ANALYSIS OF GENOTOXIC AND CYTOTOXIC EFFECTS ON THE ORAL BUCCAL MUCOSAL CELLS IN PATIENTS UNDERGOING FIXED ORTHODONTIC TREATMENT AND DUE TO TOBACCO SMOKING

A

THESIS

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ABSTRACT

Introduction: The oral mucosa is exposed to the effects of orthodontic appliances during the course of fixed orthodontic treatment. The chronic irritation from the appliances, ionic action from the metallic components and the harmful effects of bonding agents could have detrimental effects of the mucosa. Smoking tobacco is considered to be carcinogenic for the cells of the oral mucosa. Tobacco smoking could have varying effects on the oral mucosa in patients undergoing fixed orthodontic treatment.

Objective: The synergistic effects of tobacco smoking and fixed orthodontic treatment has not been described in the previous literature. Hence, this study has been designed with the broad objective of genotoxic and cytotoxic analysis of oral buccal mucosal cells in patients undergoing fixed orthodontic treatment.

Experimental work: This study was done on a group of smokers who were undergoing fixed orthodontic treatment (n=30) and compared with smokers, orthodontic patients and normal individuals. Sample were collected according to the principles of exfoliative cytology and smears stained with Rapid PAP stain. Cytomorphometry was done in 50 cells per smear using Image J analysis software and micro-nuclei count was done in 1000 cells per smear according to the criteria suggested by Tolbert et al. Data were entered in excel sheet and subjected to statistical analysis.

Results and discussion: Cytomorphometric analysis revealed that the nuclear diameter and nuclear area were significantly higher in orthodontic smokers as compared to orthodontic patients who were non-smokers [$p<0.001$ respectively]. Values were higher when compared with normal individuals but the results were not statistically significant. No differences were observed with smokers only group. Similarly, the cell diameter and cell area was significantly higher in orthodontic smokers as compared to orthodontic patients [$p=0.015$ & $p=0.001$ respectively]. These values were higher than in normal, but not significantly. No statistical was observed with smokers only group. The values of ND:CD ratio and NA:CA ratio did not show any statistical differences. The above observations suggest that the oral

mucosal cells show adaptive changes which correlated with studies done by de Arruda et al, Rafighi et al and Goregen et al. According to Goregen et al, the increase in nuclear area could be indicative of dysplastic changes related to tobacco. Few others studies observed that in response to tobacco, the cell area decreased. This was in contrast to the findings of this study and could be considered an adaptive response.

The micronuclear analysis revealed that and total number of micro-nuclei were significantly higher in orthodontic smokers as compared to normal individuals [$p<0.001$] and lower than orthodontic patients and only smokers [$p=0.022$ & $p=0.001$ respectively]. The values of total number of cells showing micro-nuclei differed similarly as the above findings. These findings suggest that genotoxic effects do occur in orthodontic patients who are smokers but these are less than only smokers and non-smoking orthodontic patients. No significant differences were seen on the basis of gender and duration of smoking.

Conclusion: Smokers undergoing fixed orthodontic treatment do exhibit some form of genotoxic and cytotoxic effects in the oral mucosal cells.

Keywords: cytomorphometry, cytotoxicity, exfoliative cytology, fixed orthodontic treatment, genotoxicity, micro-nuclei.

CHAPTER 1 - INTRODUCTION

1.0 PREAMBLE

The journey to looking good is not an easy one. It comes with lots of hardships and patience.

1.1 INTRODUCTION

Everyone wants to look good today. Correction of malocclusion has become an important need among the younger age population owing to the increased appearance based preferences in the social and professional circles (Albino Judith EN, 1994).

Alignment of mal-positioned teeth in the dental arch involves the application of optimal amount of force on the teeth. This force is applied by means of bands, brackets and wires which have to be attached to the tooth [Image 1]. However, orthodontic treatment is a lengthy process extending to over a year. Hence, the oral cavity is exposed to these foreign agents for a prolonged duration (Impellizzeri A, 2014).

