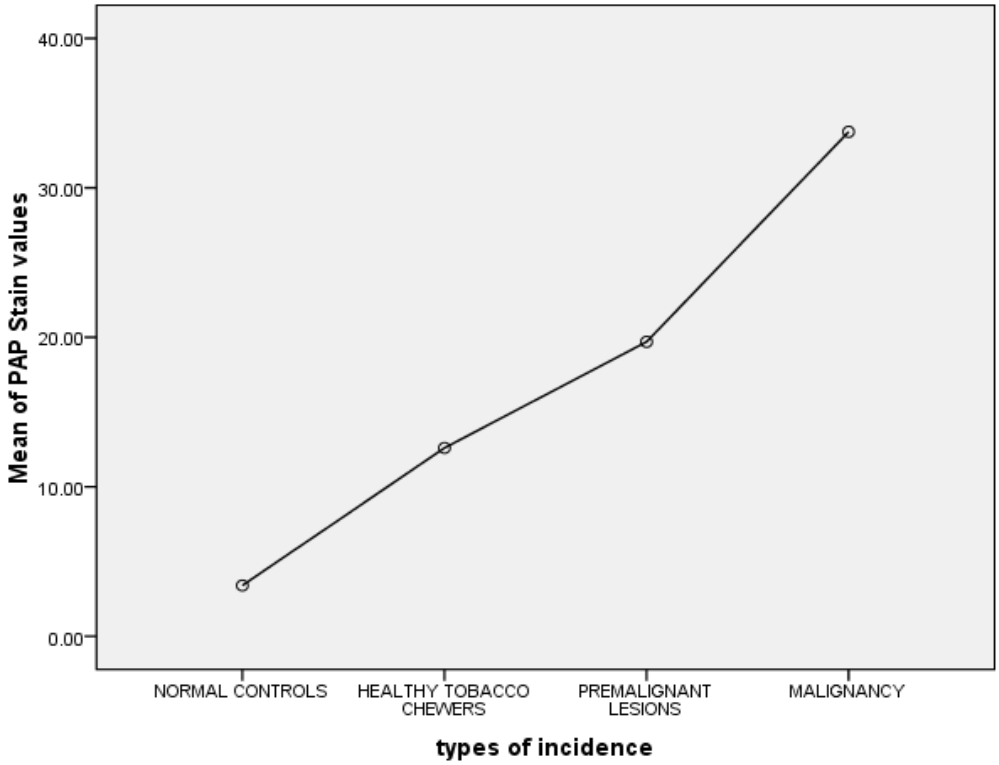
Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NORMAL CONTROLS	20	3.4000	.99472	.22243	2.9345	3.8655	2.00	5.00
HEALTHY TOBACCO CHEWERS	20	12.6000	2.28035	.50990	11.5328	13.6672	9.00	17.00
PREMALIGNANT LESIONS	20	20.2000	2.81163	.62870	18.3841	21.0159	15.00	24.00
MALIGNANCY	20	33.7500	6.53634	1.46157	30.6909	36.8091	22.00	46.00
Total	80	17.3625	11.75299	1.31402	14.7470	19.9780	2.00	46.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9832.938	3	3277.646	230.745	<.001*
Within Groups	1079.550	76	14.205		
Total	10912.488	79			

CHART 13.Multiple comparison of micronuclei in four study groups



INTERPRETATION ANALYSIS:

From table; we found H1-1: Types of incidence has significant difference to the micronuclei counting and decision factors; however, mean of micronuclei have significant difference to types of incidence as F=230.745, P=<.001*. Therefore, Hypothesis (H1) is supported; thus H1 is accepted. From Mean plot we interpret that there is maximum number of micronuclei counting in Malignancy and immediately followed by Premalignant lesions, healthy tobacco chewers and normal controls.

COLOUR PLATES

Buccal Epithelial cell with one Micronuclei in Erythroplakia

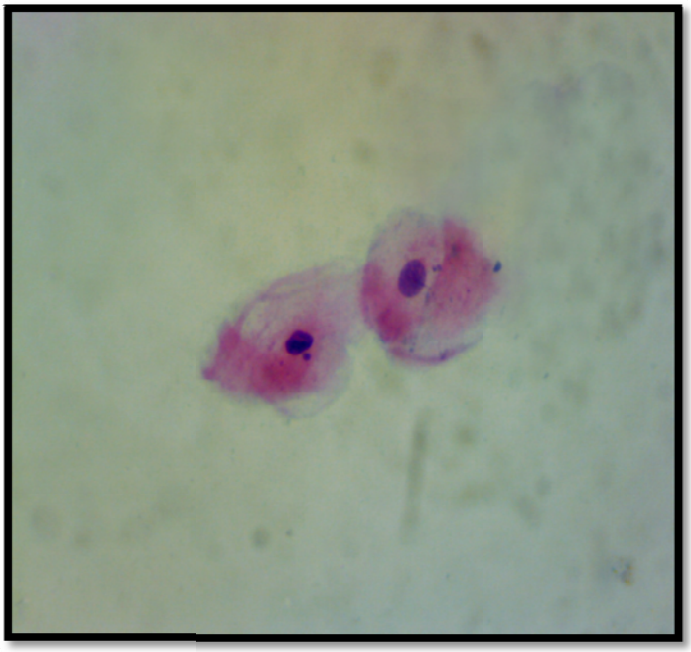


Fig 1. 40X, Pap Stain

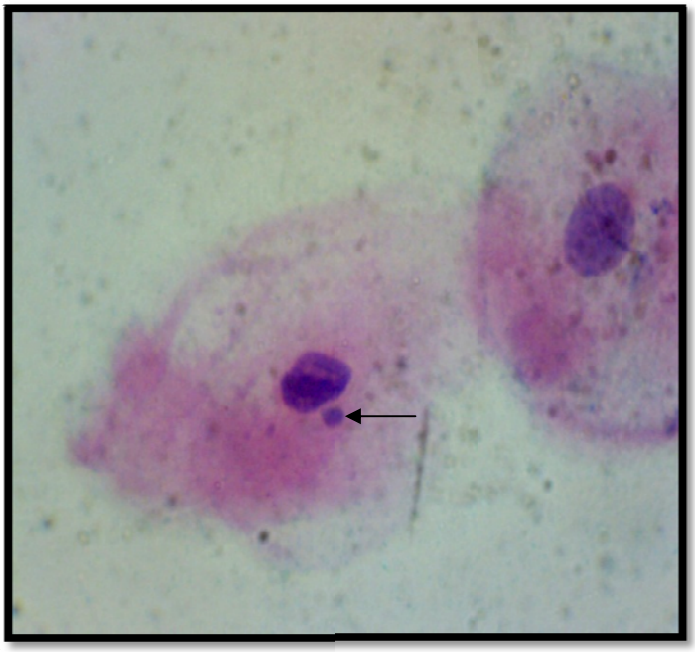


Fig 2. 100X, Pap Stain

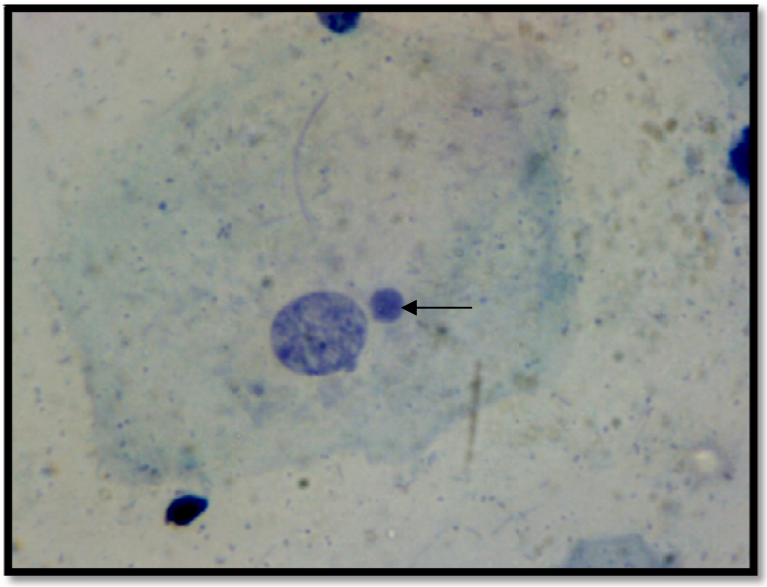


Fig 3. Leukoplakia (100X, Pap Stain)

