CYTOGENETIC CHANGES IN ORAL EXFOLIATED CELLS OF TOBACCO USERS WITHOUT LESIONS AND TOBACCO USERS WITH PRECANCEROUS LESIONS USING PAPANICOLAOU STAIN : A COMPARATIVE STUDY

DISSERTATION

Submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

BRANCH IX

ORAL MEDICINE AND RADIOLOGY

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CERTIFICATE

This is to certify that this dissertation titled **"Cytogenetic changes in oral exfoliated cells of tobacco users without lesions and tobacco users with precancerous lesions using Papanicolaou stain: a comparative study"** is a bonafide research work done by **Dr. G. Hema Mareeswari** under our guidance during her Post Graduate study during the period of 2014-2017 under THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI, in partial fulfillment for the degree of MASTER OF DENTAL SURGERY IN ORAL MEDICINE AND RADIOLOGY, BRANCH IX. It has not been submitted (partial or full) for the award of any other degree or diploma.

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(Dr. HEMA MAREESWARI G.)

CONTENTS

LIST OF ABBREVATIONS

ANOVA	:	Analysis of Variance
CA	:	Chromosomal Aberration
CBCT	:	Cone Beam Computed tomography
CC	:	Condensed Chromatin
CT	:	Computed Tomography
DNA	:	Deoxyribo Nucleic Acid
DPT	:	Dental Panoramic Tomography
D.P.X	:	Din butyl phthate in Xylene
FISH	:	Fluorescence in situ hybridization
FNAC	:	Fine Needle Aspiration Cytology
GST	:	Glutathione transferase
HPV	:	Human Papilloma Virus
HSV	:	Herpes Simplex Virus
KL	:	Karyolysis
KR	:	Karyorrhexis
LBC	:	Liquid Based Cytology
MGG	:	May Grunwald Giemsa

MNC	:	Micronuclei count
MN	:	Micronucleus
NAB	:	Nitrosoanabasine
NAT	:	Nitrosoanatabine
NNN	:	Nitrosonornicotine
OCT	:	Optical Coherence Tomography
OKC	:	Odontogenic Keratocyst
OPMDs	:	Oral Potentially Malignant Diseases
OSCC	:	Oral Squamous Cell Carcinoma
OSMF	:	Oral Sub Mucous Fibrosis
PAP	:	Papanicolaou stain
PN	:	Pyknosis
SES	:	Socioeconomic status
SPSS	:	Statistical Package for Social Sciences
SPT	:	Second Primary Tumors
TSNAs	:	Tobacco specific nitrosoamines
TB	:	Toulidine Blue

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Cancer, modern epidemic among non-communicable diseases is the second commonest cause of mortality in developed countries and remains one of the ten commonest causes of mortality in developing countries like India.[1,2]In economically developing countries the burden of cancer increases due to the increased adoption of cancer causing behaviours particularly the use of tobacco products. Nearly 5, 00,000 new oral and pharyngeal cancers are diagnosed in a year globally out of which 65000 cases were reported from developing countries, like India. The world health organisation has estimated that in India the death proportion due to tobacco related disease will rise from 1.4% in 1990 to 13.3% of all deaths in 2020.[3] According to World Health Organisation estimation in 1991 in the next quarter of the century universally the cancer cases doubles and half of which would be in the developing countries. A campaign against cancer has been launched by the World Health Organization in 1995, with a three-fold strategy: prevent all the preventable cancers, cure all that can be cured, and reduce pain and discomfort where cure is not possible.[4]

Carcinogenesis involves several processes which show genetic, epigenetic and phenotypic changes these involve genetic damage, mutation in critical genes related to the control of cell division, cell death, metastatic potential and activation of signalling or metabolic pathways that give the cells favourable growth and survival characteristics.[5]

To evaluate the genotoxic risks or effects of tobacco on buccal mucosa, deoxy-ribonucleic acid (DNA) damages can be evaluated by detecting chromosomal aberrations, sister chromatid exchanges and micronuclei (MN) test. Exposure of a tissue to genotoxic carcinogens leads to an increase in chromosomal aberrations.

Consistent with this hypothesis, karyotypic anomalies and elevated DNA content have been observed in various tobacco users with and without precancerous lesions.[2]

The biologic parameters which provide information about a physiologic or pathologic state of an individual or population are called Biomarkers. National institute of health defined the term biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathological processes or pharmacological responses to a therapeutic intervention or other health care interventions.[6] There are three types of biomarkers focused by the molecular epidemiological research studies they are ; biomarker of exposure (chromosomal aberrations, micronucleus, sister chromatid exchange), biomarker of susceptibility (genetic polymorphism) and biomarkers of disease(tumor biomarker).[7,8,9] Essentially the biomarker should be reliable and relevant minimally invasive to improve the implementation of bio monitoring, diagnostics, and treatment of diseases caused by, or associated with, genetic damage. The potentially excellent candidate to serve as such a biomarker was found to be the Micronuclei assay, which detects chromosome loss or malfunction of mitotic spindle as a result of aneugenic mechanisms.[10]

The chromosome fragments or whole chromosome, which lag behind in anaphase or metaphase during nuclear division forms the micronuclei.[11]The genomic instability is reflected in micronuclei count. An increased risk of cancer in a given population is indicated by the detection increased frequency in the occurrences of micronuclei.[10]

The first barrier for the inhalation or ingestion route is the buccal cells which are capable of metabolizing proximate carcinogens to reactive products. Nearly 92% of human cancers arise from the external and internal epithelium, that is, the skin, the

bronchial epithelium, and the epithelia lining the alimentary canal. Hence the perfect target site for detecting early genotoxic changes induced by carcinogenic agents entering the body through inhalational and ingestional route is the oral epithelial cells. The buccal cells for a given patient shows the regulatory mechanisms, signalling pathways, and genetic modulation which aids the research oppurtunities to monitor pre treatment and post treatment following antitumor therapy. It also offers the clinician opportunities for early diagnosis and an aid for smoking cessation counselling and provides a unique model for mutation research. Therefore the buccal cells permit correlating genetic alterations with histopathologic changes, also for drug discovery investigations.[10] Hence forth Micronuclei count could act as an efficient biomarker of this process.[1] Moreover, the micronuclei test is a non-invasive diagnostic test with a sensitivity of 94%, specificity of 100% and accuracy of 95%. Thereby micronuclei identification would act as an early predictor of cytogenetic changes in the oral epithelial cells of tobacco users.[2]

Aim:

To evaluate the frequency of genotoxic changes in the smears of exfoliated oral epithelial cells using PAP stain in tobacco users without lesions and tobacco users with precancerous lesions as compared with controls.

Objectives:

1. Comparison of the frequency of occurrence of genotoxic changes between tobacco users with precancerous lesions and tobacco users without clinically evident precancerous lesions compared with healthy controls.
2. To propose oral mucosal micronuclei frequency as an early potential marker of genotoxic changes in oral epithelial cells.

Cancer is the second leading cause of death in the United States; only cardiovascular diseases exact a higher toll. Even more agonizing than the mortality rate is the emotional and physical suffering inflicted by cancers. In the pre molecular era, the eminent British oncologist Willis came closest: "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change." In the modern era, a neoplasm can be defined as a disorder of cell growth that is triggered by a series of acquired mutations affecting a single cell and its clonal progeny. The causative mutations give the neoplastic cells a survival and growth advantage, resulting in excessive proliferation that is independent of physiologic growth signals (autonomous).[12]

These DNA mutations occur spontaneously, especially by the oxidation and the chemical free radicals produced. There are various cancer risk factors which vastly increase the rate of DNA mutation, among which the exogenous factors are the prime factors. They are the lifestyle factors: tobacco and alcohol appear particularly important. The other factors which relevantly involved are chemicals, radiation (e.g. sunlight, ionising radiations), infections, diet (some aspects can harm and some protect) or immuno-incompetence.[13]

Oral cancer has a multi factorial aetiology, such as tobacco, excess consumption of alcohol and betel quid usage, factors which act separately and synergistically as the main lifestyle risk factors. The oral cancer risk greatly increased by exposure to tobacco and/or alcohol or betel and therefore is potentially preventable by lifestyle change. Apart from these environmental factors, genetic factors, Human Papilloma Virus (HPV) infection may also play a role to varying degrees.[13, 14, 15] With advancing

age, the risk of developing cancer also increases occurring in people aged 50 years or over mostly. Only 6% occur in young people under the age of 45years.[16]

Life Style Factors:

Tobacco:

Usage of tobacco is widespread worldwide. Over one-third of cancer mortality is directly caused by tobacco use, and another one-third of cancer deaths have been linked to inadequate diets. Over 20 per cent of the patients who use tobacco and tobacco products develops Potentially malignant disorders (PMDS), and Second primary tumors (SPT) in the upper aero-digestive tract and also an increase in tumours elsewhere, as the carcinogens are absorbed and circulate in the blood to other organs, resulting in adverse health consequences.[17] There are various forms of tobacco and all are carcinogenic. Cigarette smoking is primarily linked to chronic obstructive pulmonary disease, atherosclerotic heart disease, and cancers of the lung, oesophagus, and bladder.[18] Tobacco smoke, which contains thousands of chemicals, many of which are either carcinogens or tumor promoters. In 1989, the United States Surgeon General released a report listing 43 carcinogenic agents found in tobacco smoke. Those carcinogens and their classification according to the International Agency for Research on Cancer (IARC) are listed below [19]

Group 1: Carcinogenic to Human

4-Aminobiphenyl

Benzene

Cadmium

Chromium

2-Naphthylamine

Nickel

Polonium-210 (Radon)

Vinyl Chloride

Group 2A: Probably Carcinogenic to Humans

Acrylonitrile

Benzo[a]anthracene

Benzo[a]pyrene

1,3-Butadiene

Dibenz(a,h)anthracene

Formaldehyde

N-Nitrosodiethylamine

N-Nitrosodimethylamine

Group 2B: Possibly Carcinogenic to Humans

Acetaldehyde

Benzo[b]fluoranthene

Benzo[j]fluoranthene

Benzo[k]fluoranthene

Dibenz[a,h]acridine

Dibenz[a,j]acridine

7H-Dibenz[c,g]carbazole

Dibenzo(a,i)pyrene

Dibenzo(a,I)pyrene

1,1-Dimethylhydrazine

Hydrazine

Indeno[1,2,3-cd]pyrene

Lead

5-Methylchrysene

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

2-Nitropropane

N-Nitrosodiethanolamine

N-Nitrosomethylethylamine

N-Nitrosomorpholine

N'-Nitrosonornicotine (NNN)

N-Nitrosopyrrolidine

Quinolineiv

Ortho-Toluidine

Urethane (Ethyl Carbamate)

Group 3: Unclassifiable as to

Carcinogenicity to Humans (Limited Evidence)

Chrysene

Crotonaldehyde

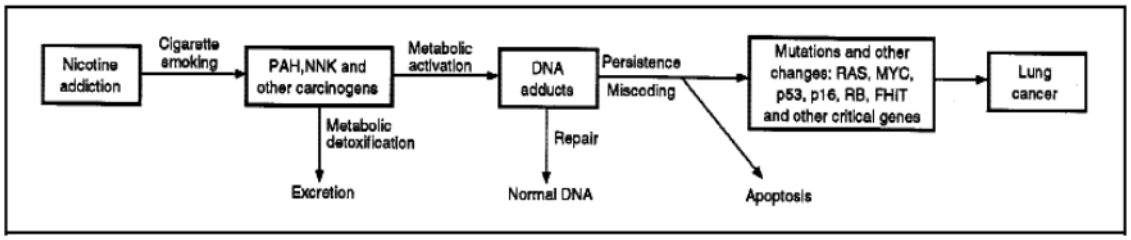
N'-Nitrosoanabasine (NAB)

N'-Nitrosoanatabine (NAT)

TSNAs - tobacco-specific nitrosamines (e.g. NNN, NAT and NAB) are the carcinogens generated by tobacco, which releases free radicals that in turn impede antioxidant enzymes (glutathione-S-transferase (GST), glutathione reductase, superoxide dismutase, catalase, and glutathione peroxidase).People who smoke tobacco expose their upper aero digestive tract to tobacco carcinogens. Genetic changes affect the whole of the aero-digestive mucosae which persists for many years, even if the patient stops smoking.[17]

Compounds in tobacco function as

- ✓ Carcinogens-Initiate tumor growth
- ✓ Tumor promoters-Stimulate the development of established tumors
- ✓ Co-carcinogens-Enhance the mutagenic potential of carcinogens; possess little or no direct carcinogenic activity
- ✓ Irritants-Induce inflammation and compromise tissue integrity.[20]



FORMS OF TOBACCO

Smoking

The smoking tobacco exists in the form of factory made cigarettes, cigars, and cheroots and loose tobacco in pipes or rolled into handmade cigarettes. There is a great variation in the tar, nicotine and nitrosamine contents, depending on species, curing, additives, and method of combustion. Benzo(a)pyrene is a powerful carcinogen found in amounts of 20-40ng/cigarette.[21]

Reverse smoking

Reverse smoking with the lightened end inside the mouth, seen particular among females is strongly associated with hyperkeratotic changes and inflammatory changes leading to dysplastic changes, potentially malignant lesions and squamous cell carcinoma.[21]

Smokeless tobacco

Much of the tobacco in the world is consumed without combustion by being placed into contact with mucous membranes, through which nicotine is absorbed to provide the pharmacological benefit.[21]

Some common forms of oral smokeless tobacco

Forms of tobacco	Ingredients
Pan/Paan/Betel quid	Areca nut, betel leaf/inflorescence, slaked lime, cathechu, condiments, with or without tobacco
Khaini	Tobacco and lime
Mishri	Burned tobacco
Zarda	Boiled tobacco
Gadakhu	Tobacco and molasses
Mawa	Tobacco, lime, areca
Nass	Tobacco,ash,cotton ,sesame oil
Naswar/Niswar	Tobacco,lime,indigo,cardoman,menthol
Shammah	Tobacco,ash,limme
Toombak	Tobacco and sodium bicarbonate

Snuff in Scandinavia and North America

Brown et al, described snuff-dippers cancer where the habit of placing snuff in the lower labial sulcus in the southern eastern states of USA.[21]

Marijuana:

Obtained from the plant extracts of cannabis sativa.it may besmoked in three ways:as marijuana derived from macerated leaves flowers of the plant mixed with tobacco in cigarette, as hashish dried resin placed in pipe, as hash oil derived from the flowers of female plant. On burning releases potential carcinogens polycyclic aromatic hydrocarbons, benzo(a)pyrene, phenols, phytosterols, acids and terpenes including nitrosamines in similar levels as in tobacco smoke.[21]

Betel

20 per cent of the world's population has the habit of chewing betel, especially among Asian communities. Similar chewing habits such as khat use may also be implicated in some communities. In people who use betel changes are found in the mouth, pancreas and also in many other organs.[22,23,24]

Alcohol

There is a widespread use of alcohol (ethanol) worldwide. It is found to be the most common drug of abuse, related to more than sixty different medical conditions such as suicide, homicide, different forms of accidents (e.g., falls, poisoning, accidents) which are the acute consequences of alcohol usage and other conditions, such as liver cirrhosis, chronic pancreatitis, haemorrhagic stroke and various cancers are the chronic consequences of alcohol usage.