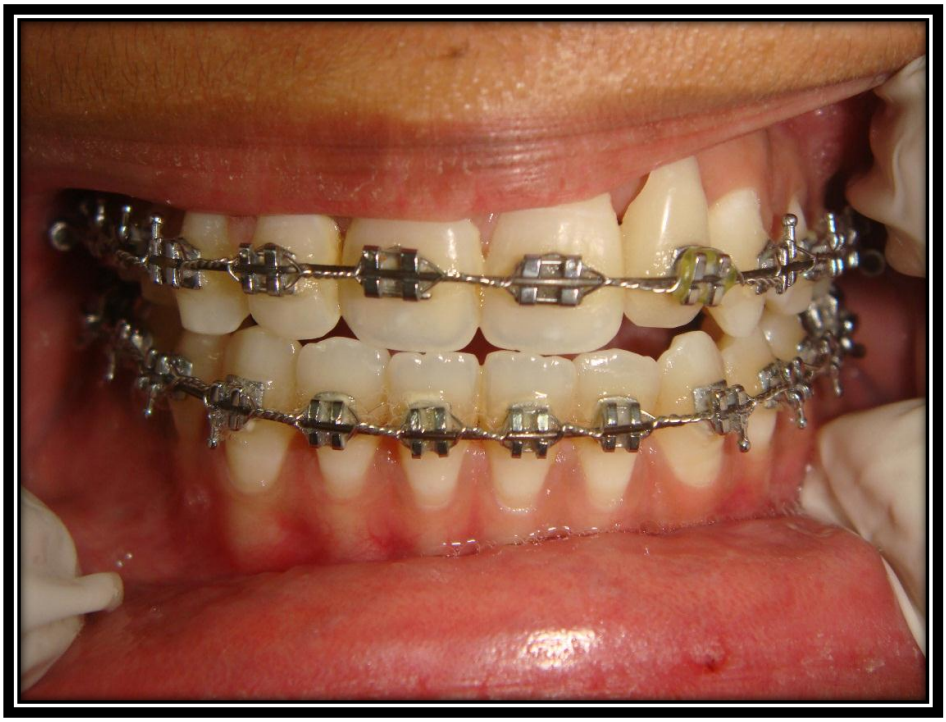

Image 1: Orthodontic appliances used for correction of malocclusion

Carcinogenesis is a multi-factorial event which involves overlapping of various etiological aspects for the initiation of a malignant process (Barrett JC, 1993). A variety of these factors can be encountered during the course of orthodontic treatment viz. poor oral hygiene, exposure to diagnostic radiation, leeching of un-polymerized resins from the orthodontic adhesive cements, ionic release as a consequence of corrosion and chronic irritation of the adjacent mucosa from the various components of orthodontic appliances (Ellis PE and Benson PE, 2002).

It is well documented in indexed literature that there is a deterioration of oral hygiene over the course of orthodontic treatment leading to increased plaque and calculus levels and alteration in the oral microbiota. Detoriorated oral hygiene has found to be correlated with the causation of oral cancer and hence should be considered as a potential risk factor (Marques LA, 2008; Behnoud F, 2011). Poor oral hygiene causes an increase and alteration in the bacterial load which provides a more conducive environment for the reduction of “nitrates into nitrites” which is an essential step in the formation of nitrosamines. In addition the inflammatory mediators released during periodontal infection are said to have a role in carcinogenesis (Dar NA, 2013).

The bands and brackets which are bonded on to the tooth surface cause consistent friction with the buccal and labial mucosa resulting in ulcerations and pain (Impellizzeri A, 2014).

The orthodontic appliances commonly used are alloys chiefly containing varying amounts/quantities of iron(Fe), nickel(Ni), chromium(Cr), cobalt(Co) and other trace metals. These components under the presence of saliva and other dynamic conditions predispose to release owing to the process of corrosion (Mikulewicz M, 2012). Various studies have shown the presence of these ionic components in the saliva. The accumulation of these ions within the mucosal cells has also been documented (Amini F, 2008; Hafez HS, 2011; Natarajan M, 2011). Of these, nickel is known to be an allergen resulting in hypersensitivity reactions. The most adverse effect of nickel is its mutagenic and carcinogenic potential. Similarly, chromium and

cobalt ions are capable of causing toxic effects on the nucleic & cytoplasmic contents of the oral epithelial cells (Chaturvedi TP, 2010).