Buccal epithelial cell with one micronuclei in Leukoplakia

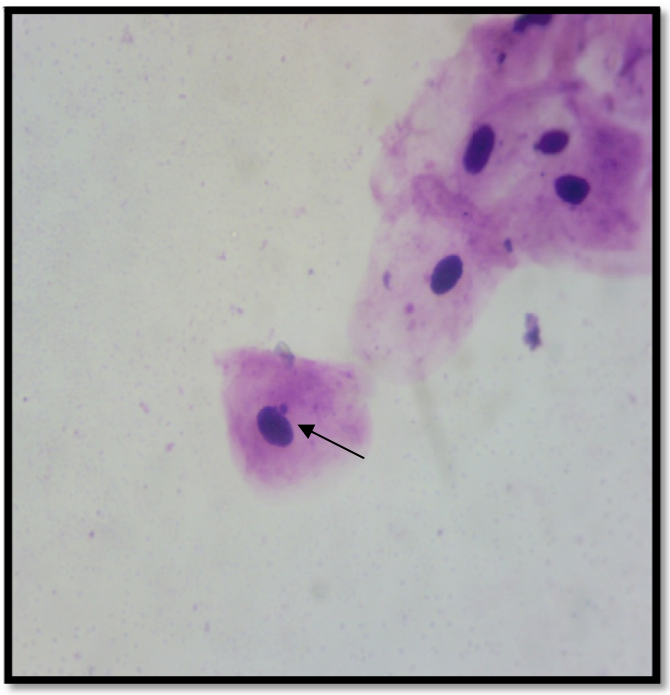


Fig 4. 40 X, Giemsa stain

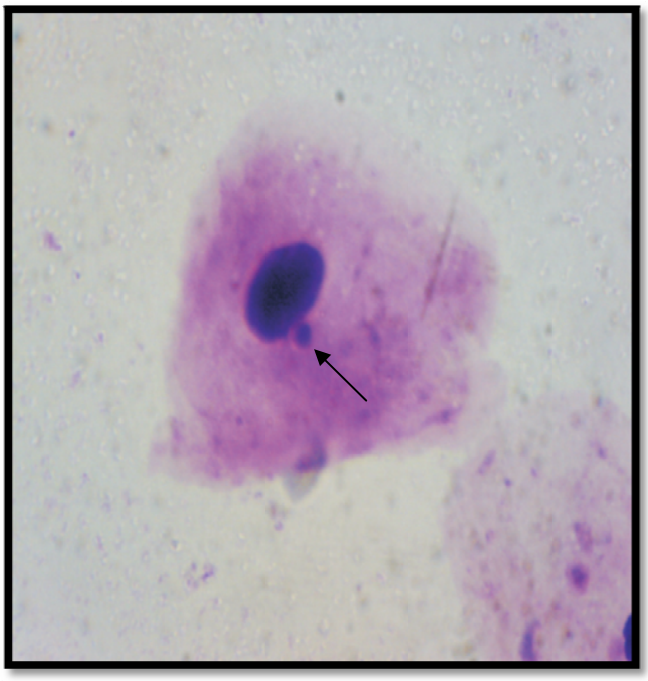


Fig 5. 100 X, Giemsa stain

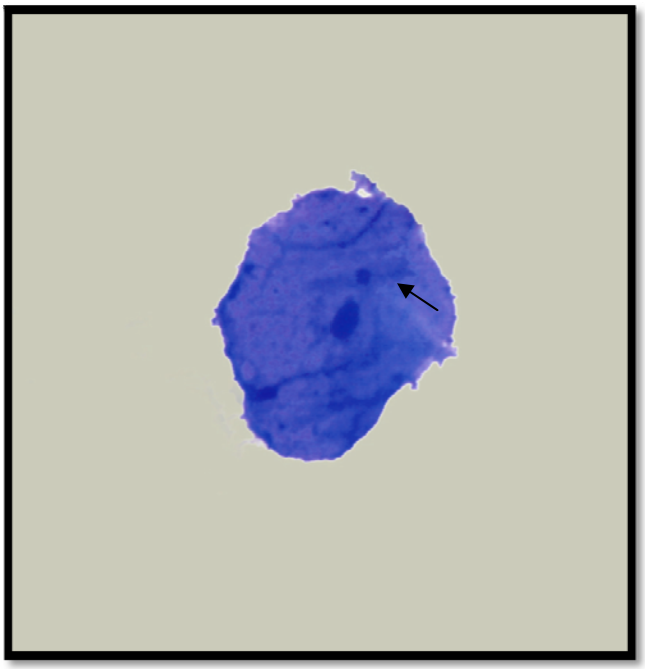


Fig 6. Healthy subject (40X, Crystal Violet Stain)

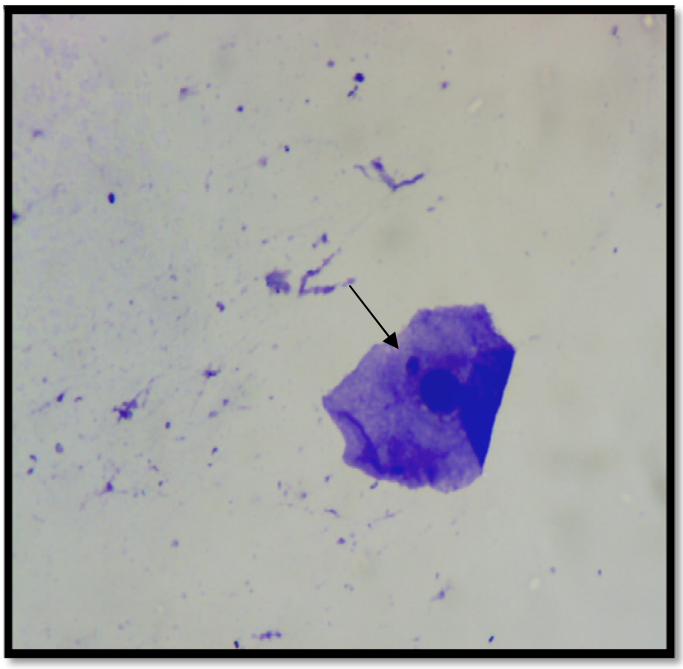


Fig 7. Tobacco chewer with normal oral mucosa (40X, Crystal Violet Stain)