Alcohol is carcinogenic. Various mechanisms are proposed the important one is the oxidization to acetaldehyde (a carcinogen) through the enzymes (alcohol dehydrogenases) which is then degraded by aldehyde dehydrogenases to acetate. The

outcome of exposure to alcohol and its carcinogenicity is influenced by the genetic variations in the activities of these enzymes.[25]

Tobacco and alcohol

Tobacco and alcohol usage have a synergistic carcinogenic effect. When these lifestyle habits often co-exist the attributable risk of oral cancer due to is estimated to be more than 80%. Smoking increases the burden of acetaldehyde following alcohol consumption and the pro-carcinogens present in tobacco is activated by alcohol-drinking. Heavy drinkers and smokers have 38 times the risk of abstainers from both products.[26]

Infectious agents

Bacterial infections

Poor oral hygiene is an independent risk factor for oral cancer. There is a risk for oesophageal cancer in patients who do not practice oral hygiene regularly also in other hand oral cancer patients often present with poor oral health. The statistical risk for head and neck cancer is increased by the periodontal diseases among the subjects who never used alcohol or tobacco. Periodontitis patients were more likely to have poorly differentiated oral squamous cell carcinomas than periodontally healthy patient.[27, 28, 29]

Candidiasis

Candida albicans is the most common commensal microflora in the oral cavity. In medically compromised patients and in oral cancer patients there is an increase in numbers of Non-Albicans Candida Albicans are seen. Yeasts may invade

oral epithelium and produce dysplastic changes via the production of nitrosamines which activate specific proto-oncogens and it efficiently converts ethanol into carcinogenic acetaldehyde. In association with other risk factors such as smoking and alcohol these can produce malignant transformation. Therefore there is a synergistic effect with candidosis and life-style factors may exist in oral carcinogenesis.[30, 31, 32]

Virus infections

Human papilloma virus infections

HPV-associated oropharyngeal cancers appear to be less associated with tobacco or alcohol use, but more associated with marijuana and oral sex. There are more than 100 types of human papilloma viruses (HPV) reported. From the oral cavity the following strains of HPV-6, -11, -16, -18, -31, -33, and -42 have been isolated of which HPV-16 and HPV-18 are regarded as carcinogenic, and the most common virus types identified in oral carcinoma.[33, 34, 35, 36, 37]

Herpes viruses

Herpes simplex viruses (HSV) have also been found to be associated with carcinogenesis. Studies showed that HSV nucleic acids have been found in lip cancer, antibody levels to HSV-1 and -2 are higher in oral cancer patients when compared with controls, also HSV seropositivity together with smoking has been associated with increased cancer risk.[34,38]

Epstein-Barr virus has also been implicated in oral cancer. [39, 40, 41, 42, 43, 44]

Dietary factors:

Studies showed that there is an inverse association between total fruit and vegetable intake and 5-year incidence of head and neck cancer. The Mediterranean diet is associated with reduced risk of cancer. Another study showed that the daily consumption of six or more plant foods, fruits, cereals, olive oil, wine and low intake of meat and dairy products over a period of 8 years gave protection against oral and pharyngeal cancer in comparison with those who do not consume as much daily. Hence an antioxidants deficient diet is a predisposition towards the development of oral cancer and for precancerous lesion/condition.[45]

Social and economic status (SES)

A meta-analysis study showed that low social and economic status (SES) and deprivation are associated with an increased risk of oral cancer.[46]

Environment

Natural or therapeutic sources or nuclear accidents (e.g. The Chernobyl accident) which emit ionising radiation may be a contributing factor to cancer risk. [26]

Genetics

There are certain protective mechanisms which include the genes for the liver enzymes (xenobiotic metabolizing enzymes) which degrade chemical carcinogens and also repair DNA mutations (DNA repair genes). In Li Fraumeni syndrome there is a genetic predisposition resulting in an inherited mutation in one allele of the p53 locus which results in susceptibility to develop sarcomas and other tumours in successive generations. In cases of Fanconi anaemia, there is a recessive inheritance

characterized by congenital anomalies and bone marrow failure, which predispose to the development of cancer.[47]

These variations in genetic factors explain well the differing susceptibilities of individual people to cancer. Hence the spectrum at one extreme end have patients with higher risk of cancer in spite of their exposure to known risk factors, with the other end of the spectrum the people are apparently unharmed by exposure to known risk factors even at larger scale.[47]

Mechanism of carcinogenesis:

Oncogenesis (carcinogenesis) is the process of progression of a normal healthy cell to a pre malignant or a potentially malignant cell - characterised by an ability to proliferate autonomously which involves a series of genetic and epigenetic changes. These changes include the aberrant expression and function of molecules regulating cell signalling, growth, survival, motility, angiogenesis (blood vessel proliferation), and cell cycle control.[26] The concept of multistep carcinogenesis was proposed by Berenblum and Schubik in 1948. This includes three main phases: initiation, promotion, and progression.[4]

Initiation:

Initiation is the first step which involves one or more stable cellular changes arising spontaneously or induced by exposure to a carcinogen. The human DNA sequences, which are responsible for transformation, are called as Oncogenes. It requires activation of more than one oncogene for neoplastic transformation, single point mutation also can induce initiation process. Thereby it leads to deregulation of genes responsible for cellular communication, development and differentiation. The

transformed cell undergoes continuous division with further mutations and malignancy being manifested.[4]

Promotion:

The initiated cell remains passive, unless and until it is aggravated to undergo further proliferation, creating the cellular imbalance. On prolonged exposures to the offending stimuli the initiated cell undergoes multistep process to form neoplasia.The intra and extracellular environment influence the neoplastic development. Initial mutation will depend on both interaction with other oncogenic mutations and factors that may temporarily change the patterns of specific gene expression which result in an amplification of cellular growth potential and/or an uncoupling of the intercellular communication that restricts cellular autonomy and which coordinate tissue maintenance and development.[4]

Progression:

Due to the successive changes in the neoplasm, an which is increasingly malignant sub-populations are formed promulgated by repeated exposures to carcinogenic stimuli or by selection pressures favoring the autonomous clonal derivatives. There is a rapid increase in the tumor size as the initiated cells undergoes continous proliferation. As the tumor grows in size, the cells may undergo further mutations and alterations, leading to increasing heterogeneity of the cell population.

The first phase of progression is the neoplastic conversion where the pre-neoplastic cells are transformed to a state in which they are more committed to malignancy. It involves accumulation of the expanding pre-neoplastic cell clone due to further gene mutations. The dynamic cellular heterogeneity which is a feature of

malignancy, may, in many instances, be a result of the early acquisition of gene specific mutations that destabilize the genome.[4]

Tumor metastasis:

As the tumor progress, the cells lose their property of adherence, dissociate from the tumor mass and invade the surrounding tissues. In addition to this local invasion, the detached cells via circulating blood and lymph and are transported to other organs/tissues away from the site of the primary growth and develop into secondary tumors at new sites. These form the distant metastases, resulting in wide spread Cancer. Cancer metastasis consists of many steps; the main steps are common for all tumors. The progress of the neoplastic disease depends on changes that facilitate:

(a) Invasion of Local Normal Tissues,

(b) Entry and transit of neoplastic cells in the blood and lymphatic systems, and

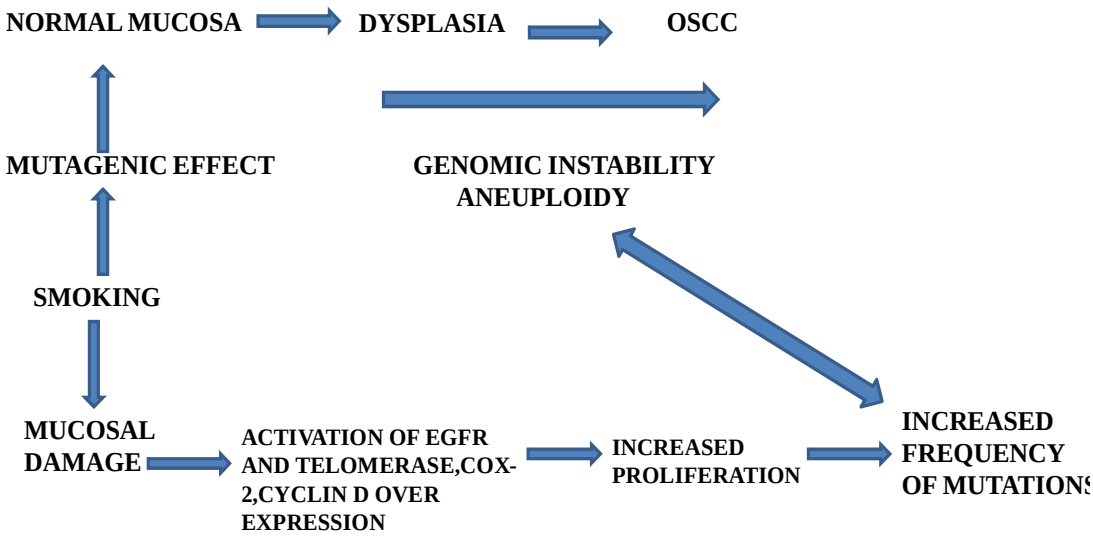
(c) The subsequent establishment of secondary tumor growth at distant sites.

Many of the steps in tumor metastasis involve cell-cell and cell-matrix interactions, involving specific cell surface molecules. Malignant cells are thought to have reduced ability to adhere to each other, so that they detach from the primary tumor and invade the surrounding tissues. The behavior of tumor is influenced by the cell adhesion molecules, one of the most important of which is cadherins. It is the metastatic process and tumors local invasion that are mainly responsible for the lethal effects of many common tumors. In many cases gene mutations are believed to be the driving force for tumor metastasis, with the development of tumor vasculature playing an important role in disease progression.[4]

Tumor angiogenesis:

Tumor growth depends on the supply of growth factors and efficient removal of toxic molecules, which is ensured through adequate blood supply. In solid tumors, efficient oxygen diffusion from capillaries occurs to a radius of 150-200nm, beyond which the cells become anoxic and die. Therefore, increase in tumor mass to more than 1-2 mm will depend on adequate blood supply through development of blood capillaries (angiogenesis).[4]

Progression Model For Oral Carcinogenesis [48]



Oral Cancers arise from pre-existing longstanding lesions, which is termed as "Oral Potentially Malignant Disorders' (OPMDS).[49]

Precancerous lesion: [49]

A precancerous lesion is a morphologically altered tissue in which Oral Cancer is more likely to occur than in its apparently normal counterpart. The precancerous lesions are:

1. Leukoplakia:

2. Erythroplakia

3. Palatal changes among smokers (smoker's palate)

Precancerous condition: [49]

A precancerous condition is a generalized state associated with significantly increased risk of Cancer.

The precancerous conditions are:

1. Oral submucous Fibrosis

2. Oral lichen planus

3. Sideropenic dysphagia

4. Discoid Lupus Erythmatosus

5. Syphilis

6. Xeroderma Pigmentosum

7. Epidermolysis Bullosa

Diagnositic aids:

Non invasive diagnostic aids

Vital staining

Toluidine Blue (TB)

The toluidine blue (tolonium chloride) staining provide information on lesion margins accelerate the decision to biopsy, and guide biopsy site selection and treatment of oral potentially malignant and malignant lesions.[50]

Methylene blue

The technique of MB staining was originally described by Japanese investigators for improving the diagnosis of early gastric cancer. MB is indicated for early detection of oral cancer and precancerous lesions and to identify the areas of incomplete excision during peripheral osteotomy of aggressive lesions like odontogenic keratocyst (OKC) and ameloblastoma. This technique has been claimed to ensure complete removal of the lesion and hence decrease in the recurrence.[50]

Lugols Solution

Lugol's iodine has been used for studying cervical and esophageal epithelium. During colposcopic examination of uterine cervix, Lugol's iodine is applied to identify dysplastic epithelium and this test is called as Schiller's test. The basic principle with iodine staining is its affinity for carbohydrates and starch in the tissues. As the malignancy is associated with reduction in the glycogen content of the tissues, the malignant tissue remains unstained and on the contrary the normal epithelium gets stained brown or black.[50]

Light-Based Detection Systems

Carcinogenesis is associated with various structural and metabolic changes, which are presumed to generate a distinct profile of absorption and refraction when exposed to different types of light or energy; light-based detection systems are designed to utilize such profiles for the detection of any abnormality (Epstein and Guneri, 2009).[51]

Chemiluminescence

Vizilite® (Zila Pharmaceutical, Phoenix, Arizona) is the most well-known high sensitivity system. This procedure appears to potentiate certain visual aspects of the lesion, such as brightness and sharpness of margins. Vizilite does not help overall in the identification of malignant and premalignant oral lesions, and therefore the combination with TB (vizilite Plus®) was subsequently proposed to reduce the number of false positives. [51]

Tissue Fluorescence Imaging

The Velscope® system (Visually Enhanced Lesion Scope; LED Dental, Inc., White Rock, British Columbia, Canada) uses direct fluorescence for any loss of fluorescence in visible and nonvisible high-risk oral lesions, such as cancers and precancers. This procedure has shown to be helpful in obtaining safer surgical margins in tumor excision (Huber, 2009). Its utility in screening low-risk patients with malignant or premalignant lesions is not yet reported (Fedele, 2009).[51]

Tissue Fluorescence Spectroscopy

This is based on an automated system that uses small optical fibers to produce excitation wavelengths, and a spectroscope that records the resulting spectral data on a computer (Fedele, 2009). This technique eliminates the subjective interpretation of changes in tissue fluorescence. However, its main application is limited to the study of small lesions previously diagnosed by visual examination. This system has been shown to have high sensitivity and specificity when differentiating healthy mucosa from malignant oral lesions (De Veld et al., 2005).[51]

Laser Capture Microdissection

LCM harvest cells from native tissue environment with the help of high dentisty oligonucleotide probe arrays,identifies the gene expression differences between normal and malignant oral epithelial cells which provide powerful means to decode the molecular events involved in the genesis and progression of oral cancer.[52]

Oral Brush Biopsy

Oralcdx® brush biopsy (oralcdx Laboratories Inc., Suffern, New York) is an oral transepithelial "biopsy" system that uses computer-assisted brushing (Sciubba, 1999). This technique was designed to detect any abnormality in oral epithelia, which may point toward the presence of dysplasia or cancer (Bhoopathi et al., 2009). Oralcdx seems to overestimate the presence of dysplastic lesions even in normal samples (Bhoopathi et al., 2009) so its practical clinical use remains debatable.[51]

Lab on a Chip

Smaller quantities of media, reagents, and even nano particles are steered through channels on the device from which they are delivered manipulated and analysed by fluorescent detection. An automated immunoassay with a single cancer marker or a panel of cancer markers with a specific gene implemented in a micro fluid cassette for detection is used. [53]

Saliva Based Oral Cancer Diagnosis

Salivary Genomics

Genomics-based diagnostic techniques are designed to monitor genomic level changes in abnormal physiological conditions like OSCC.The analysis of salivary DNA can also give an overview of epigenetic changes associated with OSCC (like hypermethylation of the promoter region of the p16 gene) and can be monitored by methylation arrays (Viet and Schmidt, 2008; Franzmann et al., 2007).[51]

Salivary Transcriptomics

The stability of endogenous cell-free mRNA in saliva is somewhat similar to plasma (Park et al., 2006), which could be possibly due to stabilizing interaction with certain macromolecules. This is used to identify the transcripts that are significant in malignancies, when compared with normal conditions.[51]

Salivary Proteomics

Saliva can be considered as a potent medium to give first hand information about the health of the oral cavity (Wong, 2006). The pro-inflammatory, pro-angiogenic cytokines (tnfa, IL-la, IL-6, and IL-8) were significantly elevated in the

whole saliva of subjects with OSCC compared patients with oral premalignant lesions and the control (Rhodus et al., 2005). Mass spectrometry based systems are currently been pursued for development of proteomics biomarker-based system for OSCC diagnosis.[51]

Imaging

Dental panoramic tomography (DPT), computed tomography (CT), and magnetic resonance imaging (MRI) are frequently used to supplement the clinical evaluation and staging of the primary tumor and regional lymph nodes. CT is the technique of choice to evaluate bone invasion by the tumor. The introduction of cone beam computed tomography (CBCT) provides an alternative for the preoperative study of patients with oral cancer to determine the degree of invasion and extension of the lesion toward the jawbone (Closman and Schimidt, 2007), Optical coherence tomography (OCT) is yet another imaging technique that is based on low-coherence interferometry using broadband light. This technique provides cross-sectional, high-resolution subsurface tissue images, and is a noninvasive method to study macroscopic characteristics of epithelial and sub epithelial structures, which can be used to detect and diagnose oral premalignant lesions (Wilder-Smith et al., 2009).[51]

Invasive Diagnostic Aids:

Surgical biopsy.[51]

Micronucleus

Micronucleus is a small additional nucleus and is readily identifiable in light microscopy. It is generally used as biomarker of chromosomal damages, genome instability and cancer risk. MN test provide a reliable measure of chromosome

breakage and chromosome loss.[54] The level of baseline chromosome damage in untreated cancer patients and in patients with preneoplastic lesions and conditions is much higher when compared to cancer free individuals. Therefore MN scoring can be used as biomarker to identify the different preneoplastic lesions and conditions much earlier than the manifestation of clinical features and might specifically be exploited in the screening of high risk population group for cancer and also used to identify the underlying genotoxicity basic mechanisms.[55,56]

Historical Perspective

Howell and Jolly described Feulgen - positive nuclear bodies in human reticulocytes, known as Howell - Jolly bodies in the late 1800s and early 1900s which represents the chromosomes separated from the mitotic spindle.[57] Evans et al first described the micronucleus (MN) assay in vitro in radiation experiments with roots of Vicia faba. [58, 59]

Boller in the early 1970s suggested the term micronucleus test and Schmidt and Heddle showed that this assay detect the genotoxic potential of mutagens after in vivo exposure of animals using bone marrow erythrocytes.[10] In 1982, Stich & co-workers first used exfoliated cells of buccal mucosa to describe the suitability of MN test for human bio monitoring studies .[60]

An international collaborative project, the Human Micronucleus [HUMAN] Project established in 1997 aimed at studying the frequency of micronucleus in human populations and assessing the effects of protocol and scoring criteria on the values obtained which currently involves more than 35 laboratories worldwide. It has three main goals; [61]

a) Compilation and comparison of base line micronucleus frequencies in human population to establish "normal base-line frequencies of DNA damage and determine the main demographic, environmental and methodological variables that impact on this index;
b) Comparison of variable methods used to measure MN frequencies in human blood and epithelial cells to identify the important methodological variables and establish standard protocols to enable more reliable comparison of data among laboratories and among populations; and
c) To establish prospective epidemiological studies aimed at determining whether the MN frequency predicts risk of cancer and other degenerative diseases associated with DNA damage and ageing.[61,62,63,64,65]

The oral epithelium (non keratinized mucosa) is composed of four strata of structural, progenitor, and maturing cell populations, that is, the stratum basale , stratum spinosum, stratum granulosum and stratum corneum. The oral epithelium maintains itself by continuous cell renewal whereby new cells produced in the basal layer by mitosis migrate to the surface replacing those that are shed. The genetic damage is expressed by the stem cells in the basal cell layer (chromosome breakage or loss) as MN during nuclear division. The daughter cells with or without MN, before exfoliation in to the oral cavity differentiate into the prickle cell layer and the keratinized superficial layer, while some of these cells may degenerate into cells with condensed chromatin, fragmented nuclei (Karyorrhectic cells), Pyknotic nuclei, or completely lose their nuclear material (karyolitic or "ghost" cells). Rarely some cells may be blocked in a binucleated stage or may exhibit nuclear buds (also known as “broken eggs” in buccal cells) which act as a biomarker of gene amplification. These biomarkers of genome damage (Eg: MN, nuclear buds) and cell death (Eg: apoptosis,

karyolysis) can be observed in both the lymphocyte and buccal cell systems, and thus provide a more comprehensive assessment of genome damage and MN in the context of cytotoxicity and cytostatic effects. Micronuclei derived from acentric chromosome fragment can be distinguished from those derived from whole chromosome by FISH using a pancentromeric probe, which will detect the centromere in the micronucleus. Thus, clastogenic and aneugenic events can be detected by the MN technique.[66]

Theories of Origin of MN

The two predominant mechanisms involved in the formation of MN in a mitotic cell: chromosomal breakage and dysfunction of the mitotic apparatus. Clastogens yield acentric fragments by inducing chromosome breaks where these chromosomal fragments are directly included into micronuclei. Secondly, aneugens prevent the spindle formation during mitosis, the whole chromosomes lag behind at anaphase and is surrounded by the nuclear envelope, forming micronuclei. Henceforth the daughter cells have micronuclei with whole chromosomes.