Also, unpolymerized resins leeching out of cements used for banding and bonding procedures are known to have toxic effects on the mucosal cells (Ellis PE and Benson PE, 2002). The cytotoxic and genotoxic effects of various formulation of adhesive cements used during orthodontic treatment have been studied using cytological and immunohistochemical techniques and the results indicates a positive correlation (Angiero F, 2009; Ozturk F, 2012; Toy E, 2014).

The mutagenic and carcinogenic effects of ionizing radiations are elevated, especially at higher doses. These result in the formation of reactive compounds which could affect the epithelial cells either directly or indirectly (Feinendegen LE, 2004). An orthodontic patient is subjected to multiple diagnostic radiographic tests for formulating a definitive plan. The buccal mucosal cells are candidates for direct exposure to these radiations and also to the other discussed factors during the course of the treatment procedure. The genotoxic and cytotoxic effects of panoramic radiation on oral mucosal cells is well documented (Pai A, 2012; Arora P, 2014; Vidya KB, 2014).

Chronic irritation has been considered as a predisposing factor for premalignant and malignant conditions. Current literature suggests the association of chronic irritation mainly from faulty dentures in causation of malignancy. Chronic irritation has been considered to be a promoter of carcinogenesis according to the multistage model wherein tobacco and alcohol can be considered as initiators. Few studies have also indicated the effects of sharp tooth, edentulousness and para-functional habits in malignant lesions. The irritation of oral mucosa during orthodontic treatment needs attention in this regard (Piemonte ED, 2010; Perry JB, 2015).

Worldwide, tobacco use is the cause of deaths amounting to above five million per year, and based on the current scenario it can be assumed that tobacco usage would cause around eight million cases of mortality per years by the year 2030. Smoking has been known to cause cancer, cardiovascular diseases, stroke, pulmonary diseases (including the likes of emphysema,

bronchitis, and chronic airway obstruction), and diabetes mellitus (CDC, 2014). Cancer of the oral cavity is one of the common known types of cancer in this world and occurs due to multiple etiologies. Seventy five percent of squamous cell carcinomas of the head and neck region are attributed to tobacco and alcohol use (Jindal S, 2013). Cigarettes contain numerous carcinogenic substances, which have toxic effects on the DNA. It is common knowledge that these substances have the potential to cause mutations in genes, abnormalities of the chromosome and even formation of micro-nuclei (Nefic H, 2013). Tobacco smoking can cause carcinoma in different parts of the oral cavity, including the labial mucosa, tongue, palatal mucosa, gingiva, and the buccal mucosa (Kamath VV, 2014). Smoking is causes an increased rate of keratin production in the oral mucosa and its effects are also observed in keratinized areas of the oral cavity suggestive of the risk potential of tobacco smoking (Yerlagudda K, 2012).

The habit of smoking is generally picked up at a younger age mainly due to peer pressure and as a result of the stress encountered due to social or professional life stresses (Hashmi S, 2013). The effect of smoking in patients undergoing fixed orthodontic treatment needs to be warranted. Persistent irritation of the oral mucosa due to the brackets and wires used for orthodontic correction [Image 2] combined with the already known effects of tobacco smoke could have grave consequences. There are no studies in the indexed literature evaluating the effects of tobacco smoking on the cells of the buccal mucosa(BM) in patients undergoing fixed orthodontic treatment.