Buccal epithelial cell with two micronuclei in Carcinoma Buccal mucosa

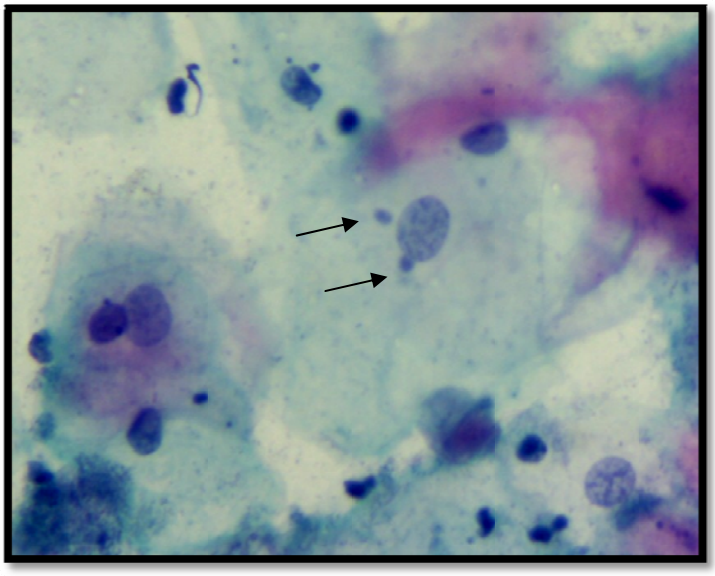


Fig 8. 40X, Pap Stain

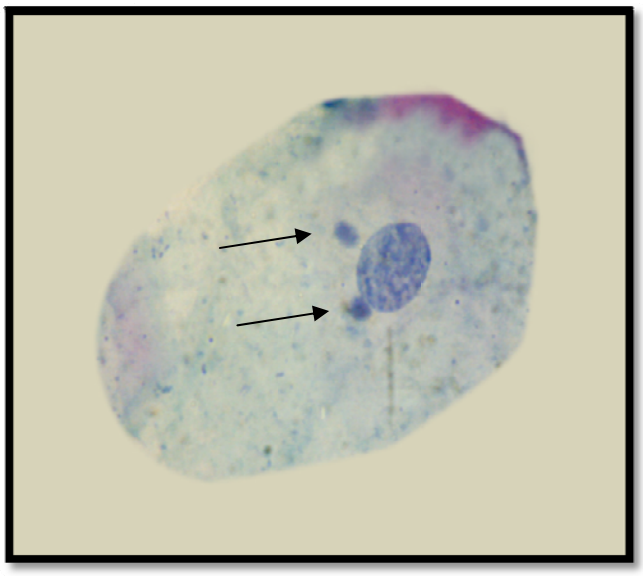


Fig 9: 100X, Pap Stain

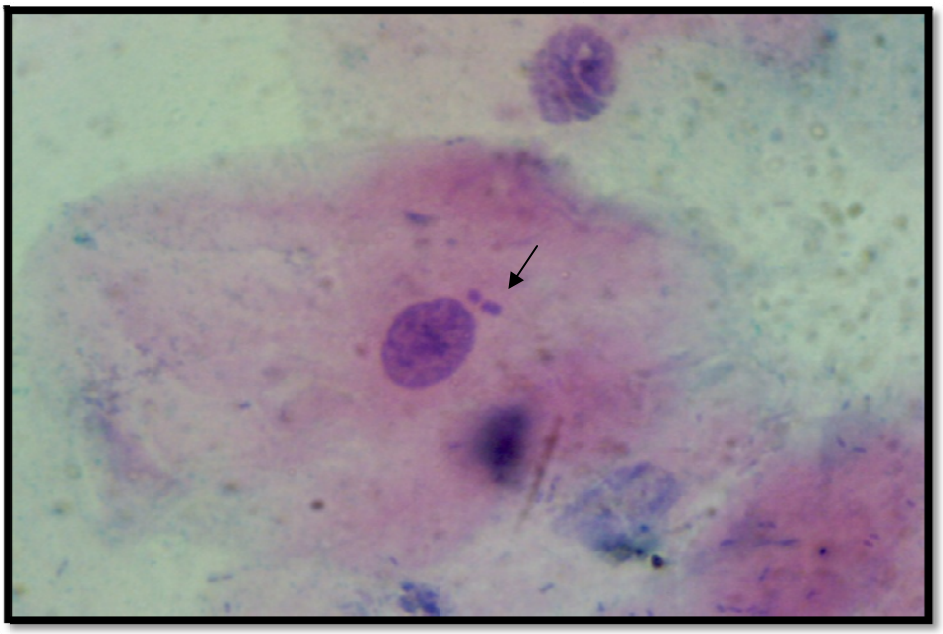


Fig 10. 100X, Pap Stain

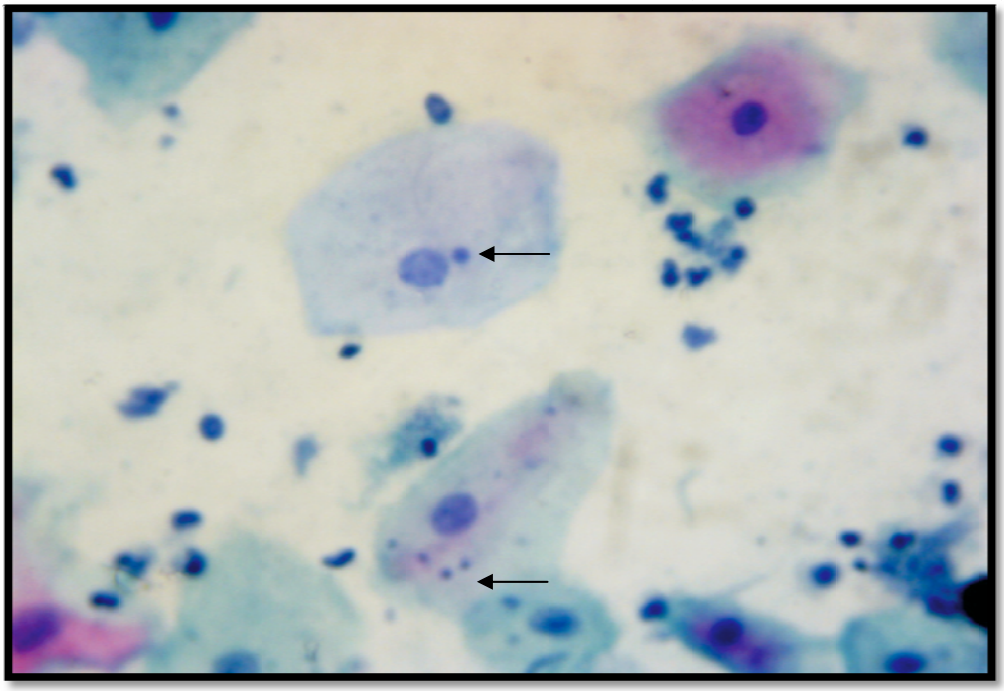


Fig 11. Buccal epithelial cell with many micronuclei in Carcinoma Buccal Mucosa (40X, Pap Stain)