Besides these important mechanisms, the broken anaphase bridges forms MN. As MN may be also be formed due to amplified DNA budding it doesn't always denote loss of chromosomal material.[67,68,69,70,71,72]

Causes of MN Formation

There are many causes of MN formation. There MN in the normal healthy individual spontaneously due to exposure to environmental pollutants, radiation, bio-hazard materials, and drugs, other poisonous chemicals and free-radical injuries, also long standing chronic inflammation, heavy metal poisoning, chemotherapy, radiation injuries, infections, nutritional deficiency and various preneoplastic and neoplastic

conditions forms MN. The possible explanations for MN formation are direct DNA damage or breakage, chromosomal aberrations, mitotic apparatus dysfunctions, and interference with DNA synthesis.[73] The cells which probably displaced at metaphase and gets predispose to "lag" even after other chromatids have moved to the other spindle poles for MN.[74] Micronuclei also can perform DNA synthesis and mitotic condensation synchronously with the main nucleus further producing chromosome loss and gain at successive mitotic cycles.[75]

Details of the Methodology of MN Scoring on Smear

Either MGG or Papanicolaou's stained smear can be used for MN scoring. All the cells with intact cell membrane should be included. Degenerated cells, cells with obscured or altered morphology and large cell clusters or clumped groups should better be avoided. Bi or multinucleated cells may show MN and should be counted and given a score of one. Cells with multiple MN should be carefully looked for a possibility of keratohyaline granules and if MN is confirmed morphologically, it should be given a score of one. Overall score is usually expressed as number of micronucleated cells per 1000 or 500 or 100 cells. With strict criteria, MN can be identified with confidence in Papanicolaou's stained smear.[73]

Morphology of micronucleus:

Location – seen within inner half of the cytoplasm near the main nucleus with MN generally close to one end of it and is always separated from the main nucleus.

Size - 1/1 6th to l/3rd the diameter of the main nucleus

Staining - same intensity or of more intensity with the main nucleus. Occasionally paler.

Texture - Same as the main nucleus, sometimes more clumped. Perimeter is smooth suggesting a membrane and non-refractile.

Shape - Mostly oval or round; may be pyramidal, hemispheric, elliptical, cylindrical or very rarely irregular.

Number - mostly occurs singly in a cell. Very occasionally double. Triple or more not seen or rare.

Others - Plane of focus coincides or nearly same as the main

Cells of occurrence - Seen in the benign-appearing cells as well as frankly malignant or dysplastic cell

Mimickers - Stain deposits, bacteria, nuclear dusts, clumped cytoplasmic fragments, partial Karyorrhexis or necrotic nucleus, carried over nuclear fragment from other cells.[73]

MN Scoring Criteria

In 1976, Heddle introduced the scoring of micronucleus in lymphocytes after exposure to genotoxic agents.[76] Criteria for identifying micronuclei as given by Heddle & Countryman (1976) are:

1. Diameter less than l/3rd the main nucleus.
2. Non-refractility (to exclude small stain particles).
3. Color same as or lighter than the nucleus (to exclude large stain particles).
4. Location within 3 or 4 nuclear diameters of a nucleus; and not touching the nucleus (to make frequency measurements meaningful).
5. No more than 2 micronuclei associated with one nucleus.

A standardization protocol for the counting of micronuclei in exfoliated buccal cells was tried by Belien et al [1995].[77]

Paige E. Tolbert et al [1992] developed Criteria for the inclusion in the total cell count which are the following: (1) cytoplasm intact (2) little or no overlap with adjacent cells; (3) little or no debris; and (4) nucleus normal and intact, nuclear perimeter smooth and distinct.[78]

For a cell to be considered micronucleated, the cell must satisfy the above criteria regarding inclusion in the total cell count and the putative micronucleus is required to meet the following criteria: Fenech et al (2003).[70]

1. The diameter of the MN should be less than 1/3''' of the main nucleus
2. MN should be separate or marginally overlap from the main nucleus as long as there is a clear identification of the nuclear boundary.
3. Micronucleus have similar staining as the main nucleus

The International Collaborative Project on Micronucleus Frequency in Human Populations was organized to collect data on micronucleus [MN] frequencies in different human populations and different cell types in 1999 & 2003. The information will be used to:

1. Determine the extent of variation of 'normal' values for different laboratories and the influence of other factors potentially affecting baseline MN frequency. Eg: age, gender and life-style.
2. Provide information on the effect of experimental protocol variations on MN frequency measurements.
3. Design and test optimal protocols for the different cell types.

4. Determine the extent to which MN frequency is a valid biomarker of ageing and risk for diseases such as cancer.[61]

B J. Majer et al [2001] reviewed the use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials and concluded that the MN assay is a useful biomarker for the detection of human cancer risk in organs.[60]

Baseline frequencies for micronucleated cells in the BM are usually within the 0 5-2.5 Micronuclei/1,000 cells range.[73]

PAP Stain

George Papanicolaou in 1942 developed a multi-chromatic staining cytological technique and in 1952 and 1960 modified subsequently by him. Classically it consists of 5 dyes and 3 solutions. A haematoxylin used for staining nucleus. Orange - G counter stain to stain keratin. Second counter stain consists of 2 dyes, Eosin Y to stain superficial epithelial squamous cells. Light Green SF which stains cytoplasm of other cells including non-keratinized squamous cells. Advantages of this staining technique are chromatin patterns are well visible in it. It also has increased cytoplasmic transparency.[79] Even though the conventional staining method takes 40 minutes rapid PAP staining kits are available which finishes total staining in 3 minutes.

A study conducted by Sarto et al., among 50 clinically normal individuals in 1987 and divided into two groups having smoking habit and non- smokers. The frequencies of micronuclei were about double in smokers as compared with non-

smokers and also found the frequencies of micronucleus from spindle disturbances were insignificant.[80]

A study was carried out in 1997 by Ozkul et al., analyzed the genotoxic effects of smokeless tobacco, smoking and non-smokers/non-users among 54 clinically healthy individuals and found that there is a similar and significant number of micronucleus in smokeless tobacco users and smokers.[81]

A study conducted by Kassie et al., in 2001 found there is an increase in the number of micronucleus among patients using khat (Catha Edulis- a psycho stimulant plant leaf) consumers, tobacco users and alcohol habits. They also added that the number was higher in khat users and use of tobacco and alcohol had an added effect in the course of genetic damage.[82]

In a comparative study conducted by Znaor et al., in 2003 in normal, pre malignant and malignant epithelium using exfoliated buccal cells and found an increase in their number from normal to premalignant then to malignant epithelium.[83]

In 2005 a study carried out by Kumari et al., among oral Squamous cell carcinoma patients suggested the use of micronucleus assay as a marker to identify the tumor sensitivity. They also found that the number of micronucleus was high at the time of diagnosis and there was an increase in their number immediately after exposure to radiation therapy.[84]

In 2007 a study conducted by Buajeeb et al., among patients with erosive and atrophic lichen planus for the assessment of micronucleus in exfoliated cells and found a significant increase in the MN frequency in these lesions than in normal individuals.[85]

A study conducted by Saran et al., in 2008 among cancer patients, patients in pre-cancer stage and normal patients using MN assay. As there was a step wise increase in the number of MN from normal to premalignant and from premalignant to malignant patients they concluded tha the MN assay can be used as a prognostic indicator in this lesions.[86]

In 2011, a study conducted by Francielli et al., among 20 patients with white lesions in the oral cavity. Both Toluidine Blue staining and samples for cytological evaluation were taken and found that the frequency of MN were higher in those patients, irrespective of TB staining.[87]

A study conducted in 2011 by Devi et al., among patients three groups of patients who have potentially malignant disorders and malignant lesions compared with control group and found that the number of micronucleus was higher in malignant group than in patients who are diagnosed with potentially malignant disorders with the control group.[88]

In 2011, a study by Ramakrishnan et al., was done among the patients with pan chewing habits and gutkha habit to find out the genotoxic effects on these agents using an MN assay. They found a significant increase in the micronucleus and chromosomal aberration in those patients and suggested the increased chances of cancer development in these patients.[89]

A prospective study conducted by Dey et al., in 2012 among breast cancer patients diagnosed by FNAC with benign cases as control group used Acridine Orange, a fluorescent dye for the identification of micronucleus and found an increase in their frequency.[90]

A cohort study was carried out by Naderi et al., in 2012, assessed the correlation of frequency of micronucleus in smokers with the duration of habit. They found that there is a direct correlation of number of micronucleus with the duration of habit noted as their number increase with duration of habit and the frequency of micronucleus was higher in smokers than non- smokers.[91]

A study conducted by Kaur et al., in 2013 among 10 tobacco users, to evaluate the presence of micronuclei and other nuclear abnormalities, concluded that age, duration of exposure and other factors like smoking and intake of alcohol affects the frequencies of nuclear abnormalities. They found no increase in the number of micronucleus in these patients.[92]

A study conducted by Jindal et al., in 2013 to find out the alterations in buccal cells due to alcohol, tobacco and combination of two. The results showed that these habits can produce cellular alterations which may cumulatively lead to carcinomatous changes. They also found that there is a significant increase in the number of micronucleus in the patients with tobacco and alcohol habit than in control group. There is no significant difference when compared with alcohol alone group with control group.[93]

A study done by Uma et al., among patients who were diagnosed with tobacco related potentially malignant disorders using MN assay during the years 2014 included the sample collection from the exfoliated buccal epithelial cells of 8 leukoplakia,7 OSMF patients and 15 healthy controls and found a significant increase in their number in patients diagnosed with OPMDS compared to control group.[94]

In 2014 study conducted by Khanna et al., among patients with leukoplakia, Squamous cell carcinoma along with normal healthy individuals using PAP and found

that MN were higher in SCC than in leukoplakia and healthy centre. Hence, MN assay can be used as an important biomarker for cytogenetic damage in oral leukoplakia and OSCC.[95]

A study conducted in 2015 by Pratheepa Sivasankari et al., to evaluate the genotoxicity of tobacco and alcohol on the buccal mucosa of alchoholics, smokers and betel nut chewers among persons having the habit of consuming alcohol and smoking and betel nut chewing were compared with controls and found that the present micro nuclear study shows a feasible and economical method which could be used as a screening test in population having the habit of alcohol and smoking or betel nut chewing for identifying the effects of genomic instabilities and to introduce timely interventional strategy in order to treat and control the epidemic.[96]

The study was planned & conducted in the department of Oral Medicine and Radiology, Sree Mookambika Institute of Dental Sciences to find out the cytogenetic changes in tobacco uses with and without precancerous lesions.

Sample Size & Its Characteristics

The patients for the study were selected among the outpatients who visited the Department of Oral Medicine and Radiology. The study group comprised of

Group 1 - Clinically healthy subjects

Group 2 - Tobacco users (smokers of 2 years smoking history) without precancerous lesions

Group 3 - Tobacco users with precancerous lesions

The sample size was calculated using software SPSS version. The sample size of each group is as follows

Group1 - 20

Group 2 - 40

Group 3 - 40

Exclusion criteria for controls (healthy volunteers)

1. Tobacco users
2. Alcohol users
3. Known history of systemic illness
4. History of malignancy

5. History of previously treated precancerous or cancerous lesions

6. History of viral infection

7. History of use of antibiotics within two months before sample collection.

8. Recent history of exposure to potential genotoxic agents, including X rays, chemotherapy and potential occupational exposures

Inclusion criteria for tobacco users without lesions:

1. Tobacco user for a minimum period of 2 years

Exclusion criteria for tobacco users without lesions:

1. Alcohol users

2. Known history of systemic illness

3. History of malignancy

4. History of previously treated precancerous or cancerous lesions

5. History of viral infection 6 months prior to sample collection

6. History of use of antibiotics within two months before sample collection.

7. Recent history of exposure to potential genotoxic agents, including X rays, chemotherapy and potential occupational exposures

Inclusion criteria for tobacco users with precancerous lesion:

1. Histo-pathologically proven cases of precancerous lesions associated with tobacco use

Exclusion criteria for tobacco users with confirmed precancerous lesions:

1. Alcohol users

2. Known history of systemic illness

3. History of malignancy

4. History of previously treated precancerous or cancerous lesions

5. History of viral infection in the past six months

6. History of antibiotics use two months prior to sample collection.

7. Recent history of exposure to potential genotoxic agents, including X rays, chemotherapy and potential occupational exposures

After screening, information on the nature and potential benefits of the study was explained to the patient. They were made to understand that the participation in this study was purely voluntary and they had the option of exiting the study at any point of time. And among the consenting volunteers who fit the inclusion and exclusion criteria were selected. A formal informed written consent was taken from all of them. A study protocol was approved by the Research Committee and the Institutional Human Ethics Committee of Sree Mookambika Institute of Medical Sciences Kulasekharam. A detailed case history of the patient was taken and a thorough clinical examination was done and recorded. Serial number was also assigned to each patient.

Armamentarium

For sample selection and case history

- A pair of sterile disposable gloves and disposable mouth masks
- Stainless steel kidney tray, mouth mirror, straight probe and tweezer
- Glass tumbler with water

For sample collection

- Microscopic slide
- Sterile wooden spatula
- Bio spray { in the RAPID - PAP™ kit} for **fixing the sample**

For staining the sample slide

- RAPID - PAP™ staining kit
- Coplin jars
- Filter/tissue paper
- Cover slip

For cytological examination

- Light microscope
- AP View software

Procedure in detail:

Sample collection

Patients were asked to rinse their mouth with water before taking smear. This helps to remove any food or artifacts that may interfere with the analysis. Buccal epithelial cells were obtained using a wooden spatula. Oral mucosa is the first line of contact with hazardous agents and the first barrier against potential carcinogens and is therefore susceptible to damage by these agents before reflecting its effects systemically. It also provides an easy access to sample collection. This technique is minimally invasive and painless and for these reasons it is well tolerated among patients. After drying, the slides were fixed using Biofix spray which comes along with the RAPID - PAP kit

Nearly 60% of oral mucosal surface is stratified non-keratinised epithelia which allow cells in the most superficial layer to maintain their nuclei well defined and almost intact which favours colorant absorption, ease of observation, proper identification of nucleus and morphological characteristic of cell with the help of microscope.

Staining of slides:

The colorant used should be basic and must have a high affinity to DNA in order to obtain a contrast so that artifacts can be easily differentiated. In this study DNA specific Papanicolaou stain was used. It is an acidic - basic stain which can produce a contrast between the cytoplasm and nucleus and there by helps in reducing the bias produced when non specific DNA stains are used.

The sample slides were stained using RAPID PAP™ {Papanicolaou's stain kit) by BIOLAB DIAGNOSTICS. Unlike the conventional PAP staining, this kit allows the staining of slides in 3 minutes. It requires minimum skills and laboratory aids for good results.

The RAPID - PAP™ staining kit consists of

Biofix spray: for cell fixation

RAPID - PAP™ NUCLEAR STAIN {Haematoxylin solution}

RAPID - PAP™ CYTOPLASM STAIN {OG-6 Solution) [2A]

RAPID - PAP^{IM} CYTOPLASM STAIN {Light Green SF-Eosin}[21»]

D.P.X GLASS MOUNTING MEDIUM

RAPID - PAP™ DEHYDRANT {Propanol}

XYLENE

RAPID - PAP™ WASH BUFFER {Scotte's tap water buffer}

Haematoxylin nuclear stain is a natural stain which has an affinity for chromatin, attaching to sulfate group in DNA molecule. It acts as acidic stain for basic neutroproteins. Its pH is 2.5-3

Scotte's tap water buffer: the pH of this solution varies from 8-8.5. This subjects haematoxylin to alkaline conditions and changes its color from red to blue.

Alcohol acts a dehydrant which helps to minimize cellular distortion, reduce cell lose from glass slide.