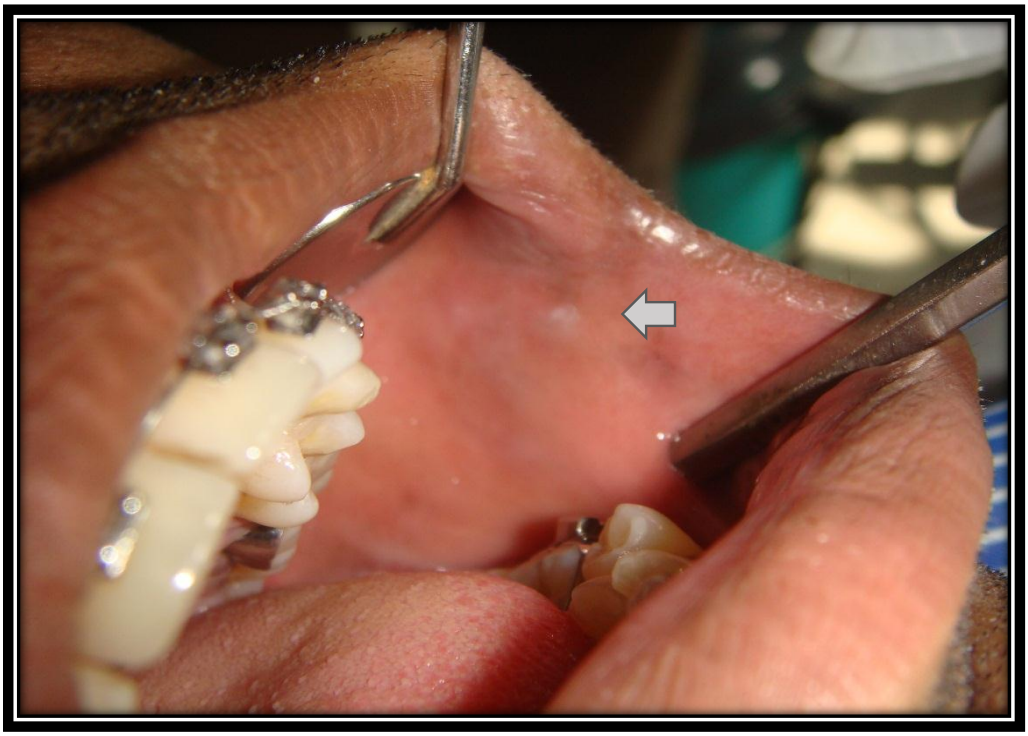

Image 2: Ulceration in oral mucosa due to chronic irritation from orthodontic brackets and wires.

The cytological preparations can be stained using different stains (Choudhary, 2012). However, Papanicolao stain (PAP stain) continues to remain the most preferred one for studying cells (Izhar, 2014). The utility of PAP stain in the laboratory has further increased with the introduction of rapid PAP stain with which staining duration is considerably reduced as opposed to the conventional technique (Choudhary, 2012; Asthana, 2014). Other factors which adds to its effectiveness includes its cost effectiveness and its ability to give good nuclear and cytoplasmic details in the sample (Izhar,2014;Asthana, 2014).

The reasoning for the use of oral exfoliative cytology is related to the physiological process of desquamation, wherein the superficial most cells are collected following exfoliation from the surfaces of different types oral mucosa and later examined (Kumaresan GD, 2014). Miller et al. in 1951 were the pioneers to evaluate the cells of the unaffected oral mucosa on the basis of cytology (Miller SC, 1951). The cells which are present in the superficial-most cell layer are known to retain the nuclei, and as such, any alterations in these

cells can be relied upon as a valid indicator of dysplastic or neoplastic changes. Exfoliative cytology is can be utilized for preliminary diagnosis of many oral mucosal diseases but it is not a substitute for biopsy which is considered to be the gold standard for definitive diagnosis. Lesions that are reactive in nature and inflammatory reactions are non-specific when studied cytologically and does not lead to definitive diagnosis. The diagnostic capability of cytology can be generally increased by addition of any quantitative parameter which should be precise, objective and reproducible (Kazanowska K, 2014).

Genotoxicity refers to any form of damage to the genetic content of a cell viz DNA & chromosomes (Shah SU, 2012). Various substances or events are considered to have to potential of causing such effects and are considered as carcinogenic (Philips DH and Arlt VM, 2009). Most notable among these are tobacco, alcohol, radiations etc (Anand P, 2008). The genotoxic effects on the somatic cells may be measured by various assays which can be considered to be predictors of carcinomatous changes in the tissues (Philips DH and Arlt VM, 2009). The micro-nuclei assay is one such procedure which can be relied upon for detecting any genotoxic effects on cells (Shashikala R, 2015). Micro-nucleus is described as a fragmented DNA observed within the cytoplasm and is considered to be a biomarker of mutagenesis. The micro-nucleus assay can be utilized to predict individuals with a potential for malignant transformation in the cells of the oral mucosa. It has been used on a very large number of accounts to evaluate the extent of chromosomal alterations in individuals who are exposed to the genotoxic agents due to different reasons [Figure 1] (Jois HS, 2010). The criteria set by Tolbert et. al. is commonly used for evaluation of micro-nuclei [Image 3] (Tolbert PE, 1991). Various studies have established the association of occurrence of micro-nuclei in cells exposed to genotoxic agents like tobacco, alcohol etc and also in pre-malignant lesions and oral-squamous cell carcinoma(OSCC) (Stich HF, 1982; Salama SA, 1999; Casartelli G, 2000).