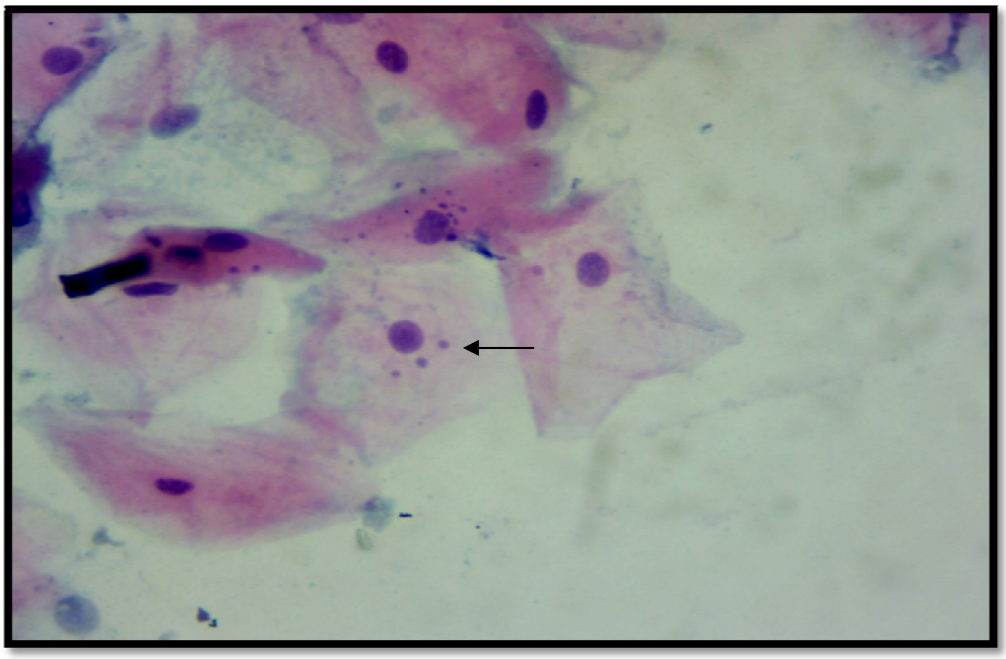


Fig 12. Buccal epithelial cell with many micronuclei in Carcinoma Tongue (40X, Pap Stain)

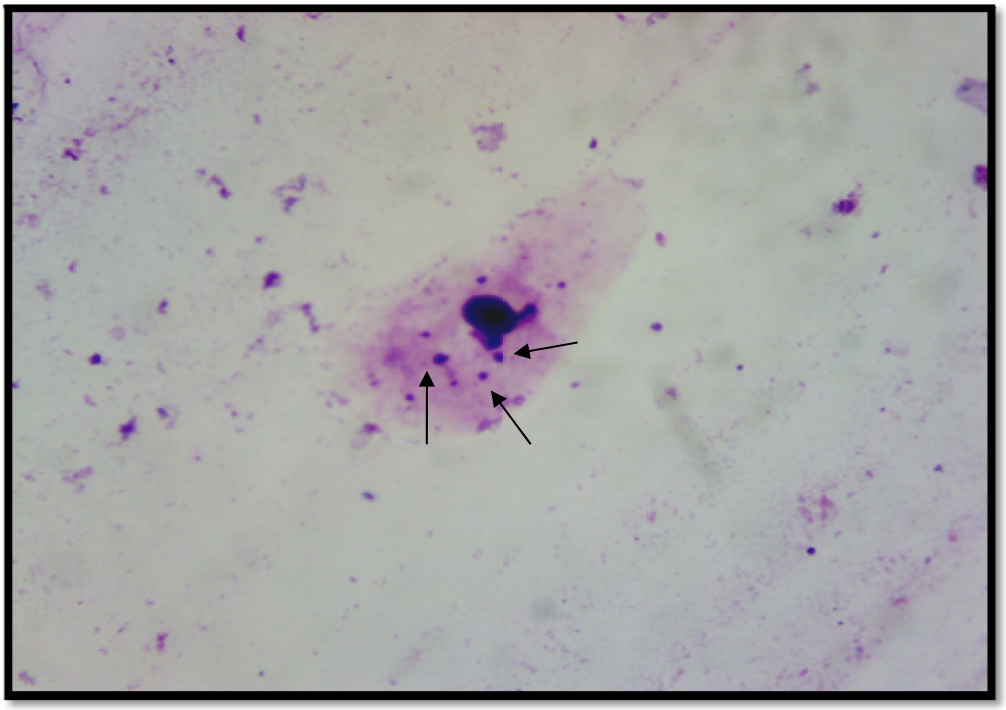


Fig 13. Buccal epithelial cell with many micronuclei in Carcinoma Buccal Mucosa (40X, Giemsa Stain)

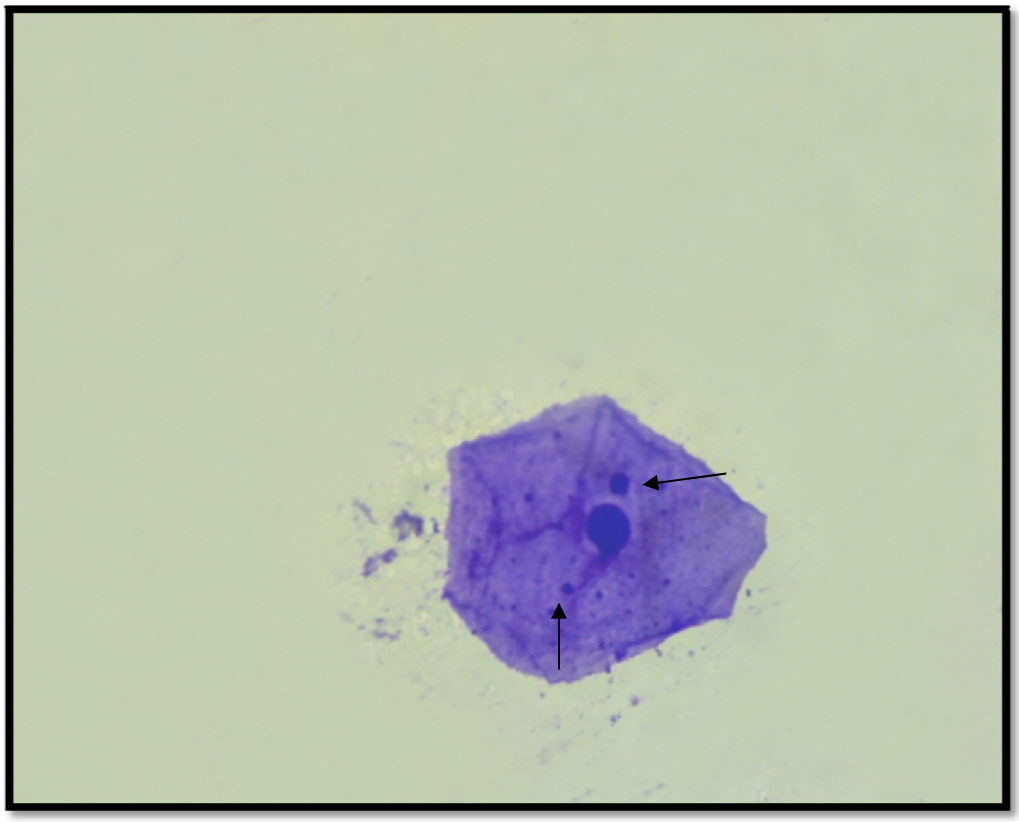


Fig 14. Buccal epithelial cell with many micronuclei in Carcinoma Soft Palate (40X, Crystal violet Stain)