OG-6: it is an acidic dye that demonstrates attraction to the basic proteins like parakeratin. It is a monochromatic stain which colors keratin brilliant green. The effect of OG - 6 is evident if the smear has keratinized cells.

Light Green SF- Eosin: Eosin gives pink color to cytoplasm of mature squamous cells and nucleoli. Light green stains cytoplasm of metabolically active cells like parabasal cells intermediate squamous cells in blue. It has a C_2H_2Nreactive group which possesses an affinity for RNA of ribosomes.

Xylol: It acts as a clearing agent. It is a colorless, chemically non- reactive and has almost the same refractive index as that of glass. Thus it helps to give transparency to image.

D.P.X Mountant: it acts as a permanent bond between the slide and cover slip, protects the cell material from air drying and shrinkage. It also acts as a seal against oxidation and fading of stain.

Reagent Preparation:

Cytoplasm stain is prepared by mixing equal volume of cytoplasm stain 2A and 2B. It is then stored in an air tight bottle to prevent contamination from water and other pollutants.

Steps in slide staining using RAPID PAP™ kit

1. Dip the fixed smear for 3 minutes in tap water and blot the excess water from the slide.
2. Dip 60 seconds in RAPID PAP™ Nuclear Stain.

3. Add 3 drops of Scott Tap Water buffer and wash after 10 seconds. Blot out the excess water from the slides.
4. Dip with the two changes in RAPID PAP™ dehydrant for 30 seconds each
5. Dip in 45 seconds in working cytoplasm stain.
6. Wash in tap water and blot out excess water from the slide
7. Repeat dehydration in a second bath of RAPID PAP™ dehydrant for 30 seconds and dry.
8. Dip in xylene, dry and mount with cover glass using a drop of D.P.X.

Cytological examination:

The stained slides are examined using light microscope first in 10X magnification and then 40X magnification. AP viewer software is used for the visualization of cells and for taking photomicrographs. The cells are selected for scoring as per **Tolbert's criteria** (1992) (1) cytoplasm intact and lying relatively flat; (2) little or no overlap with adjacent cells; (3) little or no debris; and (4) nucleus normal and intact, nuclear perimeter smooth and distinct.

The micronucleus is identified with the help of the suggested criteria by Fenech el al {2003}

1. The diameter of the MN should be less than 1/3 rd of the main nucleus
2. MN should be separate or marginally overlap from the main nucleus as long as there is a clear identification of nuclear boundary.
3. MN has similar staining as the main nucleus

The purpose of this study was to evaluate the cytogenetic changes seen in smokers of two years duration and in tobacco users with precancerous lesions in comparison with the healthy controls by the frequency of occurrence of micronuclei using PAP stain. On examination of the stained slides under light microscope of 40X magnification, the numbers of micro nucleated cells were counted per 500 cells.

The data was analyzed by Statistical Package for Social Sciences (SPSS 16.0) version. ANOVA (Post hoc) followed by Dunnet t test applied to find statistical significant between the groups. p value less than 0.05 ($p<0.05$) considered statistically significant at 95% confidence interval.

The results are as follows:

Group 1 consisted of normal healthy individuals showed MN mean value **M= 4.25** (P=0.001) with the standard deviation value of **SD=0.96**.(Table 1)

Group 2 consisted of smokers of two years duration showed the mean value **M=13.30** (P=0.001) and the standard deviation value of **SD=2.98** there by indicating a significant rise in the mean value when compared to the control group.(Table 1)

Group 3 consisted of histopathologically proven precancerous lesion (leukoplakia) showed the mean value **M=35.70** (P=0.001) and the standard deviation value of **SD= 6.68** which shows a significant rise when compared to the other two groups.(Table 1)

Among the three groups, the healthy controls exhibited values ranging from **3-6 cells** with micronuclei /500 cells. The healthy smokers of two year smoking history showed a range between **10-18 cells** with micronuclei /500cells.

Histopathologically proven precancerous lesion (leukoplakia) group patients showed values ranging from **31-41 cells** with micronuclei / 500 cells. The increase in the frequency is very evident from the values obtained which correlates with the increased and abnormal mitotic process at cellular level.

Table-1: Mean number of micronuclei seen per 500 cells of different groups

Groups	Group description	Number of micronuclei seen per 500 cells (MEAN±SD)
Group-I	**Controls**	4.25±0.96
Group-II	**Smokers for 2 years**	13.30±2.98
Group-III	**Tobacco users with precancerous lesion**	35.70±6.68

Table-2: Comparison of mean number of micronuclei seen per 500 cells Group-I with other groups

Groups	Number of micronuclei seen per 500 cells (MEAN±SD)	P value
Group-I	4.25±0.96	
Group-II	13.30±2.98*	**0.001**
Group-III	35.70±6.68*	**0.001**

(*p<0.05 significant compared Group-I with other groups)

Table-3: Comparison of mean number of micronuclei seen per 500 cells Group-III with other groups

Groups	Number of micronuclei seen per 500 cells (MEAN±SD)	P value
Group-III	35.70±6.68	
Group-I	4.25±0.96*	**0.001**
Group-II	13.30±2.98*	**0.001**

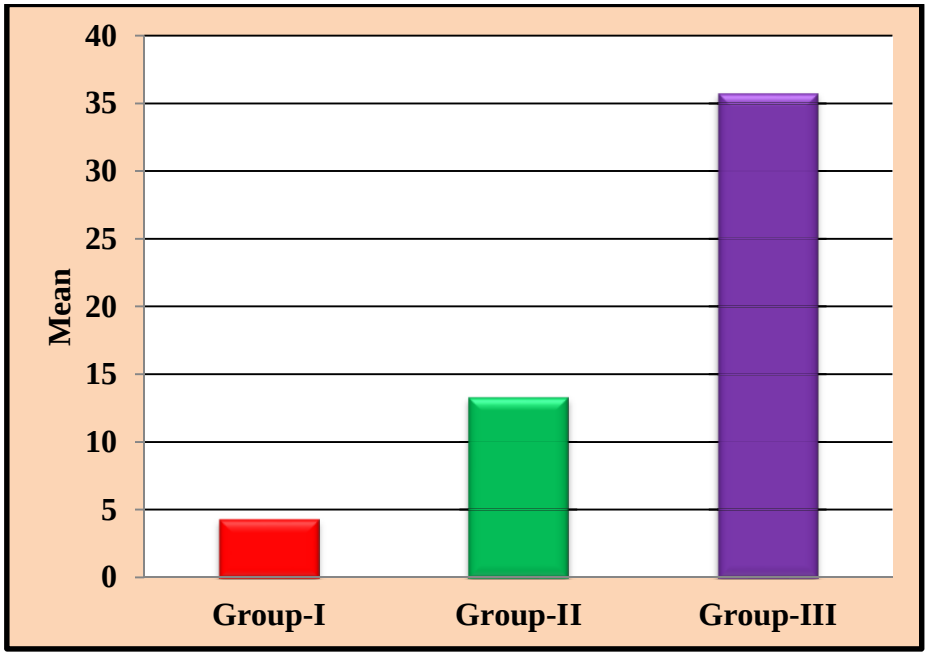
(*p<0.05 significant compared Group-III with other groups

Table-4: Multiple comparison of mean number of micronuclei seen per 500 cells between the groups

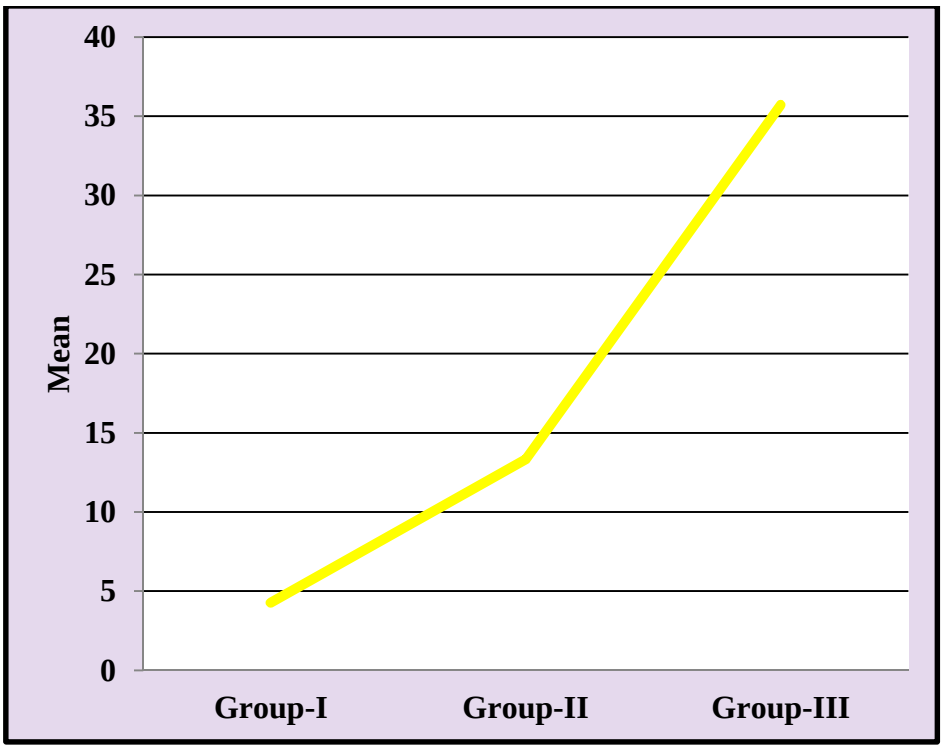
Groups	Number of micronuclei seen per 500 cells (MEAN±SD)	P value
Group-I	4.25±0.96	
Group-II	13.30±2.98*	**0.001**
Group-III	35.70±6.68*,#	**0.001**

(*p<0.05 significant compared Group-I with other groups, #p<0.05 significant compared Group-II with other groups)

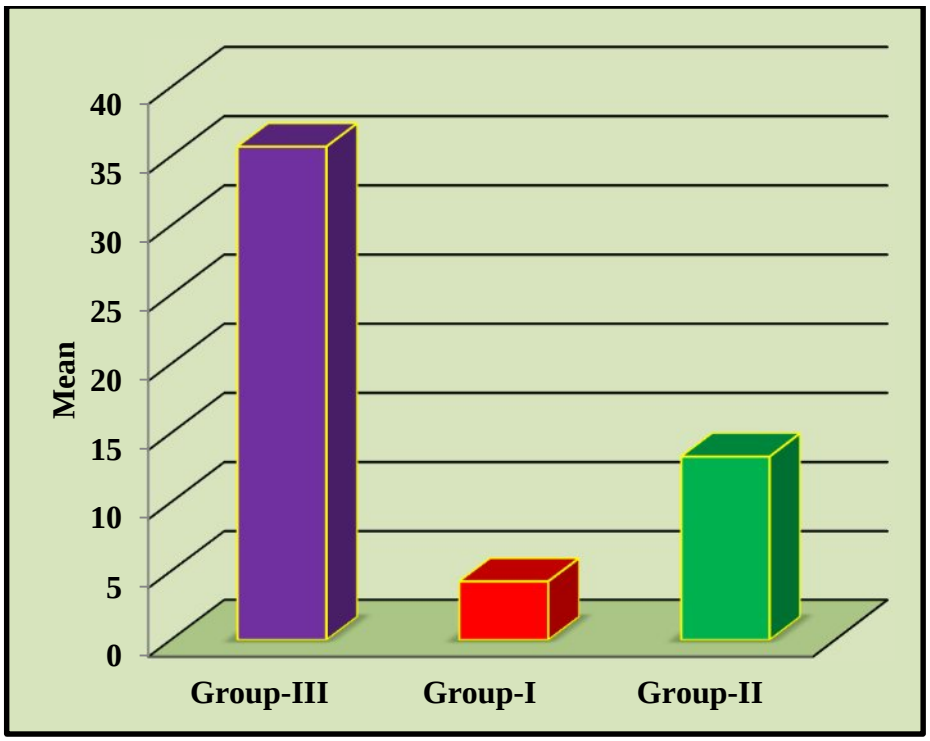
Graph-1: Mean number of micronuclei seen per 500 cells of different groups



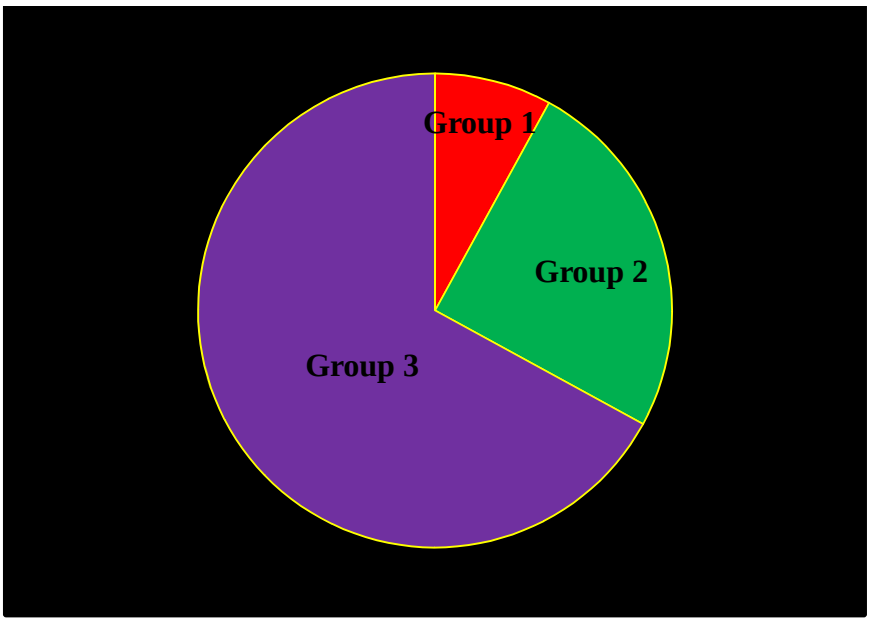
Graph-2: Comparison of mean number of micronuclei seen per 500 cells Group-I with other groups



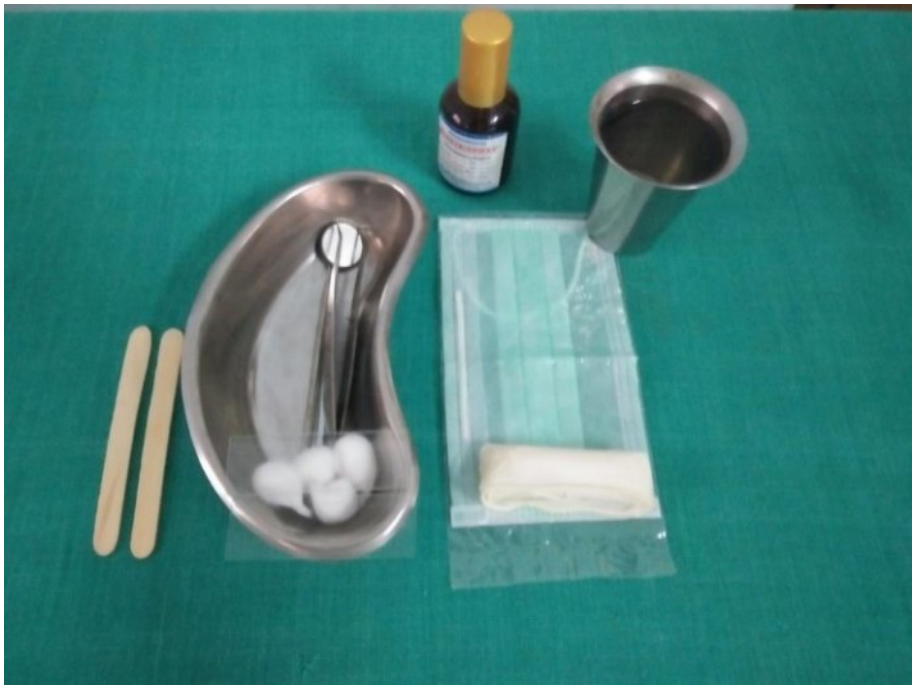
Graph-3: Comparison of mean number of micronuclei seen per 500 cells Group-III with other groups



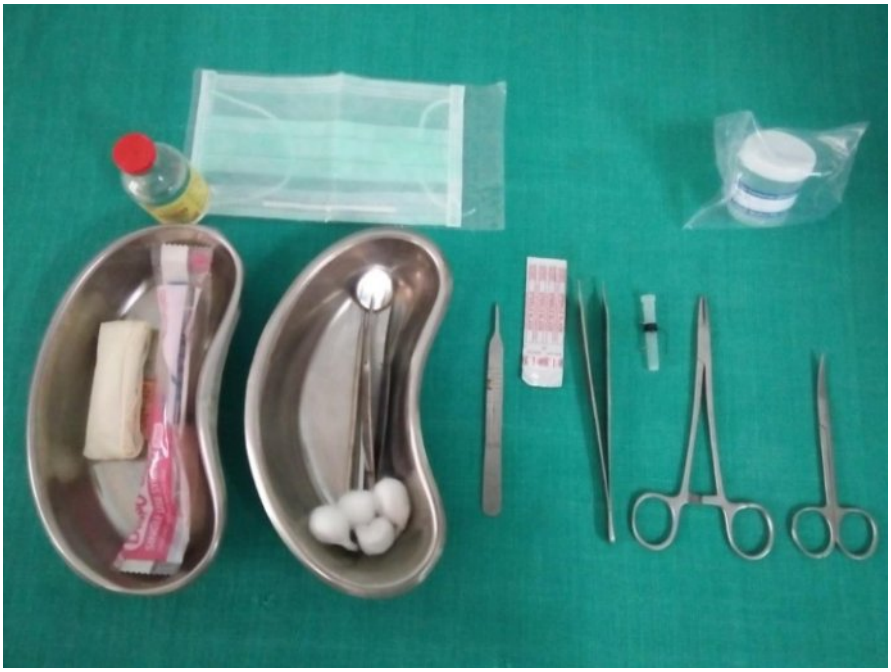
Graph-4: Multiple comparison of mean number of micronuclei seen per 500 cells between the groups



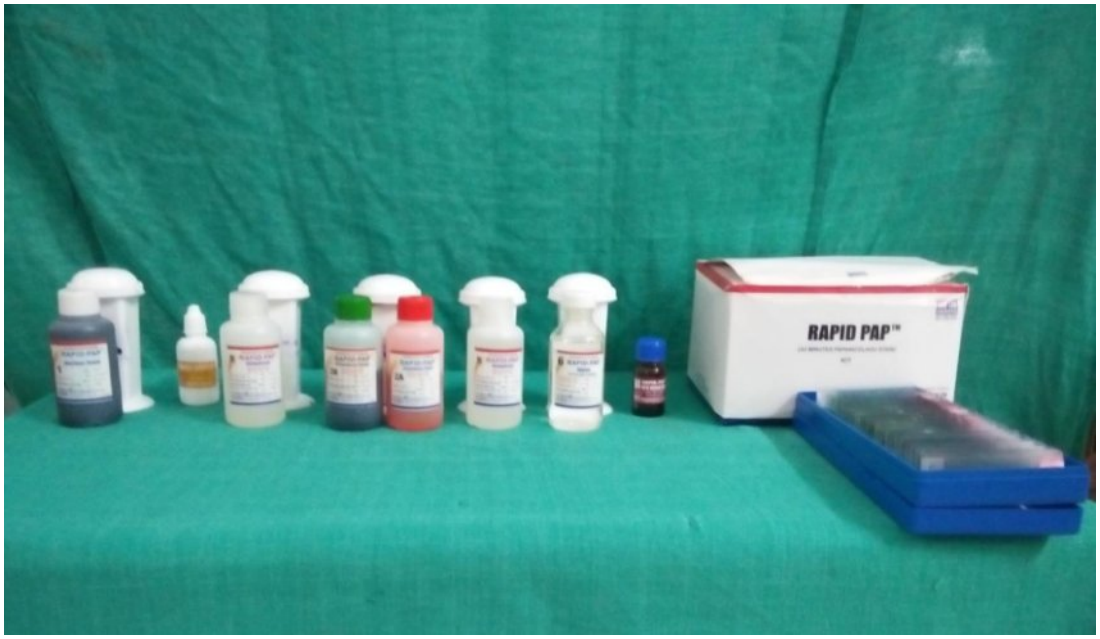
Colour plate 1: Armamentarium for sample collection



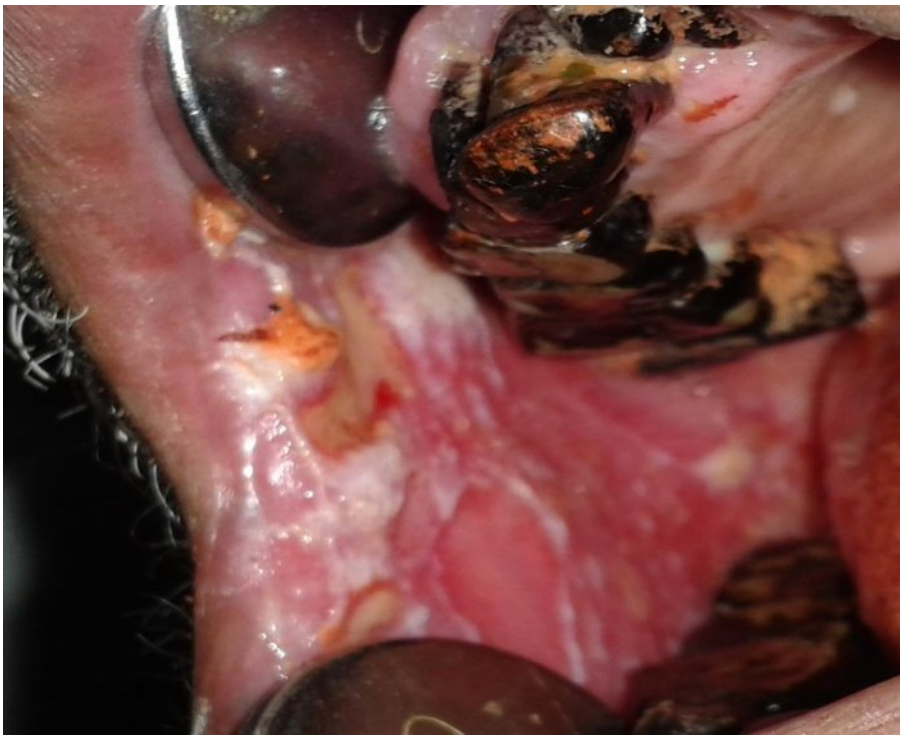
Colour plate 2: Armamentarium for biopsy



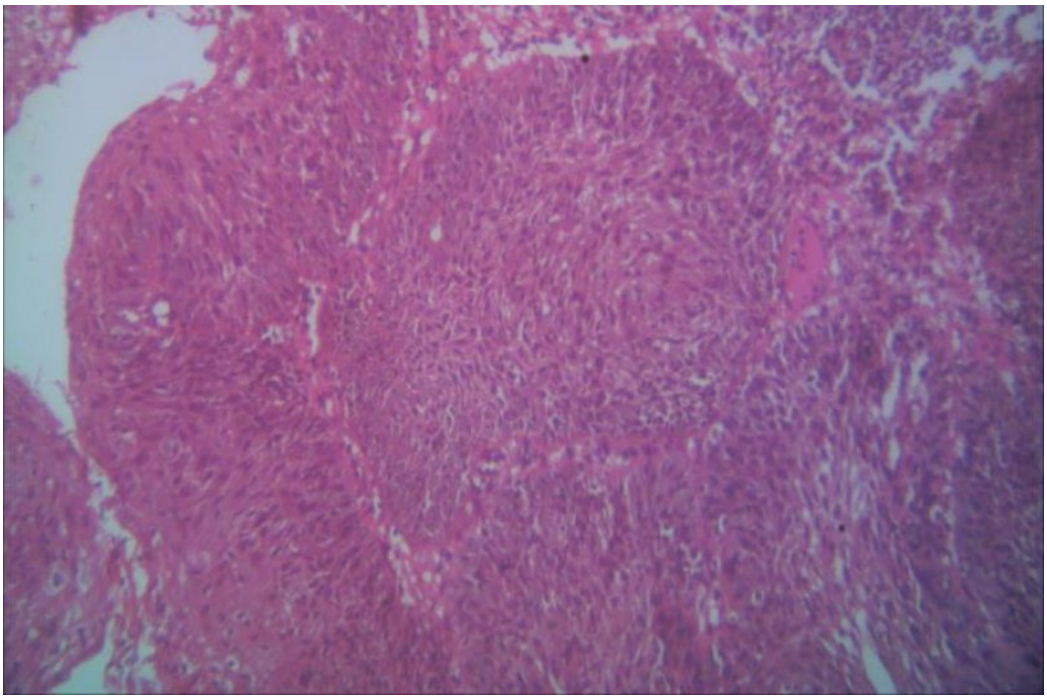
Colour plate 3: Rapid PAP^{TM} staining kit



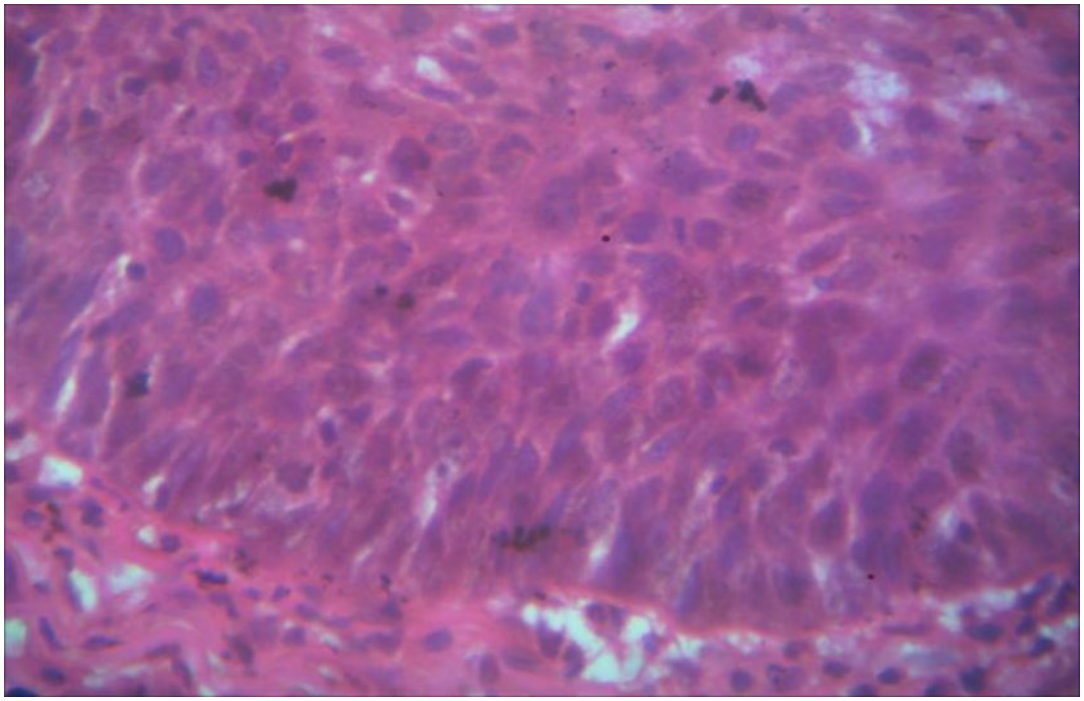
Colour plate 4: Clinical picture of Leukoplakia



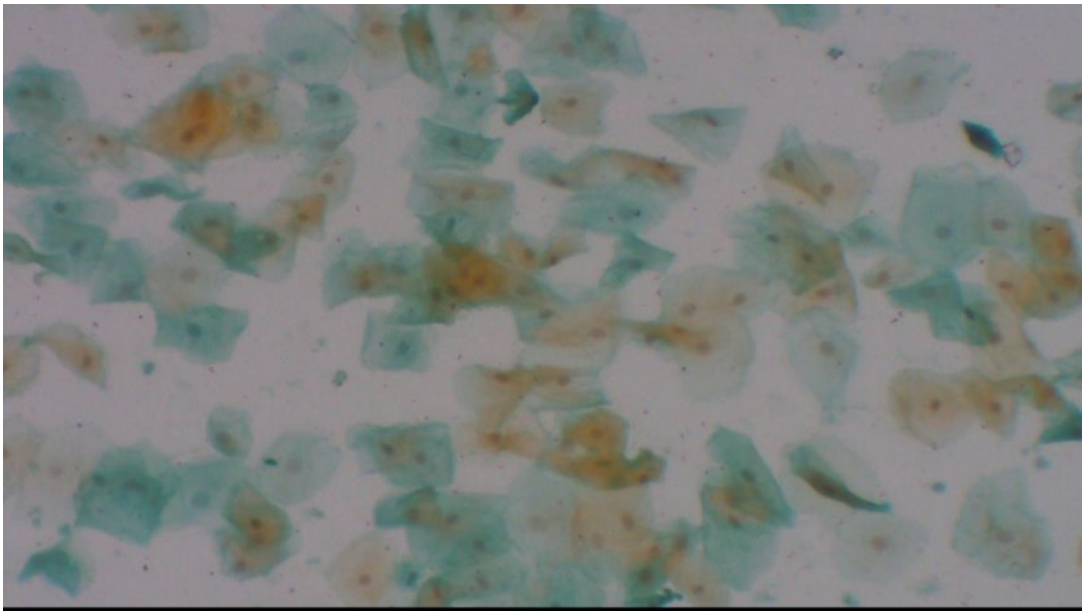
Colour plate 5: Photomicrograph showing Haematoxylin and Eosin stained section of oral epithelial severe dysplasia x100



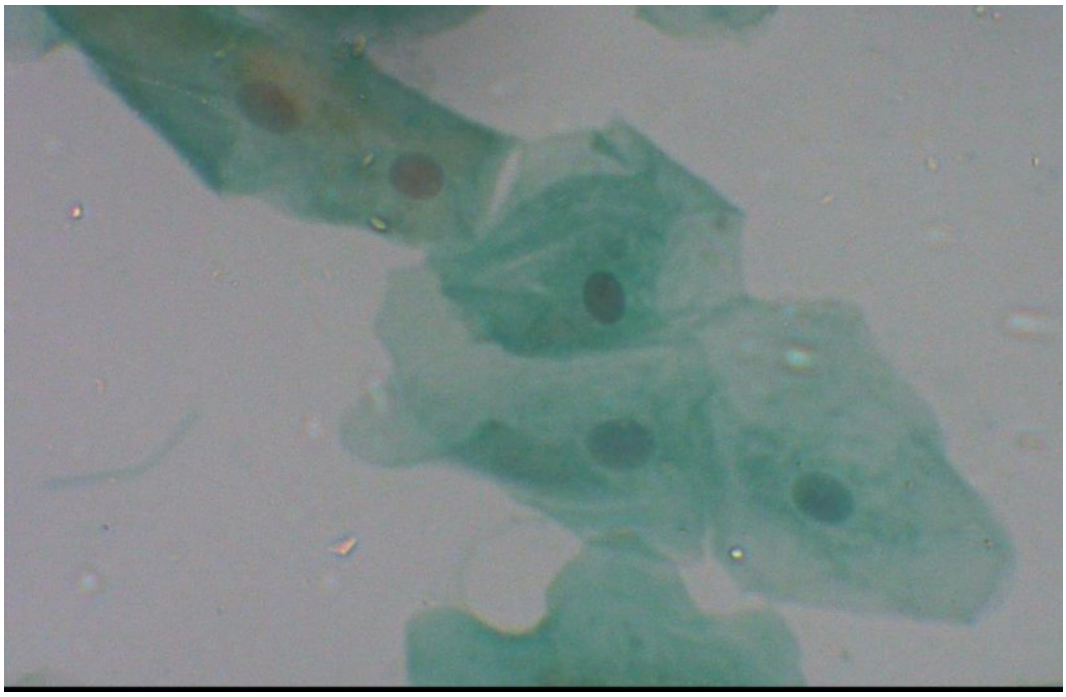
Colour plate 6: Photomicrograph showing Haematoxylin and Eosin stained section of oral epithelial severe dysplasia x400



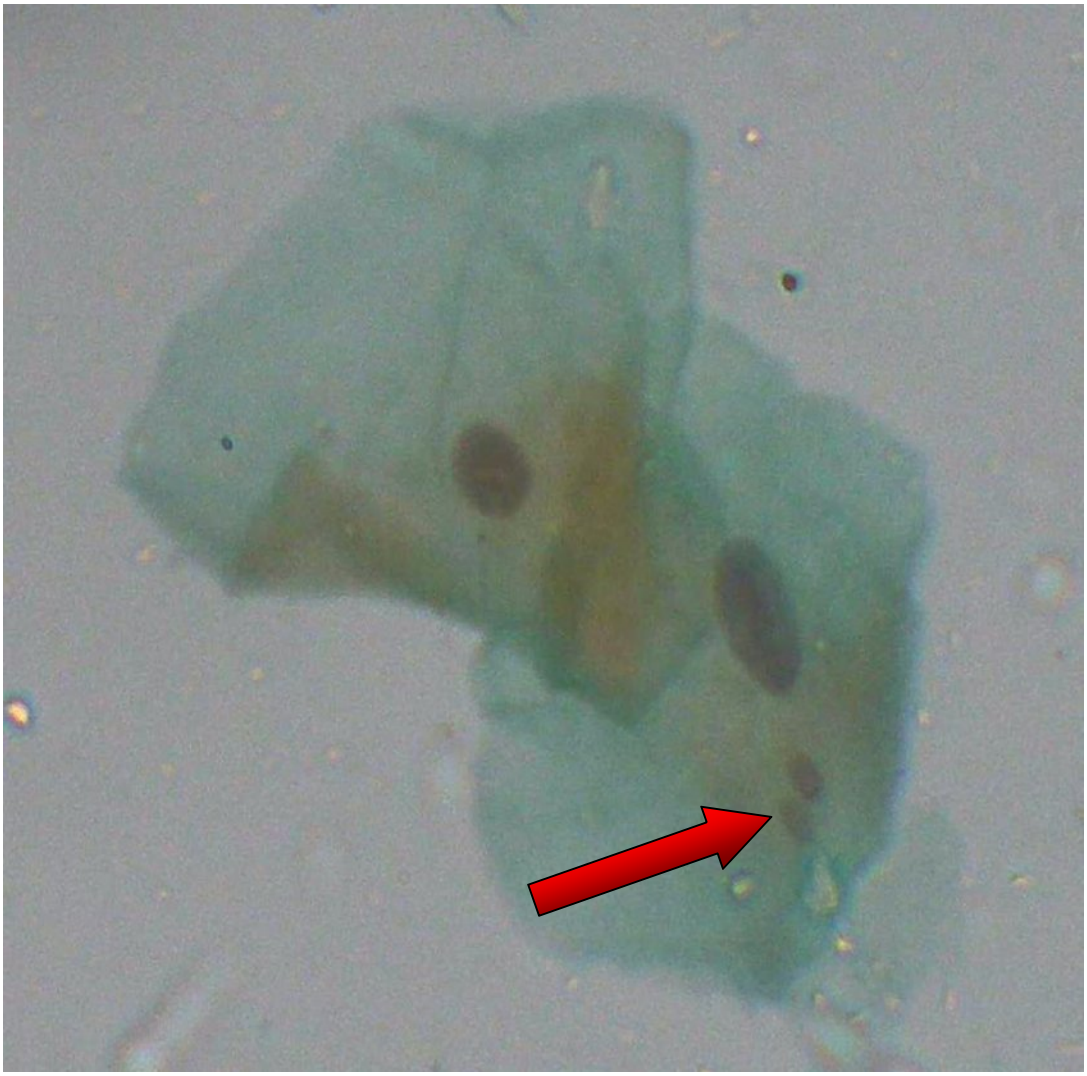
Colour plate 7: Photomicrograph showing normal exfoliated cell (Buccal smear, PAP stain x100)