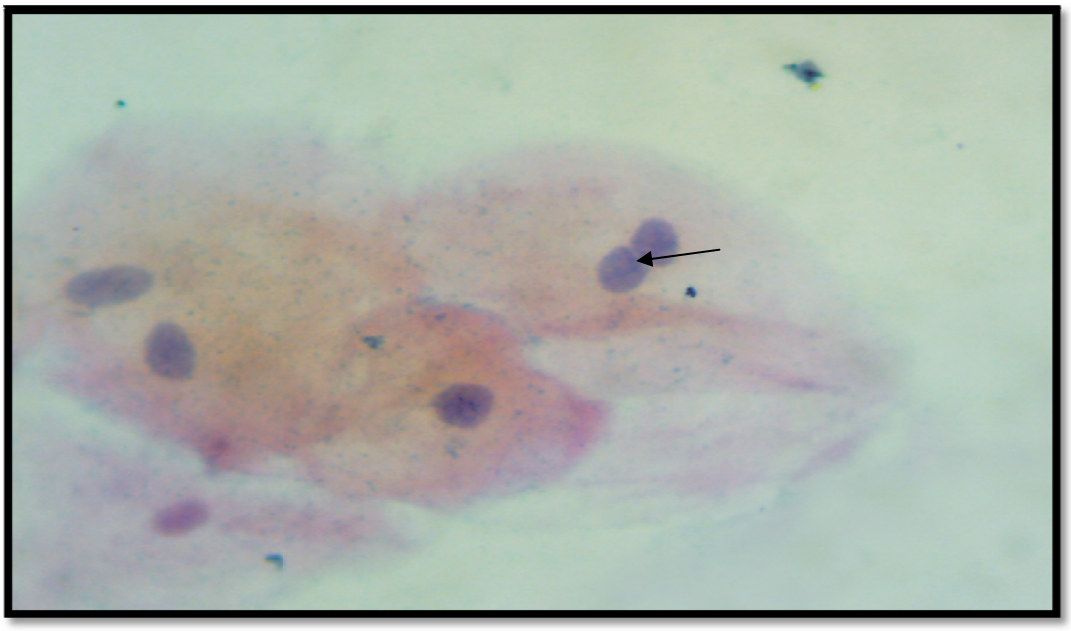


Fig 15. Binucleated Buccal Epithelial Cell in Carcinoma Buccal Mucosa (40X, Pap Stain)

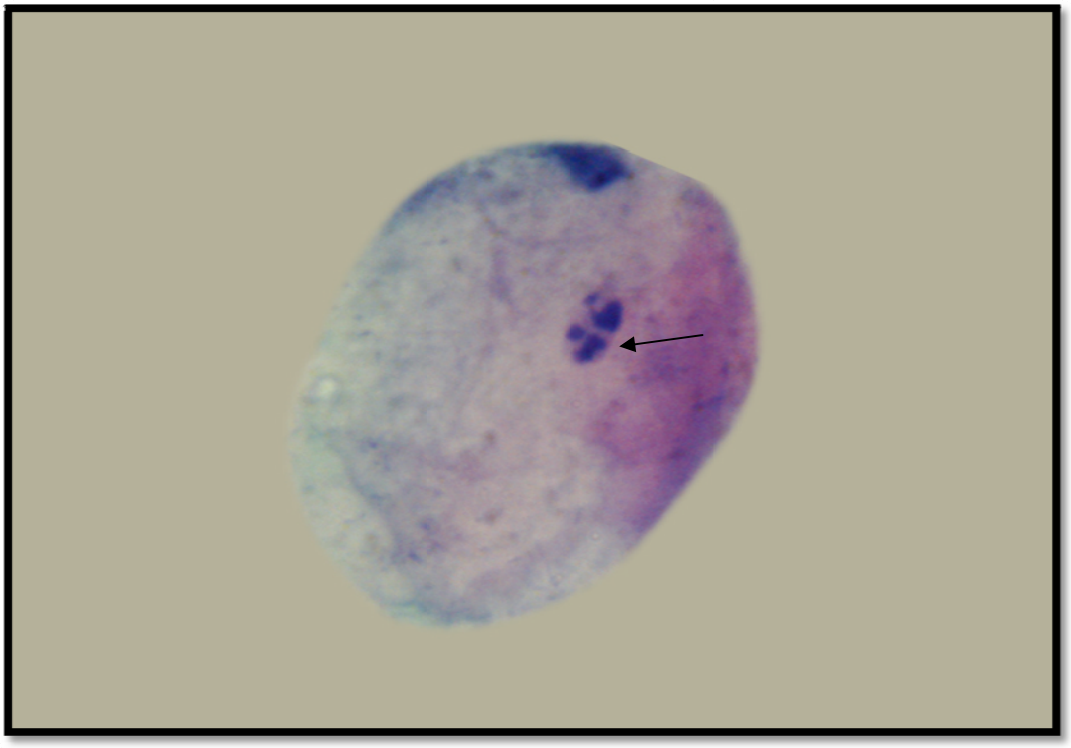


Fig 16. Buccal Epithelial Cell with Karyorrhexis in Carcinoma Buccal Mucosa (100X, Pap Stain)

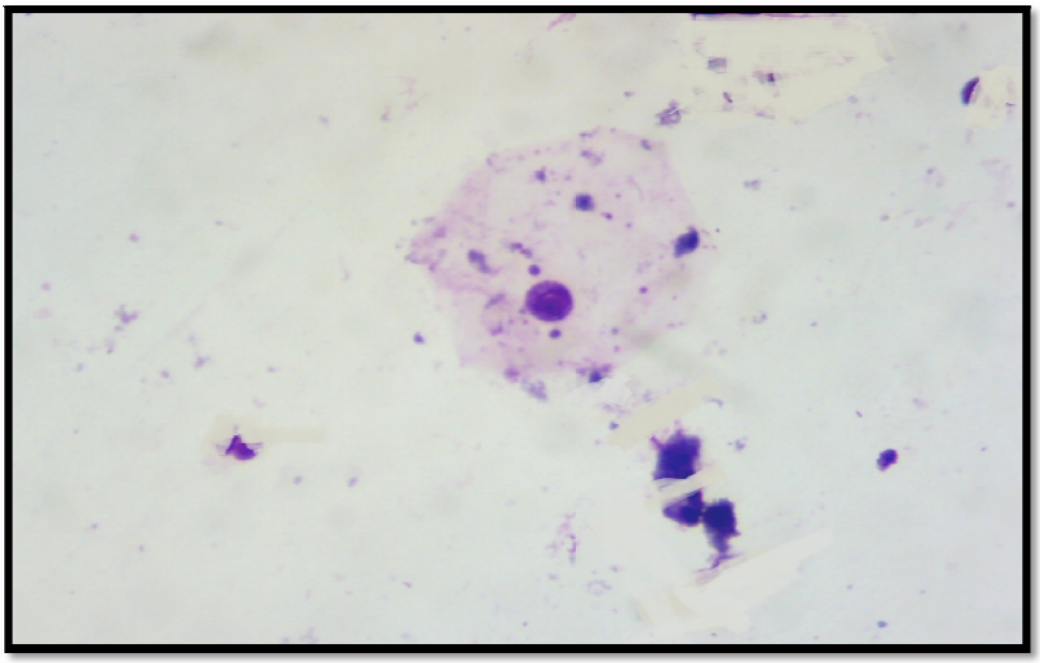


Fig 17.Stain deposits obscuring the cell (40X, Giemsa Stain)