Colour plate 8: Photomicrograph showing normal exfoliated cell (Buccal smear, PAP stain x400)



Colour plate 9: Photomicrograph showing Micronucleated cell (Buccal smear, PAP stain x400)



This study was conducted in the Department of Oral Medicine and Radiology, Sree Mookambika institute of Dental Sciences, Kulashekaram, Kanyakumari district. The total sample size was 100.This includes three groups

Group 1- consisted of 20 normal healthy controls

Group 2- consisted of 40 healthy smokers of two years smoking history

Group 3- consisted of 40 histopathologically proven precancerous lesions (Leukoplakia)

The present study consisted of three groups, Group 1 showed the values ranging from **3-6** cells with micronuclei /500 cells with a mean value M= 4.25 (P=0.001). This is concurrent with the study conducted among the population of Punjab by Bansal et al, whose control group showed the mean value of micronuclei occurrence per 1000 cells as 4.17.MN formation is induced by several other factors like systemic diseases, antibiotic therapy, radiation exposure, wearing prosthesis, etc., hence all these factors have been excluded to reduce the bias.[3] This observation was similar to those reported by Palaskar et al., Ozkul et al., and Patel et al., when compared with the mean difference, the result was highly statistically significant($P < 0.05$) which was in accordance with Palaskar et al., and Patel et al and Ozkul et al., found no difference between the mean percentage of MN cells for the group considered ($P > 0.05$). [3,81,97,98] MN in buccal cells originate from genome damage events in the basal layer of the oral mucosa, which has the turn over for every 21 days.[99] It has been shown frequently that clastogenic and aneugenic effects in somatic cells are associated with the development of cancer.[100,101] There are number of earlier studies which have shown that the MN assay with exfoliated cells is an appropriate

tool to monitor cancer risks in humans caused by exposure to environmental factors or inherited genomic instability.[102,103] It has been hypothesized about direct association between the frequency of MN in target tissues and cancer development, supported by different findings: like, increase in the frequency of MN in target tissues and lymphocytes in cancer patients.[104,105] Clinical chemoprevention trials on oral pre-malignancies have used MN in oral mucosa as a surrogate endpoint of cancer.[99] A correlation exists between carcinogenicity and genotoxicity for some agents who are able to increase MN frequencies in humans and in animals, e.g., ionizing radiation, ethylene oxide, benzene, tobacco smoke.[101] Many studies showed Chromosomal Aberrations (CA) analysis from lymphocyte culture also measures the genome damage that get accumulated while lymphocytes circulate around the body in the quiescent phase.[99] While comparing chromosomal aberrations analysis with MN count index, MN count gives index of recent damage which is tissue specific and reflects the tobacco associated mucosal damage. MN test is better indicator for genotoxicity damage than CA.[98]

Group 2 consisted of smokers with two years duration showed the range of values between 10-18 cells with micronuclei /500cells with the mean value M=13.30 (P=0.001) and standard deviation value SD = 2.98 there by indicating a 3 fold rise in the mean value when compared to the controls. This is in accordance with a previous micronuclei study conducted by Palaskar S and Jindal C, among the rural population of Haryana.[97] These findings are consistent with the studies of Sellapa et al and Patel et al.[98,106] In a study by Suhas et al.,on buccal cell changes which are associated with smoking by using the micronuclei assay, there was found to be a significant correlation between the habit of smoking and the frequency of the micro nucleated oral mucosal cells. The results of present study are in accordance with this study.[107]

Group 3 includes histopathologically proven precancerous lesion (leukoplakia) showed the values ranging from 31-41 cells with micronuclei / 500 cells with a mean value M= 35.70(P=0.001) which shows a significant rise when compared to the other two groups and this correlates with the comparative study conducted by Pratheeba Sivasankari et al, among the potentially malignant lesions has the statistical significant mean value M= MN/500 cells.[96] Recent study conducted by Pratheepa Sivasankari et al in 2015, revealed a correlation of significantly increased frequency of micro nucleus present in users of alcohol and smoking in combination and betel nut chewers as compared to normal counterparts, indicating strong cytogenetic damage which may lead to cancerous proliferation.[108] Carcinogenic and mutagenic compounds, including tobacco-specific nitrosamines, are believed to be responsible for the induction of micronuclei.[109] These compounds are produced from nicotine by bacterial or enzymatic activity. The same formation occurs in the mouth under the influence of saliva.[110] There are other non invasive investigations of saliva which could be used to diagnose oral malignancies, where the apoptotic cells among the exfoliated epithelial cells is evaluated using immunoassay method as there is a dysregulation in apoptosis of the epithelial cells which is seen in the pathogenesis of smoking related oral changes.[111] Also Cytology with DNA-cytometry has emerged as a highly sensitive and non-invasive method for the early diagnosis of oral epithelial neoplasia.[112] Although the salivary laboratory investigations are noninvasive (no needles) ,as is the collection of saliva (any time of day/month, any place) is optimized, but requires specialised processing (eg, centrifugation, ice-packs) ,also technically more challenging: and needs 10-20 x sensitivity. Interference of substances-food, beverages and its cost, are the disadvantages faced.[113] There are also many other cytogenetic and molecular genetic techniques providing information about

the specific and subtle genetic changes. Somatic mutations are promising biomarkers for cancer risk as these can capture genetic events that are associated with malignant transformation.[114]Tumor markers may be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm are released by cancer cells or produced by the host in response to cancerous substances .[115]Of which the use of cytokeratin markers are used in detecting Oral Squamous Cell Carcinoma helps in analyzing the altered keratin expression in the oral site especially the buccal mucosa.[116] Flow cytometric analysis of DNA content is an automated technique which rapidly allows large number of cells to be measured, has been extensively used for the assessment of proliferation status. The challenges in the biomarkers using genomic and proteomic diagnostic technology like previously stated analysis include the development of complex mathematical algorithms to handle simultaneous analysis of many parameters to aid the diagnosis instead of a single parameter. Further, issues regarding quality control methods and procedures also need to be developed for using these markers with reliability and reproducibility.[117] Several staining methods have been used for the evaluation of MN. Although DNA specific stains are preferred for staining MN, the most commonly used staining procedure is Feulgen staining reaction. Armen Nerseyan et al., reported almost identical outcome in a study carried out on heavy smokers and non-smokers using Feulgen stain. They manifested pronounced rise in mean MN cells in heavy smokers with non-specific DNA stain as compared with DNA specific stain and also observed two times the number of MN in non specific stain than the specific stain. This variation is due to the turnover time period, MN cells produced in the basal cells could lyse and go missing before making it to the top layers. Therefore, the frequency of MN may be wrongly estimated in DNA specific stain too.[118] Liquid based cytology is an improvement in cytology

technique that can compensate many disadvantages of conventional exfoliate cytology. Many artifacts of conventional cytology do not occur in LBC technique. By employing this technique unsatisfactory slides and false negative results have been reduced and the diagnostic value of cytology has improved.[119]Liquid-based cytology with DNA-specific stains (Feulgen/fast green) could be suggested for the best results. However the staining procedure is lengthy and also technique sensitive and may lead to under scoring of MN. [120] The problem with LBC is the studies in oral cavity are usually performed using cervical or dermatological tools for sample collection and never a specialized oral tool (e.g. CDx brush) has been employed. Hence LBC technique employing a specialized oral brush (OralCDx® Brush), simultaneously was developed called a modified liquid-based cytology using OralCDx® Brush. Even though this has better diagnostic agreements, it is technique sensitive, time consuming and expensive and also needs specialised armamentarium.[119] In the present study, PAP stain is used. It is done using RAPID-PAP ^{TM} kit. It is rapid, easy to read and the fixative used has a bactericidal effect and the nuclear stain stains both intra nuclear and extra nuclear DNA and cytoplasmic stain help to maintain the transparency of cytoplasm and makes the boundary well demarcated. [1] The added advantage between cytoplasm of non keratinised and keratinised cells where it appears as blue/green in former and pink /orange latter. The nucleus appears blue/black.[79]

A screening test must be evaluated with respect to their diagnostic value. This value includes sensitivity, specificity, positive predictive value and negative predictive value.[119] Oral exfoliative cytology has been used extensively for screening cellular alteration. An accuracy of 95% and a reliability of more than 96% in detection of squamous cell carcinoma in mass screening have been reported in the literature.[121] Thus the minimal invasiveness of cell collection, low cost, ease of

storage and slide preparation make the MN assay with buccal epithelial cells the ideal choice for molecular epidemiological studies.[63] Hence in molecular epidemiology and cytogenetics the frequency of MN in exfoliated cells is extensively used as a biomarker to evaluate the presence and the extent of chromosomal damage in human populations exposed to genotoxic agents or bearing a susceptible genetic profile and genomic stability in human populations.[122,123,124]

Our study proves the efficacy of MN detection as an effective predictor of cytogenetic changes and could be considered a useful tool in the assessment of cellular changes in the oral cavity. Additionally, the test is sensitive enough to detect cellular changes well before clinically appreciable changes have occurred and hence possibly improving prognosis by early detection and an effective patient education and motivation kit to deter further continuation of deleterious habits.

The purpose of this study was to evaluate the cytogenetic changes seen in smokers of two years duration and in tobacco users with precancerous lesions in comparison with the healthy controls by the frequency of occurrence of micronuclei using RAPID PAP stain. Among the three groups, the healthy controls exhibited values ranging from **3-6 cells** with micronuclei /500 cells. The healthy smokers of two year smoking history showed a range between **10-18** cells with micronuclei/500cells. Histopathologically proven precancerous lesion (leukoplakia) group patients showed values ranging from **31-41** cells with micronuclei / 500 cells. The increase in the frequency is very evident from the values obtained which correlates with the increased and abnormal mitotic process at cellular level and the results were statistically significant. Analysis of the data obtained showed that MN were present in all groups and the frequency increases in tobacco users without lesions and further increase in MN was noticed in tobacco users with lesions. We conclude that Micronuclei assay can be used to detect early genotoxic changes and could be used as an effective tool to improve patient care, enhance prognosis and educate patients well before clinically appreciable changes have occurred.

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SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

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Institutional Human Ethics Committee (IHEC)

{CDSCO Reg No: ECR/446/Inst/TN/2013}

Ref. No: SMIMS/IHEC/2015/A/30 **Date: 17th February 2016**

CERTIFICATE

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2015/A/30** entitled "Cytogenetic Changes in Oral Exfoliated Cells of Tobacco Users without Lesions and Tobacco Users with Precancerous Lesions Using Papanicolaou Stain: A Comparative Study" submitted by Dr. Hema Mareeswari G, Postgraduate of Department of Oral Medicine and Radiology, SMIDS has been approved by the Institutional Human Ethics Committee at its meeting held on 15th December 2015.

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor and HOD of Pharmacology
SMIMS, Kulasekharam (K.K District)
Tamil Nadu-629161

[This Institutional Human Ethics Committee is organized and is operating according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES

KULASEKHARAM, KANYAKUMARI DIST., TAMIL NADU, INDIA.

INSTITUTIONAL RESEARCH COMMITTEE

Certificate

This is to certify that the research project protocol, ***Ref no. 09/06/2015*** titled, ***"Cytogenetic changes in oral exfoliated cells of tobacco users without lesions and tobacco users with precancerous lesions using Papanicolaou stain: a comparative study"*** submitted by ***Dr. Hema Mareeswari G., II Year MDS, Department of Oral Medicine and Radiology*** has been approved by the Institutional Research Committee at its meeting held on **23rd *June 2015.***

Convener
Dr. T. Sreelal

Secretary
Dr. Pradeesh Sathyan

CONSENT FORM

PART 1 OF 2

INFORMATION FOR PARTICIPANTS OF THE STUDY

Dear Volunteers,

We welcome you and thank you for your keen interest in participation in this research project. Before you participate in this study, it is important for you to understand why this research is being carried out. This form will provide you all the relevant details of this research. It will explain the nature, the purpose, the benefits, the risks, the discomforts, the precautions and the information about how this project will be carried out. It is important that you read and understand the contents of the form carefully. This form may contain certain scientific terms and hence, if you have any doubts or if you want more information, you are free to ask the study personnel or the contact person mentioned below before you give your consent and also at any time during the entire course of the project.

1. **Name of the Principal Investigator:**

G.Hema Mareeswari

Post Graduate student

Department of Oral Medicine and Radiology,

Sree Mookambika Institute of Dental Sciences,

Kulasekharam, KanyaKumari District-629161

2. **Name of the Guide:**

Dr.Tatu E Joy. MDS.

HOD and professor

Department of Oral Medicine and Radiology.

Sree Mookambika Institute of Dental Sciences.

Kulasekharam, KanyaKumari District-629161

3. Name of the Co-Guide:

Dr. Shashi Kiran M. MDS.

Reader

Department of Oral Medicine and Radiology.

Sree Mookambika Institute of Dental Sciences.

Kulasekharam, KanyaKumari District-629161

4. Institute:

Sree Mookambika Institute of Dental Sciences,

V.P.M Hospital complex, Padanilam,

Kulasekharam,

Kanyakumari – 629161

Tamilnadu

5. Title of the study: "Cytogenetic changes in oral exfoliated cells of tobacco users without lesions and tobacco users with precancerous lesions using Papanicolaou stain: a comparative study"

6. Background information:

Micronuclei are induced in cells by a variety of substances like UV radiation, infrared rays, X-radiation, and chemicals. Among them tobacco- specific nitrosamines have been reported to be potent mutagenic agents which are thought to be responsible for the induction of chromosomal aberrations resulting in production of micronuclei. Hence increase in micronuclei frequency before occurrence of clinically evident lesion will be a useful early indicator of cytogenic changes in tobacco users.

7. Aims and Objectives:

i. To evaluate the frequency of genotoxic changes in smears of exfoliated oral epithelial cells using PAP stain in tobacco users without lesions and tobacco users with precancerous lesions as compared with controls.

ii. Comparison of the frequency of occurrence of genotoxic changes between tobacco users with precancerous lesions and tobacco users without clinically evident precancerous lesions compared with healthy controls.

iii. To propose oral mucosal micronuclei frequency as an early potential marker of genotoxic changes in oral epithelial cells.

8. Scientific justification of the study:

Early identification of the process of carcinogenesis is challenging and when decoded, would provide an efficient diagnostic and prognostic tool in the early identification of neoplastic change in the tissues. Micronuclei could act as an efficient surrogate biomarker of this process. Moreover, the micronuclei test is a non-invasive diagnostic test with a sensitivity of 94%, specificity of 100% and accuracy of 95%. Thereby micronuclei identification could act as an early predictor of cytogenetic changes in the oral epithelial cells of tobacco users

9. Procedure for the study:

A total of 100 subjects are selected from among the outpatients who attended the Department of Oral medicine and radiology, Sree Mookambika Institute of Dental Sciences. The tobacco users without any lesions who had tobacco in any form every day for at least two years and the tobacco users with histopathologically confirmed precancerous lesions are included in their respective groups. The control group persons were not habituated to any form of tobacco consumption. Each subject was asked about his lifestyle, food consumption, infectious diseases, X-ray exposure, medication, etc.

Individuals who had a recent viral infection or had been exposed to X-rays or those who had been under medication were excluded from the study. The lifestyle (except the habit of tobacco) and dietary habits of the controls were similar to those of the users.

Cytological preparations and examination

Before sampling, each individual rinsed his/her mouth thoroughly with tap water. The exfoliated cells were obtained by scraping the buccal mucosa with a moistened wooden spatula. The scraped cells were placed onto pre-cleaned slide. The slide was wet fixed and stained with PAP.

For designating an extra nuclear body as micronucleus, the following criteria given by Tolbert will be considered.