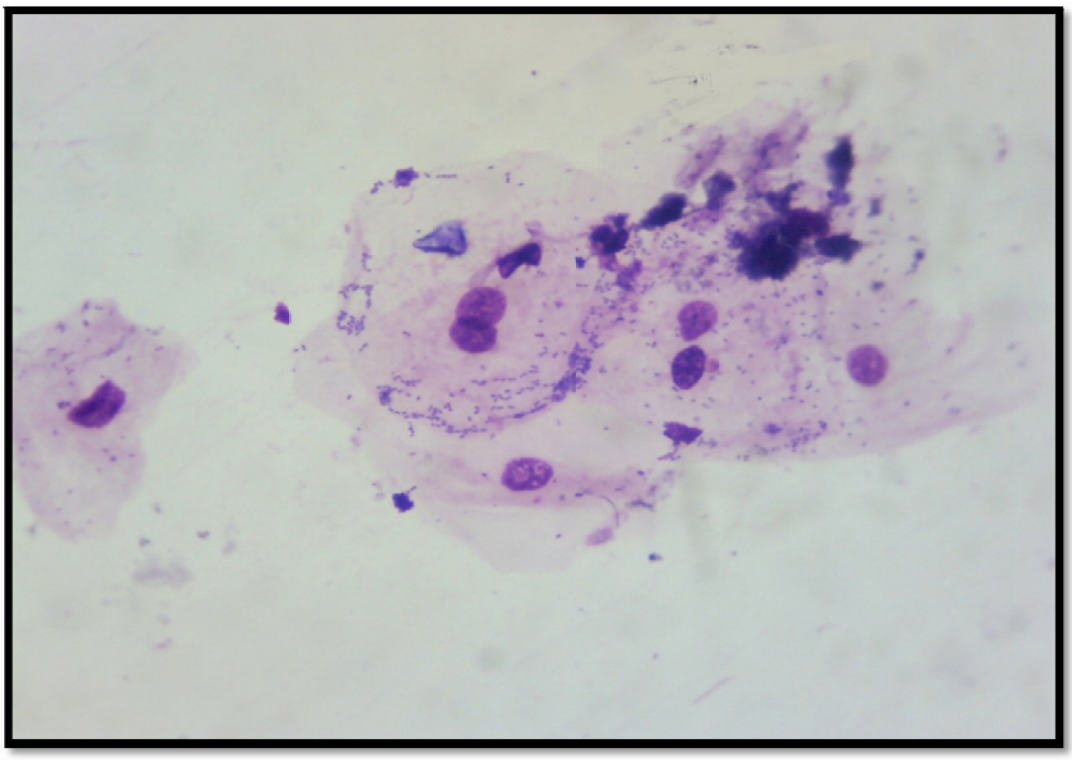


Fig 18. Bacilli and neutrophils obscuring the cell
(40X, Giemsa Stain)

DISCUSSION

DISCUSSION:

The annual incidence of oral cancer in India is around 75,000 to 80,000. Squamous cell carcinoma is the commonest tumour in the oral mucosa accounting for 90% to 95%. Around one third of the cancer mortality in India is said to be related to tobacco. Oral exfoliative cytology is used for screening cellular alterations in preneoplastic and neoplastic oral lesions. Mass screening by cytology has been reported to have 96% reliability and 90% accuracy in detecting squamous cell carcinoma. The sensitivity is about 94% and specificity is 100%.

The present study is aimed at evaluating the differences in micronuclei frequencies in various study population which includes tobacco chewers with premalignant and malignant lesions, with apparently normal oral mucosa and controls of non tobacco users.

Tobacco consumption is said to be higher in people with low socioeconomic status and illetrates. Education tends to play a major role in lifestyle modification to certain extent, that is why the poor, less educated population and illetrates become a prey to tobacco induced morbidity and mortality. But still tobacco consumption is not far from the well educated concern, only the form of consumption varies. Tobacco

chewing is common in uneducated, whereas smoking is prevalent in both population.[56-9]

In the present study, the incidence of oral malignant lesions is found to be common after the age of 50 years. The variation in age seems to increase from apparently healthy tobacco chewers to premalignant lesions to cancerous lesions. The age incidence correlates well with most of the studies done in oral cancer.[19-22,51] This can be disputed that as the frequency and duration of tobacco chewing increases, there is significant increase in the complication from healthy oral mucosa and premalignant state to malignant transformation.

According to WHO, the incidence of oral carcinoma is higher in males. The ratio of male to female is 3:2. But in the current study, the incidence is observed to be higher in females and the ratio is reversed. This is probably due to tobacco quid chewing is predominantly seen in women and also because of the exclusion of other lifestyle habits like smoking and alcohol.[55-60] This result is compared well with the study conducted by Piyathilake et al. who defend that the micronuclei frequencies are 2.8-fold higher in women when adjusted for age, race, smoking, alcoholism and nutritional deficiencies.[113]

In the present study, the commonest site of occurrence of malignancy is found to be buccal mucosa with 50% incidence. This is the favourable site in tobacco chewers since the betal quid is placed here for quite a long period which tends to cause chronic irritation. The tongue is the next common site to be involved with 30%. Rest of them is constituted by floor of mouth, lip, soft and hard palate each accounting for 5%. This is in contrast with the incidence of overall oral malignancies which ranks tongue malignancy as the frequent site.

The mean micronuclei frequency in the four study population is analysed and compared using different staining procedures commonly used in laboratories including Papanicolaou, Giemsa and Crystal violet stains and the results are presented in Table 8. The mean MN frequency show no difference in various staining techniques in all the four study population ($p > 0.05$).

Grover et al, 2012 studied the mean MN frequency in oral cancer cases and compared them with three different stains like Pap, Fuelgen and Hand E. They found MN frequency was significantly higher in cases ($P < 0.01$) than that of controls in each of the three stains. Feulgen stain has the least MN frequency, H and E shows the higher count, while the Pap stain shows intermediate values. The observation was similar in case of controls.[114]

Mala Khamboj and Sumita Mahajan, 2007 had studied 25 cases of histologically proven leukoplakia and Squamous Cell Carcinoma and assessed MN frequency in buccal smears using Fuelgen and Acridine Orange (fluorescent) staining methods. They concluded that the mean MN frequency showed significant increase in leukoplakia and SCC when compared to controls.[115]

As there is no significant difference in the mean micronuclei in the three staining techniques, comparison of MN frequency among the study groups is done with Pap stain. The mean MN is evaluated to be 3.4, 12.60, 20.20 and 33.75 in healthy controls, healthy tobacco chewers, premalignant lesions and malignant lesions respectively. The observed results show an overall MN frequency in tobacco chewers with malignant lesions is higher (33.75 ± 6.5) when compared to tobacco chewers with premalignant lesions (20.20 ± 3.35), healthy tobacco chewers (12.60 ± 2.20) and healthy controls (3.4 ± 0.99). The mean difference among the different study population is found to be statiscally significant ($p < 0.001$). The observation in the present study is similar to those analysed by the following studies.

Casartelli *et al*. assessed micronuclei frequencies in exfoliated buccal cells in premalignant lesions and malignant lesions of oral cavity. They contended an increase in micronuclei frequency in order from normal mucosa to premalignant lesions to carcinoma.[28]

Stich and Rosin, had predicted higher baseline micronuclei frequencies in their earlier studies done in 1983 and 1984. But this is largely due to lack of definite scoring criteria.[19-22]

Abbas et al, Dec 2012 have analysed micronuclei frequency in Toombak users (Toombak – tobacco preparation in Sudan) and found higher micronuclei frequency in Toombak users than control group indicating the toxic effects of tobacco. The micronuclei frequencies seem to raise with increase in the duration and frequency of Toombak use and this was found to be statistically significant and $p<0.000$.[116] These findings correlate well with the findings of Ozkul et al. (1997).[117]

Anila et al, 2011 observed significant increase in micronuclei in buccal exfoliated cells in betel quid chewers as against healthy individuals. The micronuclei frequency was still more higher in smokers who are also chewing a mixture of betal nut, tobacco. They illustrated MN frequency of 0.5 – 5.74% in oral submucous fibrosis cases and proved to be a significant increase compared to the controls.[118]