(a) Rounded smooth perimeter suggestive of a membrane.

(b) Less than a third the diameter of the associated nucleus, but large enough to discern shape and colour.

(c) Staining intensity similar to that of the nucleus.

(d) Texture similar to that of nucleus.

(e) Same focal plane as nucleus.

(f) Absence of overlap with, or bridge to, the nucleus.

Accordingly, 500 cells will be scored to determine the micronuclei frequency

10. Expected risks for the participants:

The previous study conducted did not report any complication or risk other than the slight pain and even that can be overcome by topical application of local anesthesia.

11. Expected benefits of research for the participants:

- You will not be required to pay for this lab test.
- You can enquire about the outcome of the procedures and your details.
- You will get a better treatment at the end of the procedure.

12. Maintenance of confidentiality:

a. You have the right to confidentiality regarding the privacy of your medical information

(Personal details, results of physical examinations, investigations, and your medical history).

b. By signing this document, you will be allowing the research team investigators, other study Personnel, sponsors, institutional ethics committee and any person or agency required by law to view your data, if required.

c. The results of clinical tests and therapy performed as part of this research may be included in your medical record.

d. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

13. Why have I been chosen to be in this study?

a. Chosen because of groping under the inclusion and exclusion criteria

b. Need of good sampling size

c. No invasive procedure that harms your health is used, it helps in diagnosis and will be helpful for the society

14. How many people will be in the study? 100 individuals

15. Agreement of compensation to the participants (In case of a study related injury): Patient will be taken care in case of complication and medical treatment will be provided in the institution.

16. Anticipated prorated payment, if any, to the participant(s) of the study: Not applicable

17. Can I withdraw from the study at any time during the study period?

- The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.
- However, it is advisable that you talk to the research team prior to stopping information.

18. If there is any new findings/information, would I be informed? Yes

19. Expected duration of the participant's participation in the study: 1 month

20. Any other pertinent information: No other information

21. Whom do I contact for further information?

For any study related queries, you are free to contact:

G.Hema Mareeswari
Post Graduate student.
Department of Oral Medicine and Radiology,
Sree Mookambika Institute of Dental Sciences,
Kulasekharam, KanyaKumari District-629161.
Mobile No: 09442092087
vidhushamayag@gmail.com

Place:

Date: **Signature of Principal Investigator**

Signature of the participant

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement or medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free **to** withdraw at any time, without giving any reason, without the medical care that will **normally** be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled

"Cytogenetic changes in oral exfoliated cells of tobacco users without lesions and tobacco users with precancerous lesions using Papanicolaou stain: a comparative study"

Serial no / Reference no:

Name of the participant: Address of the participant:

Contact number of the participant:

Signature / thumb impression of the participant / Legal guardian

Witnesses:

1.

2.

Date:

Place:

ஒப்புதல் வாக்குமூலம்

முதல் பாகம்

ஆராய்ச்சியில் பங்குபெறுவோருக்கான தகவல் குறிப்பு

அன்பார்ந்த பங்கேற்பாளர்களே,

இந்த ஆராய்ச்சியில் தங்களை ஈடுபடுத்திக்கொள்ள மிகுந்த ஆர்வத்துடன் முழுமனதுடன் கலந்துகொள்ள வந்த வரவேற்பாளர்களை வரவேற்கிறேன். நீங்கள் இந்த ஆராய்ச்சியில் பங்கெடுத்துக்கொள்வதற்கு முன் இந்த ஆராய்ச்சி எதற்காக நடத்தப்படுகிறது என்பதை தெளிவாக புரிந்துகொள்ளவேண்டும். உங்களுக்கு தேவையான அனைத்து விபரங்களும் கீழே கொடுக்கப்பட்டுள்ளது. இந்த ஆராய்ச்சியின் மூலம் ஏற்படும் நன்மைகள், ஏதேனும் ஆபத்துகள் மற்றும் அதற்காக மற்றும் எவ்வாறு இந்த ஆராய்ச்சி மேற்கொள்ளப்படும் முறைகளையும் தெரிவிக்கப்பட்டுள்ளது. இதில் கொடுக்கப்பட்டுள்ள விபரங்களை தெளிவாக படித்து புரிந்து கொள்ளவேண்டும். நீங்கள் ஆராய்ச்சியில் பங்கேற்பாளர்களாக ஒப்புதல் வழங்குவதற்கு முன்பு உங்களுக்கு ஏற்படும் அறிவியல் சார்ந்த சந்தேகங்கள் மற்றும் ஆராய்ச்சி சம்பந்தப்பட்ட சந்தேகங்கள் அனைத்தும் இந்த ஆராய்ச்சியின் எந்த காலகட்டத்திலும் நீங்கள் படிவத்தில் குறிப்பிட்ட நபரிடம் கேட்டு தெளிவுபடுத்திக்கொள்ளலாம்.

1. தலைமை ஆய்வாளர் : ***டாக்டர். ஹேமா மாரீஸ்வரி. ஜி***
   தகுதி : முதுகலை மாணவி (MDS)
   பிரிவு : ஓரல் மெடிசின் மற்றும் ரேடியோளஜி
   நிறுவனம் : ஸ்ரீ முகாம்பிகா இன்ஸ்டிட்யூட் ஆப் டென்டல் சயன்ஸஸ்,
   இடம் : குலசேகரம் - 629 161.

2. வழிகாட்டி : ***டாக்டர். டாட்டு இ. ஜாய்***, MDS.
   தகுதி : தலைமையாளர், பேராசிரியர்
   பிரிவு : ஓரல் மெடிசின் மற்றும் ரேடியோளஜி,
   நிறுவனம் : ஸ்ரீ முகாம்பிகா இன்ஸ்டிட்யூட் ஆப் டென்டல் சயன்ஸஸ்,
   இடம் : குலசேகரம்- 629 161

3. இணைவழிகாட்டி : ***டாக்டர். சசி கிரண். எம்.*** MDS.
   தகுதி : பேராசிரியர்
   பிரிவு : ஓரல் மெடிசின் மற்றும் ரேடியோளஜி,
   நிறுவனம் : ஸ்ரீ முகாம்பிகா இன்ஸ்டிட்யூட் ஆப் டென்டல் சயன்ஸஸ்,
   இடம் : குலசேகரம் - 629 161.

4. ***கல்லூரி :*** ஸ்ரீ முகாம்பிகா இன்ஸ்டிட்யூட் ஆப் டென்டல் சயன்ஸஸ், படநிலம் குலசேகரம்- 629 161.

5. ஆராய்ச்சியின் தலைப்பு

பேப் சாயம் பயன்படுத்தி புகையிலை பயன்படுத்துபவர்களுள் புற்றுநோயற்றவர்கள் மற்றும் புற்றுநோயின் ஆரம்பகால அறிகுறியுள்ளவர்களின் மைக்ரோ நியூக்கிளியையின் எண்ணிக்கையும் புகையிலை பயன்படுத்தாதவர்களின் மைக்ரோ நியூக்கிளியை எண்ணிக்கைகளோடு ஒப்பிடுதல்.

6. நோக்கம் மற்றும் கொள்கைகள் :

மைக்ரோ நியுக்கிளியையின் எண்ணிக்கை பேப் சாயம் பயன்படுத்தி புகையிலை பயன்படுத்துபவர்களுள் புற்றுநோயற்றவர்கள் மற்றும் புற்றுநோயின் ஆரம்பகால அறிகுறிகள் உள்ளவர்களை புகையிலை பழக்கம் இல்லாதவர்களோடு ஒப்பிட்டு ஆராய்தல்.

7. ஆய்வினைப் பற்றிய அறிவியல் விளக்கம்

புற்றுநோய் ஆரம்பகாலத்தில் கண்டறிதல் ஒரு மிகப்பெரிய சவாலாகவே இன்றுவரை அமைந்துள்ளது. அவ்வாறு ஆரம்பகால புற்றுநோயின் வேறுபாடுகளை செல்களின் அளவில் கண்டறிய மைக்ரோநியுக்கிளியையின் எண்ணிக்கை கண்டறிதல் ஒரு எளிய ஆய்வு முறையாக திகழ்கிறது. மைக்ரோ நியூக்கிளியை கொண்டுசெய்யப்படும் ஆய்வு முறைகள் 95% துல்லியமாக அமைந்துள்ளது. ஆதலால் புகையிலை பயன்படுத்துபவர்களில் ஆரம்பகால மாற்றங்களை மைக்ரோ நியூக்கிளியை எண்ணிக்கை கொண்டு செய்யப்படும் ஆய்வு முறை சிறந்த மற்றும் எளிய ஆய்வு முறையாக திகழ்கிறது.

9. ஆய்வின் செயல்முறை

- தகவல் குறிப்பு எண் வழங்கப்படும்
- வாயை சலைன்கொண்டு சுத்தம் செய்யவும்
- ஒரு மரத்தட்டையான் ஸ்பேச்சுலாவைக் கொண்டு ஸ்மியர் எடுக்கப்படும்
- அது மைக்ரோஸ்கோப் ஸ்லைடில் பூசப்படும்
- பின்னர் ஒன்றில் பேப் சாயம் பூசப்படும்.
- அதன்பின் கவர்ஸ்லிப் வைக்கப்பட்டு மைக்ரோஸ்கோப்பில் மைக்ரோ நியூக்கிளியை தனி தனியாக கணக்கிடப்படும்.

10. ஆய்வில் கலந்து கொள்பவர்களுக்கு எதிர்பார்க்கப்படும் ஆபத்துகள் ? ஒன்றும் இல்லை

11 . பங்கேற்பாளர்களுக்கு எதிர்பார்க்கப்படும் பயன்கள்?

இந்த ஆய்வின் மூலம் மைக்ரோ நியூக்கிளியையின் எண்ணிக்கையை வைத்து மக்களுக்கு புகையிலையினால் வரும் மாற்றங்களை ஆரம்பகாலத்திலேயே எடுத்துரைக்கப்படும். மற்றும் இவ்வாய்வு முறை சிறந்த விழிப்புணர்வு ஏற்படுத்தும்.

12 இரகசியத்தன்மை காத்தல் ?

உங்களிடம் இருந்து சேகரித்த எந்த விபரமும் இரகசியமாக வைக்கப்படும். இதன்மூலம் கிடைக்கும் புள்ளிவிபரம் மட்டும் வெளியிடப்படும். மற்றபடி தனிநபரின் சொந்த விபரங்கள் வெளியிடப்படமாட்டாது.

13. . எதனால் இந்த ஆய்வில் நான் பங்கேற்க தேர்ந்தெடுக்கப்பட்டேன் ?

அ, எனது கல்வி நிறுவனத்தின் நிபந்தனைகளுக்கு இது உட்பட்டது.

ஆ. தாங்கள் இந்த ஆய்வின் சேர்ப்பு மற்றும் விடுப்பு கட்டளையின் உள் அமையப்பெறுவதால்

இ. சமூகத்தின் உதவிக்கு

14. இந்த ஆய்வில் எத்தனைபேர் பங்கேற்கிறார்கள் ? 100

15. இந்த ஆய்வின் மூலம் ஏதேனும் பின்விளைவுகள் ஏற்பட்டால் ஆராய்ச்சியாளர் பொறுப்பு ஏற்பாரா ? ஆம்

16. இந்த ஆராய்ச்சியில் பங்குபெறுவோருக்கு எவ்வித தொகையும் வழங்கப்படுமா ? இல்லை

17. நான் இந்த ஆராய்ச்சியிலிருந்து விருப்பப்பட்டால் எந்த காலகட்டத்திலும் விலகலாமா ? நோயாளியின் எந்த ஒரு கட்டுப்பாடு / நிபந்தனைகளின் கீழ் இந்த ஆய்விற்கு உட்படுத்தப்படவில்லை. அவர்களின் முழு ஒத்துழைப்பு மற்றும் சம்மதத்தின் பேரில் மட்டுமே பங்கெடுத்துள்ளனர்.

18. ஏதேனும் புதிய செய்தி, புதிய கண்டுபிடிப்பு பற்றி நான் அறிவிக்கப்படுவேனா ? ஆம்

19. ஆராய்ச்சியின் எதிர்பார்க்கப்படும் பங்குகால அளவு ? ஒரு நாள்

20. வேறு ஏதேனும் பொருத்தமான விபரங்கள் உண்டா ? இல்லை

21. இவ்வாராய்ச்சியைப் பற்றிய விவரங்களை யாரிடம் கேட்டு தெரிந்துக்கொள்வது ?

தலைமை ஆய்வாளர் : *டாக்டர். ஹேமா மாரீஸ்வரி. ஜி*

தகுதி : முதுகலை (MDS)

பிரிவு : ஓரல் மெடிசின் மற்றும் ரேடியோளஜி

நிறுவனம் : ஸ்ரீ மூகாம்பிகா இன்ஸ்டிட்யூட் ஆப் டென்டல் சயன்ஸஸ்,

இடம் : குலசேகரம்

Cell: 9442092087, Email:vidhushamayag@gmail.com

இடம் :

தேதி : முதன்மை ஆராய்ச்சியாளரின்

கையொப்பம்

ஒப்புதல் படிவம்

பாகம்-2

இந்த ஆராய்ச்சியின் தகவல்கள் அனைத்தும் என்னிடம் தெளிவாக எழுத்துமூலம் விளக்கப்பட்டுள்ளது. இந்த ஆராய்ச்சியின் முடிவுகள் எனக்கு நேரடியாக பயன்பராவிட்டாலும் மருத்துவத்துறையின் முன்னேற்றத்திற்கு பயன்படும் என்பதை அறிவேன். இவ்வாராய்சியைப் பற்றி நான் தெளிவாக புரிந்துக் கொண்டுள்ளேன். நான் தானாக முன்வந்து இதில் பங்குப் பெறுகிறேன். என்பதை அறிவேன். இதிலிருந்து எந்த நேரமும் எக்காரணமும் கூறாமல் வந்தாலும் இந்த மருத்துவமனையில் எனக்கு கிடைக்கும் மருத்துவ உதவி எவ்விதத்திலும் பாதிக்கப்படாது என்பதையும் அறிவேன். இவ்வாராய்ச்சியின் மூலம் வரும் முடிவுகள் மற்றும் தகவல்களை அறிவியல்துறையின் பயன்பாடுகளுக்கு (மட்டுமே) உபயோகப்பட்டிக்கொள்ள சம்மதிக்கிறேன். எனக்கு இவ்வாராய்ச்சியைப் பற்றிய விரிவான தகவல்கள் அடங்கிய படிவம் தரப்பட்டுள்ளது.

நான் "***பேப் சாயம் பயன்படுத்தி புகையிலை பயன்படுத்துபவர்களுள் புற்றுநோயற்றவர்கள் மற்றும் புற்றுநோயின் ஆரம்பகால அறிகுறியுள்ளவர்களின் மைக்ரோ நியூக்கிளியையின் எண்ணிக்கையும் புகையிலை பயன்படுத்தாதவர்களின் மைக்ரோ நியூக்கிளியை எண்ணிக்கைகளோடு ஒப்பிடுதல்.***" என்கிற ஆராய்ச்சியில் பங்கேற்க முழுமனதுடன் சம்மதிக்கிறேன்.

பங்கு கொள்பவரின் தொடர், மருத்துவ எண் :

பங்கு கொள்பவரின் பெயர் :

18 வயதிற்கு கீழ் உள்ளவர்களுக்கு பாதுகாவலரின் கையொப்பம்:

முகவரி

தொலை தொடர்பு எண் :

பங்கு கொள்பவர் பராமரிப்பவர் கையொப்பம்/பெருவிரல் சுவடு :

சாட்சி 1

சாட்சி 2

தேதி:

இடம்: குலசேகரம்

സമ്മത പത്രം – ഭാഗം – 1

പഠനവുമായി സഹകരിക്കുന്ന വ്യക്തികളുടെ അറിവിലേയ്ക്ക്

പ്രിയപ്പെട്ട സന്നദ്ധ സേവകരേ,

ഞങ്ങൾ നിങ്ങളെ സ്വാഗതം ചെയ്യുന്നു. അതോടൊപ്പം ഈ പഠനവുമായി സഹകരിക്കാനുള്ള സന്നദ്ധതയോട് നന്ദി രേഖപ്പെടുത്തുന്നു. നിങ്ങൾ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതിനു മുൻപ് ഈ പഠനം എന്തിനാണ് നടത്തപ്പെടുന്നത് എന്ന് അിറയേണ്ടതുണ്ട്. അതിനാൽ ഈ ഫോറത്തിൽ ഗവേഷണ പഠനത്തിന്റെ വിവരങ്ങളും മറ്റും വിശദമായി രേഖപ്പെടുത്തിയിരിക്കുന്നു. ഈ പഠനത്തിന്റെ രീതി, ഉദ്ദേശം, പ്രയോജനം, അപകടസാദ്ധ്യത, ക്ലേശം, മുൻകരുതൽ, എങ്ങനെ ഈ പഠനം മുൻപോട്ടു കൊണ്ടുപോകുന്നു എന്നിങ്ങനെ എല്ലാ വിവരങ്ങളും ഫോറത്തിൽ രേഖപ്പെടുത്തിയിരിക്കുന്നു. സദയം ഈ വിവരങ്ങൾ വായിച്ചു മനസ്സിലാക്കുവാൻ അഭ്യർത്ഥിക്കുന്നു. ഈ വിവരങ്ങളിൽ ശാസ്ത്രപരമായ പദങ്ങൾ ഉള്ളതിനാൽ സംശയനിവാരണത്തിനു പ്രധാന പഠനകർത്താവിനോടോ താഴെ രേഖപ്പെടുത്തി യിരിക്കുന്ന വ്യക്തികളോടോ ഫോറം ഒപ്പിടുന്നതിനു മുൻപോ അല്ലെങ്കിൽ ഈ പഠനത്തിന്റെ കാലാവധി തീരുന്നതുവരേയോ സമീപിക്കാവുന്നതാണ്.