Pratheepa Sivasankari et al, 2008 evaluated 25 cases of chronic tobacco users with premalignant and malignant oral lesions. They found similar results in micronuclei frequency.[119]

Desai et al. (1996) noted similar results in his study on the exfoliated buccal cells of patients with precancerous oral lesions including leukoplakia, oral submucous fibrosis, and lichen planus.[120] They predicted an increase in MN frequency in the study group compared to the healthy individuals. This also agrees with the findings of Saran et al. (2008).[121]

Ahmad et al. (2006) point out a good correlation of increased micronuclei frequency in gutkha users with oral submucous fibrosis. They also observed that gutkha chewing induced OSMF in a shorter duration of 4years when compared to other causes. This is probably explained by various ingredients of the quid and frequency of quids per day.[122]

The HUMN project is a valuable tool developed for evaluating and assessing the micronuclei in buccal cells. It validates various procedures of collecting samples, different staining modalities, risk strategies, comparison of MN frequencies in various study population and the diagnostic criteria. HUMN validation project speculates the variability of MN frequency in human lymphocytes. Further it implies on

the evaluation of more than 1000 epithelial cells to validate micronuclei and for better results.[51,123]

Devendra Palve and Jagdish Tupkari, 2008 had insisted that MN frequency as a valuable prognostic marker in oral SCC. They found good correlation of histological grades of squamous cell carcinoma and micronuclei in increasing proportions. The MN was found to be increased than in controls and similarly in increasing grades of the tumour. Here, the micronuclei was assessed by Pap stain.[124]

Veerendra Kumar et al, 2000 had evaluated 86 cases of oral squamous cell carcinoma and correlated the micronuclei frequency with different grades of SCC. They concluded a good association of cytogenetic damage with micronuclei frequency and carcinogenic effects of tobacco and paan.[125]

Halder et al, 2004 had analysed 50 cases of oral premalignant and malignant lesions and compared them with healthy controls. They observed that MN frequency seem to be increased in preoperative patients and tend to decrease postoperatively. Similarly, the MN frequency is increased in premalignant lesions than in healthy controls.They implied micronuclei frequency as a biomarker of carcinogenesis as well as a prognostic indicator.[126]

SUMMARY AND

CONCLUSION

SUMMARY AND CONCLUSION

Oral carcinoma is one among the top three cancers of the country showing an increasing trend for few decades. Squamous cell carcinoma, constituting about 90% of the tumour burden, needs to be diagnosed and treated at the earliest. So, it has become essential to innovate minimally invasive techniques like micronuclei assay in diagnostic modalities for primary and secondary prevention in tobacco users. The micronuclei assay holds good as an upcoming research tool for biomonitoring.

The present study is a case control study carried over a period of one year from July 2013 to July 2014 at Coimbatore Medical college and hospital, Coimbatore. The study population comprises of four groups of each 20 cases viz., tobacco chewers with malignancy, tobacco chewers with premalignant lesions, apparently healthy tobacco chewers and healthy controls without tobacco habit. Exfoliative oral cytology was analysed in these groups.

The micronuclei frequency of the four groups are studied using Pap, Giemsa and crystal violet stains. There is no significant difference of MN frequency observed in these staining techniques. Though many studies recommend Fuelgen as the optimal staining

procedure, this study aims in evaluating cost effective, simple, rapid and readily available staining techniques that suit well for mass screening.

The present study concludes that there is a significant difference in the mean micronuclei frequency of the study groups. The MN frequency is found to be higher in malignant lesions as compared in order with premalignant lesions and healthy tobacco chewers, and controls.

Thus, micronuclei in oral exfoliative cells is a good promising domain in detecting cytogenetic damage and aiding in early diagnosis, treatment and prognosis. However, the buccal MN assay has to be standardized in view of sample collection, staining modalities and diagnostic criteria followed. Further, the whole of the smear should be screened for obtaining exact MN frequency and preferably at least 1000 cells should be validated. As this is time consuming and has high chance of interobserver variations, MN assay needs to be automated. Many studies are being done in the scenario and more valuable improvements are expected.

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ANNEXURE - I

ANNEXURE I

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

COIMBATORE.

Particulars of the patient

Name : IP/OP No :

Age : Ward No :

Sex : Occupation :

Address :

Presenting Complaints :

H/o oral ulcer or growth – duration

H/o treatment taken for the growth – radiotherapy/ chemotherapy details

H/o sharp dentures

Family History :

Personal History :

- H/o Tobacco habit – Form of tobacco usage
  Frequency of use / day
  Duration of addiction

- H/o smoking & alcoholism
- H/o intake of spicy foods

General Physical Examination:

Built/ Nourishment : Febrile / Afebrile :

Pallor : Vital Signs :

Jaundice :

Lymphadenopathy :

Local Examination of Oral Cavity :

- Ulcer / patch / growth
- Site of ulcer / growth
- Dentition – caries / sharp tooth

Clinical Diagnosis :

Tumour Staging :

Cytological Examination :

- Number of Micronuclei assessed using Pap Stain
- Number of Micronuclei assessed using Giemsa Stain
- Number of Micronuclei assessed using Crystal Violet stain

Histological findings and diagnosis :

ANNEXURE - II

ANNEXURE II – MASTER CHART

S.No	CASE NO	HPE NO	AGE/ SEX	IP/ OP NO	UNIT	SITE	CLINICAL DIAGNOSIS	BIOPSY REPORT	PAP	GIEMSA	CV
1	M1	3634	63/M	71947	ENT	Tongue	Carcinoma	SCC MD	25	24	25
2	M2	3882	75/M	73956	ENT	Buccal mucosa	Carcinoma	Verrucous carcinoma	34	36	37
3	M3	3846	47/M	68804	ENT	Tongue	Carcinoma	SCC MD	28	25	27
4	M4	3934	72/F	731359	ENT	Buccal mucosa	Carcinoma	SCC WD	27	26	30
5	M5	41	68/F	70694	ENT	Tongue	Carcinoma	SCC MD	27	27	28
6	M6	89	70/F	76229	S2	Buccal mucosa	Carcinoma	SCC WD	31	30	32
7	M7	143	80/F	38645	S5	Buccal mucosa	Carcinoma	SCC PD	46	41	45
8	M8	164	65/F	40982	S6	Lower Lip	Carcinoma	SCC WD	33	34	37
9	M9	322	42/M	59903	S6	Buccal mucosa	Carcinoma	SCC MD	39	38	40
10	M10	425	36/F	3887	S2	Buccal mucosa	Carcinoma	SCC WD	32	32	32
11	M11	554	62/M	119950	S3	Buccal mucosa	Carcinoma	SCC WD	35	34	41
12	M12	605	47/F	9138	S.oncology	Buccal mucosa	Carcinoma	SCC WD	34	32	35
13	M13	702	65/F	12642	s.oncology	Hard palate	Carcinoma	Sarcomatoid SCC	22	22	24
14	M14	728	60/M	156257	Dental	Floor of mouth	Carcinoma	SCC MD	30	29	31
15	M15	952	64/F	13742	S6	Buccal mucosa	Carcinoma	SCC MD	41	38	43
16	M16	1138	39/M	17729	s.oncology	Buccal mucosa	Carcinoma	SCC WD	37	37	39
17	M17	1139	48/F	13050	s.oncology	Tongue	Carcinoma	SCC MD	30	28	30
18	M18	1690	54/F	24165	s.oncology	Tongue	Carcinoma	SCC WD	38	35	42
19	M19	1733	60/F	361721	s.oncology	Tongue	Carcinoma	SCC PD	44	42	45
20	M20	2176	60/F	422745	s.oncology	Buccal mucosa	Carcinoma	SCC PD	42	43	44