1. മുഖ്യ ഗവേഷകൻ : **ഡോ. ഹേമ മാരീശ്വരി. ജി**
പോസ്റ്റുഗ്രാജുവേറ്റ് വിദ്യാർത്ഥിനിമമ
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ & റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസ്,
കുലശേഖരം - 629 161.

2. പ്രധാന മാർഗ്ഗദർശി : **ഡോ. റ്റാറ്റു ജോയ്. ഇ, എം. ഡി. എസ്.**
പ്രൊഫസർ & ഹെഡ് ഓഫ് ദി ഡിപ്പാർട്ടുമെന്റ്,
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ & റേഡിയോളജി
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
കുലശേഖരം.

3. സഹ മാർഗ്ഗ ദർശി : **ഡോ. ശശി കിരൺ എം. ഡി. എസ്.**
പ്രൊഫസർ
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ & റേഡിയോളജി
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
കുലശേഖരം.

4. ഇൻസ്റ്റിറ്റ്യൂട്ട് : **ശ്രീ. മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്**
പടനിലം, കുലശേഖരം, കന്യാകുമാരി - 629 161.
തമിഴ്നാട്.

5. പഠനത്തിന്റെ ശീർഷകം

പാപ്പ് സ്മേയർ ഉപയോഗിച്ച് ധൂമപാന ഉപയോക്താക്കളുടെ വായിലെ ഉരിഞ്ഞുപോകുന്ന കോശങ്ങളുടെ കണികതലത്തിലുള്ള മാറ്റങ്ങൾ നിർണ്ണയിച്ചുകൊണ്ട് അർബുദലക്ഷണങ്ങൾ ഉള്ളവരുടേയും ഇല്ലാത്തവരുടേയും തമ്മിലുള്ള താരതമ്യപ്പെടുത്തൽ.

6. ലക്ഷ്യങ്ങളും ഉദ്ദേശങ്ങളും ?

1. വായിൽ നിന്ന് എടുക്കുന്ന കോശങ്ങളിൽ നിന്ന് പാപ്പും ജീയംസാ സ്റ്റെയിനും ഉപയോഗിച്ച് പുകയില ഉപയോഗിക്കുന്നവരുടെയും അർബുദ ബാധിതരുടേയും അർബുദ ലക്ഷണങ്ങൾ ഉള്ളവരുടേയും പുകയില ഉപയോഗിക്കാത്തവരുടേയും മൈക്രോന്യൂക്ളിയയ് അളവിനെ താരതമ്യപ്പെടുത്തുന്ന പഠനം.
2. പുകയില ഉപയോഗിക്കുന്നവരിലെ അർബുദവും അർബുദ ലക്ഷണങ്ങളും പുകയില ഉപയോഗിക്കാത്ത വരുടേയും മ്ക്രോന്യൂക്ലിയസ് അളവിന്റെ താരതമ്യ പഠനം
3. മൈക്രോന്യൂക്ലിയയ് അളവ് ജീനോടോക്സിസിറ്റി കണ്ടുപിടിക്കാനുള്ള മികച്ച പരിശോധന ആണ്.

7. ഗവേഷണം നടത്തുവാനുള്ള ന്യായീകരണം

അർബുദം ആരംഭത്തിൽ കണ്ടുപിടിക്കുന്നത് ഒരു ചോദ്യചിഹ്നമായി അവശേഷിക്കുന്നു. അങ്ങനെ അർബുദത്തേയും അർബുദലക്ഷണങ്ങളേയും തുടക്കത്തിൽ തന്നെ കണ്ടെത്തുവാൻ മൈക്രോന്യൂക്ലിയയ് പഠനം സഹായിക്കുന്നു.

8. പഠന രീതി

താങ്കളെ ഈ പഠനത്തിൽ ഉൾപ്പെടുത്തുമ്പോൾ താങ്കളുടെ പേരിനു പകരമായി ഒരു ക്രമ നമ്പർ തരുന്നതാണ്.

- പഠനത്തിനു മുൻപായി വായ് വൃത്തിയായി കഴുകേണ്ടതാണ്.
- കവളിന്റെ ഉൾവശങ്ങളിൽ നിന്നും ഒരു സ്പാച്ചുല ഉപയോഗിച്ച് സ്മിയർ എടുക്കുകയും അത് ഒരു കവർ സ്ലിപ്പ് ഉപയോഗിച്ച് ഒരു ഗ്ലാസ്സ് സ്ലയിഡിലേയ്ക്ക് മാറ്റുകയും അതിനുശേഷം 95% എത്തനോൾ ഉപയോഗിച്ച് ഫിക്സ് ചെയ്യുകയും പിന്നീട് പാപ്നിക്കോലൊജീയംസ എന്നിവ ഉപയോഗിച്ച് സ്റ്റെയിൻ ചെയ്യുകയും ചെയ്യുന്നു.

9. പ്രതീക്ഷിക്കുന്ന അപകട സാദ്ധ്യതകൾ

ചെറിയ രീതിയിൽ വേദന (മരവിപ്പിക്കാനുള്ള മരുന്നുകൾ ഉപയോഗിച്ച് ഈ വേദനയും ഇല്ലാതാക്കാവുന്നതാണ്

10. പ്രതീക്ഷിക്കാവുന്ന പ്രയോജനങ്ങൾ

1. ലാബ് ടെസ്റ്റുകൾക്ക് നിങ്ങൾ തുക നൽകേണ്ടതില്ല
2. നിങ്ങൽക്ക് ഈ ഗവേഷണത്തിന്റെ വിവരങ്ങൾ തിരക്കാവുന്നതാണ്.

11. വ്യക്തിവിവരങ്ങളുടെ സ്വകാര്യത് ?

1. രാഗവിവരങ്ങളും മറ്റ് വ്യക്തിവിവരങ്ങളും സ്വകാര്യമായി സൂക്ഷിക്കപ്പെടുന്നതായിരിക്കും
2. ഈ ഫോറത്തിൽ ഒപ്പിടുന്നത് വഴി നിയമം അനുശാസിക്കുന്ന രീതിയിൽ പഠനത്തിൽ ഉൾപ്പെടുന്ന വ്യക്തികൾക്ക് നിങ്ങളുടെ വിവരങ്ങൽ പരിശോധിക്കാവുന്നതാണ്.

3. ഈ പഠനത്തിന്റെ വിവരങ്ങൾ കാസ്ത്രാനുപാധികളായ പ്രസിദ്ധീകരണങ്ങളിലോ, കൂടി ആലോചനകളിലോ വെളിപ്പെടുത്തുമ്പോൾ നിങ്ങളുടെ സ്വാകാര്യത സൂക്ഷിക്കപ്പെടുന്നതാണ്.

12. **എന്തുകൊണ്ട് നിങ്ങൾ തിരഞ്ഞെടുക്കപ്പെട്ടു ?**

1. പഠനത്തിന് നല്ല ശതമാനം ആളുകൾ ആവശ്യമാണ്.
2. പല കൂട്ടിക്കുറച്ചിലുകൾക്കൊടുവിൽ നിങ്ങൾ ഉൾപ്പെടുന്ന വിഭാഗത്തെ തിരഞ്ഞെടുത്തു
3. നിങ്ങളുടെ സഹകരണം മൂലം സമൂഹത്തിന് സഹായവും നന്മയും ഉണ്ടുകുന്നു.

14. **എത്ര ആളുകൾ ഈ പഠനത്തിൽ ഉൾപ്പെടുന്നു.** 150

15. **നഷ്ടപരിഹാര ഉടമ്പടി ? (പഠനവുമായി ബന്ധപ്പെട്ട് എന്തെങ്കിലും പരിക്കുണ്ടായാൽ)**
പഠനവിധേയമായി ഏതെങ്കിലും തരത്തിൽ രോഗം സങ്കീർണ്ണമായാൽ രോഗിയെ ഈ സ്ഥാപനത്തിൽ വിദഗ്ദ്ധ ചികിത്സക്കു വിധേയനാക്കുന്നതാണ്.

16. **ഏതെങ്കിലും വിധത്തിൽ വേതനം ലഭിക്കുമോ** – ഇല്ല

17. **എപ്പോൾ വേണമെങ്കിലും എനിക്ക് ഈ പഠനത്തിൽ നിന്ന് പിന്മാറാമോ ?**
കാരണം വ്യക്തമാക്കാതെ എപ്പോൾവേണമെങ്കിലും നിങ്ങൾക്ക് ഈ പഠനത്തിൽ നിന്നും പിന്മാറാവുന്നതാണ്. എങ്കിലും അതിന് മുൻപായി ഗവേഷകരുമായി സംസാരിക്കുന്നത് നല്ലതാണ്.

18. **പഠനവുമായി ബന്ധപ്പെട്ട എന്തെങ്കിലും പുതിയ വിവരങ്ങൽ ഉണ്ടെങ്കിൽ എന്നെ അിറയിക്കുന്നതാണോ ?** അതെ

19. **പ്രതീക്ഷിക്കുന്ന പഠന കാലാവധി ?** ഒരു വർഷം

20. **ഇതിന്റെ ഭാഗമായി എന്തെങ്കിലും കൂടുതൽ വിവരങ്ങൾ** ഇല്ല

21. **കൂടുതൽ വിവരങ്ങൾക്കായി താഴെ പറയുന്നവരെ നിങ്ങൾക്ക് ബന്ധപ്പെടാവുന്നതാണ്.**

ഡോ. ഹേമാ മാരീശ്വരി. ജി
ബിരുതാനന്തര വിരുത വിദ്യാർത്ഥിനി,
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ & റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസ്,
കുലശേഖരം - 629 161.
മൊബൈൽ നമ്പർ : 9442092087
ഇ-മെയിൽ ഐഡി::vidhushamayag@gmail.com

സ്ഥലം: പ്രഥമ അന്വേഷകന്റെ ഒപ്പ്

തീയതി : പങ്കെടുക്കുന്ന ആളിന്റെ ഒപ്പ്

സമ്മതപത്രം

ഭാഗം – 2

ഈ പഠനത്തെ പറ്റിയുള്ള എല്ലാ കാര്യങ്ങളും എനിക്ക് പറഞ്ഞ് മനസ്സിലാക്കി തരികയും അതിന്റെ ഒരു പകർപ്പ് എനിക്കു നൽകുകയും ചെയ്തിട്ടുണ്ട്. ഈ പഠനം ഗവേഷണത്തിനായി ഉള്ളതാണെന്നും എനിക്ക് ഇതിൽ നിന്ന് നേരിട്ട് ഒരു ഫലവും ഉണ്ടാകില്ലെന്നും ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനത്തിന്റെ രീതിയും ഉദ്ദേശവും എനിക്ക് മനസ്സിലാക്കി തന്നിട്ടുണ്ട്. അതു പോലെ എനിക്ക് സംശയങ്ങൾ ചോദിക്കാൻ അവസരങ്ങൾ ലഭിച്ചിട്ടുമുണ്ട്. ഇതിൽ പങ്കെടുക്കാനും പങ്കെടുക്കാതിരിക്കാനും ഉള്ള അവകാശം എനിക്കുണ്ടെന്നും അതുപോലെ പഠനത്തിന്റെ ഏതു ഘട്ടത്തിലും ഇതിൽ നിന്ന് പിൻവങ്ങാനുള്ള സ്വാതന്ത്ര്യവും എനിക്കുണ്ടെന്ന് ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതു കൊണ്ടോ, പങ്കെടുക്കാത്തതുകൊണ്ടോ എന്റെ മറ്റു ചികിത്സകളെ ബാധിക്കുന്നതല്ലെന്ന് ഞാൻ അറിയുന്നു.

പാപ് സ്മേയർ ഉപയോഗിച്ച് ധൂമപാന ഉപയോക്താക്കളുടെ വായിലെ ഉരിഞ്ഞുപോകുന്ന കോശങ്ങളുടെ കണികതലത്തിലുള്ള മാറ്റങ്ങൾ നിർണ്ണയിച്ചുകൊണ്ട് അർബുദലക്ഷണങ്ങൾ ഉള്ളവരുടേയും ഇല്ലാത്തവരുടേയും തമ്മിലുള്ള താരതമ്യപ്പെടുത്തൽ എന്ന ഗവേഷണത്തിൽ പങ്കെടുക്കുന്നതിനും ഇതിന്റെ ഫലങ്ങൾ ശാസ്ത്രലേഖനത്തിൽ പ്രസിദ്ധീകരിക്കുന്നതിനും എനിക്ക് സമ്മതമാണെന്ന് ഞാൻ ഇതിനാൽ അറിയിച്ചുകൊള്ളുന്നു.

സീരിയൽ നമ്പർ / റഫറൻസ് നമ്പർ :

പങ്കെടുക്കുന്ന ആളിന്റെ പേര് :

മേൽവിലാസം :

ഫോൺ നമ്പർ :

ഒപ്പ് / വിരലടയാളം

സാക്ഷി :

സ്ഥലം :

തീയതി

CASE RECORD FORM

DATE OF EXAMINATION:

OP NO:

NAME: **PH NO:**

AGE: **(years)**

SEX:

EDUCATIONAL STATUS:

OCCUPATION:

INCOME: **(per year)**

ADDRESS:

PRESENTING COMPLAINT:

HABITS

Do you?	Yes	No	If yes, frequency	Age of starting the habit	Age of ending the habit	Type	Any side preference?
Smoke							
Chew tobacco							
Spicy food							
Alcohol							

FAMILY HISTORY:

a) History of cancer in the family?

PAST MEDICAL HISTORY:

Any systemic diseases?

Disease	Yes	No	Duration	On treatment?
Diabetic				
Hypertensive				
Cardio Vascular Disease				
Liver disease				
Hypercholesterolemia				
TB				
Hyper/Hypothyroidism				
Kidney disease				
Bronchial Asthma				
Epilepsy				

Surgeries?

Hospital admissions?

Long term medication?

History of Radiation Therapy within one year?

GENERAL EXAMINATION:

Built	Nourishment

Signs	Yes	No
Pallor		
Jaundice		
Cyanosis		
Clubbing		
Pedal oedema		
Regional lymphadenopathy		

VITAL SIGNS:

Pulse rate	
Respiratory rate	
Temperature	
BP	
JVP	

LOCAL EXAMINATION:

INTRA ORAL EXAMINATION:

Hard tissue:

Soft tissue:

SITE: Lip /Tongue / Cheek / Palate /Floor of the mouth/Alveolar mucosa

Clinical appearance –Homogenous /Non-homogenous/Not specified

CASE PHOTOS

PROVISIONAL DIAGNOSIS:

BLOOD INVESTIGATIONS: RBS:

Hb%: TLC: DLC:

RADIOLOGICAL INVESTIGATION:

BIOPSY:

OTHERS:

FINAL DIAGNOSIS:

STUDY OBSERVATION SHEET

TITLE : Cytogenetic changes in oral exfoliated cells of tobacco users without lesions and tobacco users with precancerous lesions using papanicolaou stain: a comparative study

GROUPS:

Group 1-Controls

Group 2-Tobacco users without clinically evident lesion

Group 3-Tobacco users with confirmed precancerous lesions

Total number of micronuclei / 500 cells in the smear (5 cells/box)

STUDY OBSERVATION SHEET

FREQUENCY OF OCCURENCE OF MICRONUCLEI IN PAP STAIN

Group 1(HEALTHY CONTROLS)

Serial Number of patient.	Number of micronuclei seen per 500 cells
1	4
2	3
3	4
4	5
5	6
6	4
7	5
8	3
9	4
10	6
11	5
12	4
13	5
14	3
15	4
16	4
17	3
18	5
19	5
20	3

Group 2(SMOKERS FOR 2 YEARS)

Number of patients	Number of micronuclei seen per 500 cells
1	15
2	17
3	12
4	12
5	16
6	14
7	11
8	14
9	15
10	12
11	16
12	14
13	13
14	12
15	12
16	11
17	18
18	17
19	13
20	10
21	12
23	17
24	11
25	13
26	15
27	17
28	14
29	11
30	15
31	12
32	13
33	14
34	11
35	14
36	12
37	16
38	14
39	12
40	15

Group 3(TOBACCO USERS WITH PRECANCEROUS LESION)

Number of patients	Number of micronuclei seen per 500 cells
1	37
2	40
3	36
4	38
5	39
6	31
7	35
8	38
9	32
10	41
11	37
12	33
13	34
14	32
15	41
16	39
17	34
18	37
19	39
20	32
21	34
23	37
24	31
25	34
26	41
27	42
28	40
29	41
30	39
31	32
32	34
33	40
34	35
35	37
36	34
37	32
38	40
39	41
40	39