S.No	CASE NO	HPE NO	AGE/ SEX	IP/ OP NO	UNIT	SITE	CLINICAL DIAGNOSIS	BIOPSY REPORT	PAP	GIEMSA	CV
21	PM1	3534	40/F	731359	ENT	Tongue	Leukoplakia	Hyperplasia	15	13	17
22	PM2	3940	47/M	853507	S2	Buccal mucosa	Leukoplakia	Hyperplasia with MiD	17	15	16
23	PM3	3977	60/F	904074	ENT	Buccal mucosa	SMF	Hyperplasia	16	14	15
24	PM4	14	40/F	4758	S3	Buccal mucosa	Leukoplakia	Hyperplasia	16	15	16
25	PM5	17	44/F	3402	Dental	Buccal mucosa	SMF	Hyperplasia	17	17	17
26	PM6	48	66/F	14400	S2	Tongue	Leukoplakia	Hyperplasia	17	14	16
27	PM7	238	51/F	59692	S6	Buccal mucosa	Leukoplakia	Non neoplastic	20	18	21
28	PM8	306	46/F	73164	S5	Buccal mucosa	Erythroplakia	Hyperplasia	20	19	21
29	PM9	462	43/F	3845	S4	Buccal mucosa	Leukoplakia	Hyperplasia	18	18	18
30	PM10	471	62/M	101758	S3	Tongue	Leukoplakia	Hyperplasia	24	20	26
31	PM11	577	47/F	120018	S4	Buccal mucosa	SMF	Hyperplasia	21	20	23
32	PM12	630	54/F	131652	S5	Tongue	Leukoplakia	Hyperplasia	23	19	24
33	PM13	633	54/M	133389	S2	Buccal mucosa	Erythroplakia	Hyperplasia with MoD	20	18	20
34	PM14	681	48/M	141304	S5	Buccal mucosa	Erythroplakia	Hyperplasia with MoD	24	20	25
35	PM15	712	48/M	147431	S1	Buccal mucosa	Leukoplakia	Hyperplasia	20	19	21
36	PM16	949	73/F	200836	S5	Tongue	Leukoplakia	Hyperplasia	21	20	20
37	PM17	983	58/M	6241	S2	Tongue	Leukoplakia	Hyperplasia	18	18	20
38	PM18	999	32/M	212670	S3	Buccal mucosa	SMF	Hyperplasia	22	21	21
39	PM19	2122	40/M	260117	Dental op	Buccal mucosa	Lichen Planus	Lichen Planus	23	22	25
40	PM20	2218	68/F	37980	Dental op	Buccal mucosa	Leukoplakia	Hyperplasia	22	21	22

S.No	CASE NO	HPE NO	AGE/ SEX	IP/ OP NO	UNIT	SITE	CLINICAL DIAGNOSIS	BIOPSY REPORT	PAP	GIEMSA	CV
41	NT1		30/M	161043	S3				10	8	10
42	NT2		36/F	100161	S2				9	7	9
43	NT3		32/F	182351	Dental				12	10	15
45	NT5		45/F	75912	Dental				11	10	10
46	NT6		46/M	308415	Dental				13	11	13
47	NT7		47/F	47921	ENT				10	9	11
48	NT8		27/F	193274	Dental				11	9	9
49	NT9		60/M	291032	S2				15	13	17
50	NT10		58/F	13751	ENT				13	12	15
51	NT11		65/F	1410	Dental				10	8	11
52	NT12		27/M	352598	S4				12	10	14
53	NT13		42/F	160976	S3				10	9	12
54	NT14		52/F	11825	Dental				13	12	14
55	NT15		71/F	240592	S2				17	15	17
56	NT16		48/F	387114	S1				14	13	13
57	NT17		62/M	175691	ENT				16	15	15
58	NT18		42/M	308490	S2				14	14	14
59	NT19		72/M	20829	S3				12	10	13
60	NT20		35/F	152021	Dental				15	14	14
61	N1		37/M	455688					3	3	2
62	N2		48/F	38476					3	2	4
63	N3		48/M	256333					4	2	2
64	N4		55/F	23398					3	2	3
65	N5		44/M	338146					5	4	5
66	N6		51/F	337176					5	3	2

S.No	CASE NO	HPE NO	AGE/ SEX	IP/ OP NO	UNIT	SITE	CLINICAL DIAGNOSIS	BIOPSY REPORT	PAP	GIEMSA	CV
67	N7		65/M	259249					4	4	4
68	N8		71/M	38752					3	2	2
70	N10		32/F	36538					3	2	2
71	N11		43/F	256238					2	3	2
72	N12		49/F	36271					2	2	2
73	N13		52/M	447459					3	2	3
74	N14		62/F	392739					5	4	6
75	N15		35/F	35851					4	3	3
76	N16		63/M	334204					2	2	2
77	N17		65/F	38405					3	3	2
78	N18		60/F	38234					4	5	4
79	N19		72/F	38197					3	4	4
80	N20		60/F	17201					4	4	4

ANNEXURE – III

ANNEXURE III

ABBREVATIONS TO MASTER CHART

SCC : Squamous Cell Carcinoma

WD : Well Differentiated

MD : Moderately Differentiated

PD : Poorly Differentiated

MiD : Minimal Dysplasia

MoD : Moderate Dysplasia

SMF : Submucous Fibrosis

MN : Micronuclei

PAP : Papanicolaou stain

CV : Crystal Violet stain

ANNEXURE – IV

ANNEXURE IV

CONSENT FORM

Dr.R.Suganya, postgraduate student in the Department of Pathology, Coimbatore Medical College is conducting a study on " **Role of Micronuclei as a diagnostic tool in exfoliative cytology of oral preneoplastic and neoplastic conditions among tobacco chewers**". The study and test procedures were explained to me clearly. I hereby give my consent to participate in this study and to give buccal smear. The data obtained herein may be used for research and publication.

Name:

Age / Sex :

Address :

Place:

Date : Signature

ஒப்புதல் படிவம்

பெயர் :

வயது : பாலினம் :

முகவரி :

அரசு கோவை மருத்துவக் கல்லூரியில் நோய் குறியியல் துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி மரு.ர.சுகன்யா அவர்கள் மேற்கொள்ளும் ‘‘புகையிலை மெல்வதால் வாயில் ஏற்படக்கூடிய புற்றுநோய் மற்றும் புற்றுநோயாக மாறும் நிலைமைகள் போன்றவற்றை உறிசெல்லியில் மூலம் கண்டறிய மைக்ரோநியூக்ளியஸ் என்பது பயன்படுகிறது’’ என்ற ஆய்வில் மேற்கொள்ளும் பரிசோதனை பற்றிய செய்முறை மற்றும் அனைத்து விவரங்களையும் கேட்டு எனது சந்தேகங்களை தெளிவுபடுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

எனது வாயின் உட்பகுதியிலிருந்து ஐஸ் குச்சி மூலம் வாயின் மேற்புற திசுவை எடுத்து பரிசோதனை செய்வதற்கு முழுமனதுடன் சம்மதம் தெரிவிக்கிறேன்.

இந்த ஆய்வில் என்னுடைய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதையும் தெரிவித்துக் கொள்கிறேன்.

இடம் : இப்படிக்கு

நாள் :

(கையொப்பம் / ரேகை)