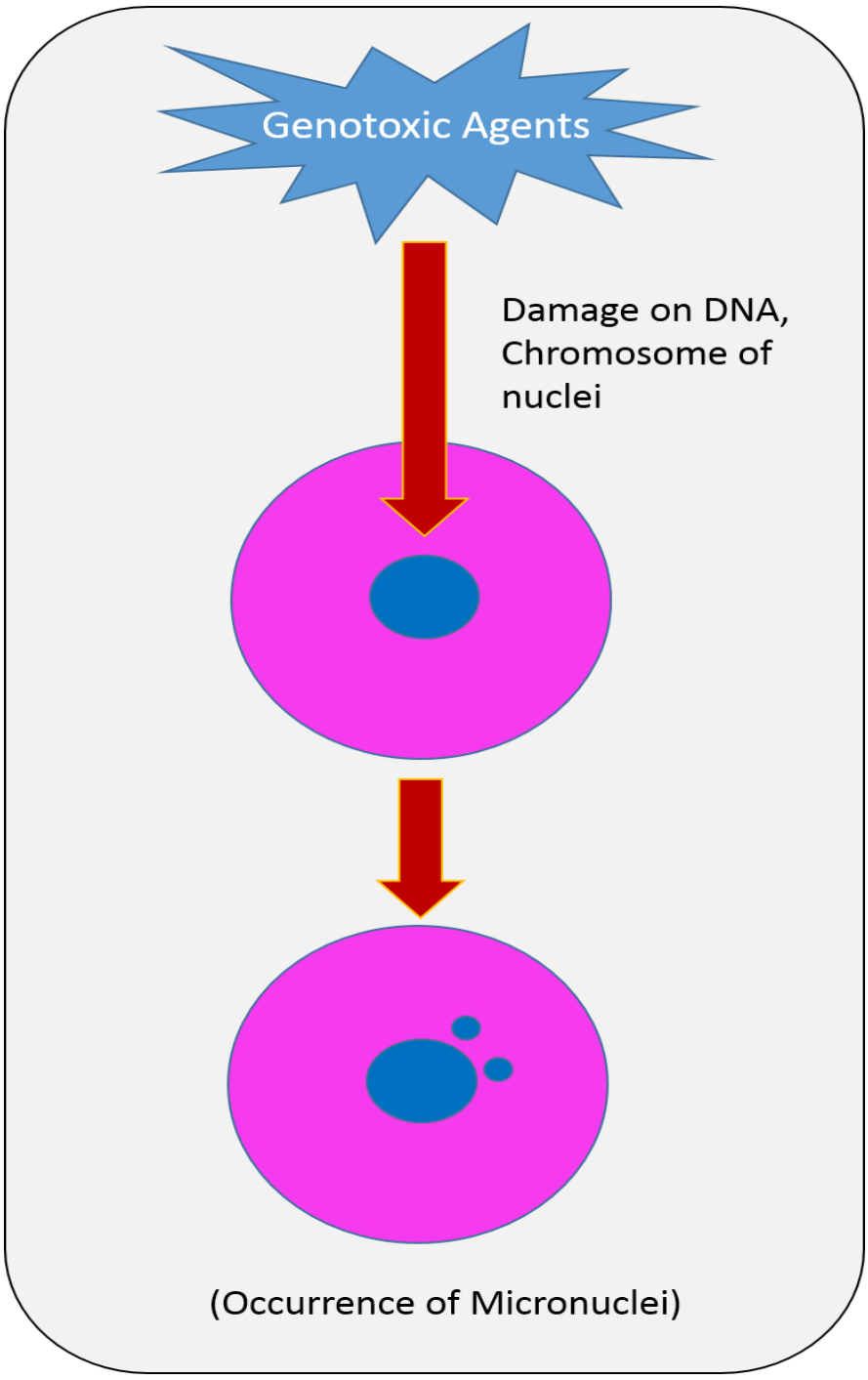

Figure 1: Phenomenon of occurrence of micro-nuclei

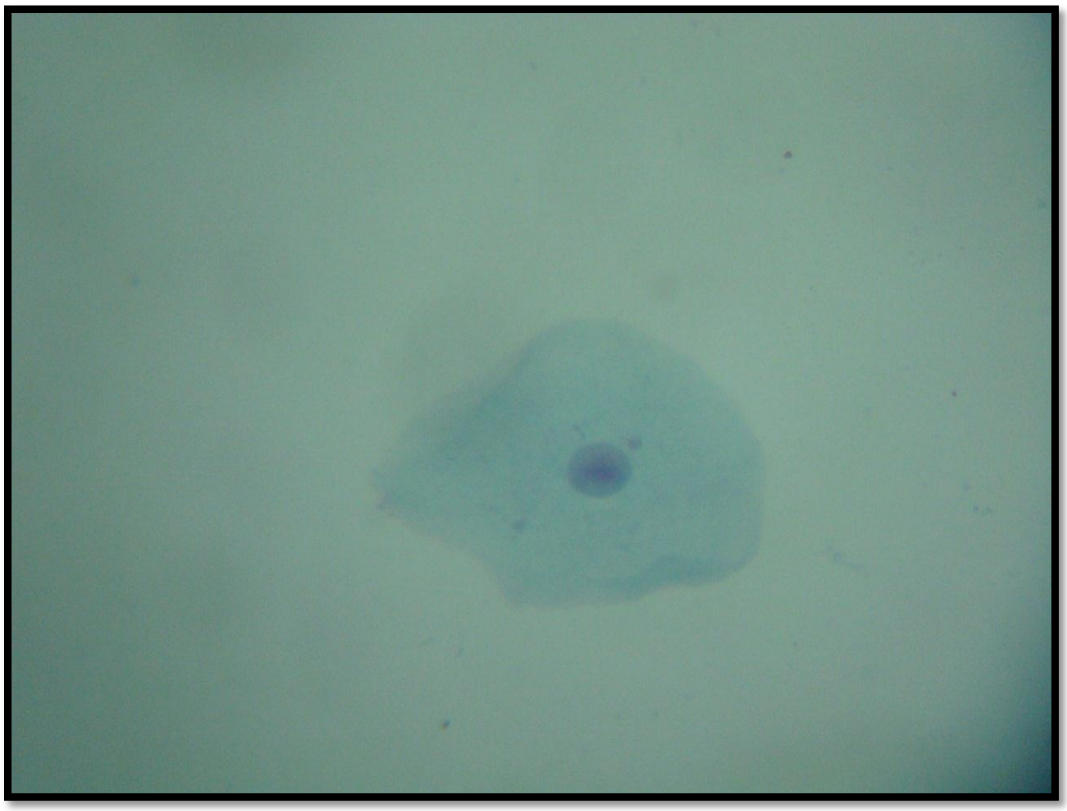

Image 3: Exfoliated Buccal cell showing micro-nucleus within the cytoplasm [PAP, 400x magnification]

Cytomorphometry is the most widely used method of oral exfoliative cytology, and assesses parameters [Figure 2] such as diameter of the cell [C.D.], diameter of nucleus [N.D.], area of nucleus[N.A.], area of cytoplasm [C.A.], N.A./C.A. ratio, nuclear shape, nuclear membrane continuity, optical density, and nuclear texture. These parameters, especially N.A. and N.A./C.A. ratio, have been shown to provide meaningful results in the diagnosis of oral lesions (Kazanowska K, 2014). Cytomorphometrical analysis of oral exfoliated cells from normal healthy individuals have been categorized according to different variables like age, gender and location within the oral cavity and used to create a baseline data which can be used in the future for comparison with pathologically altered cells (Cowpe JG, 1985).

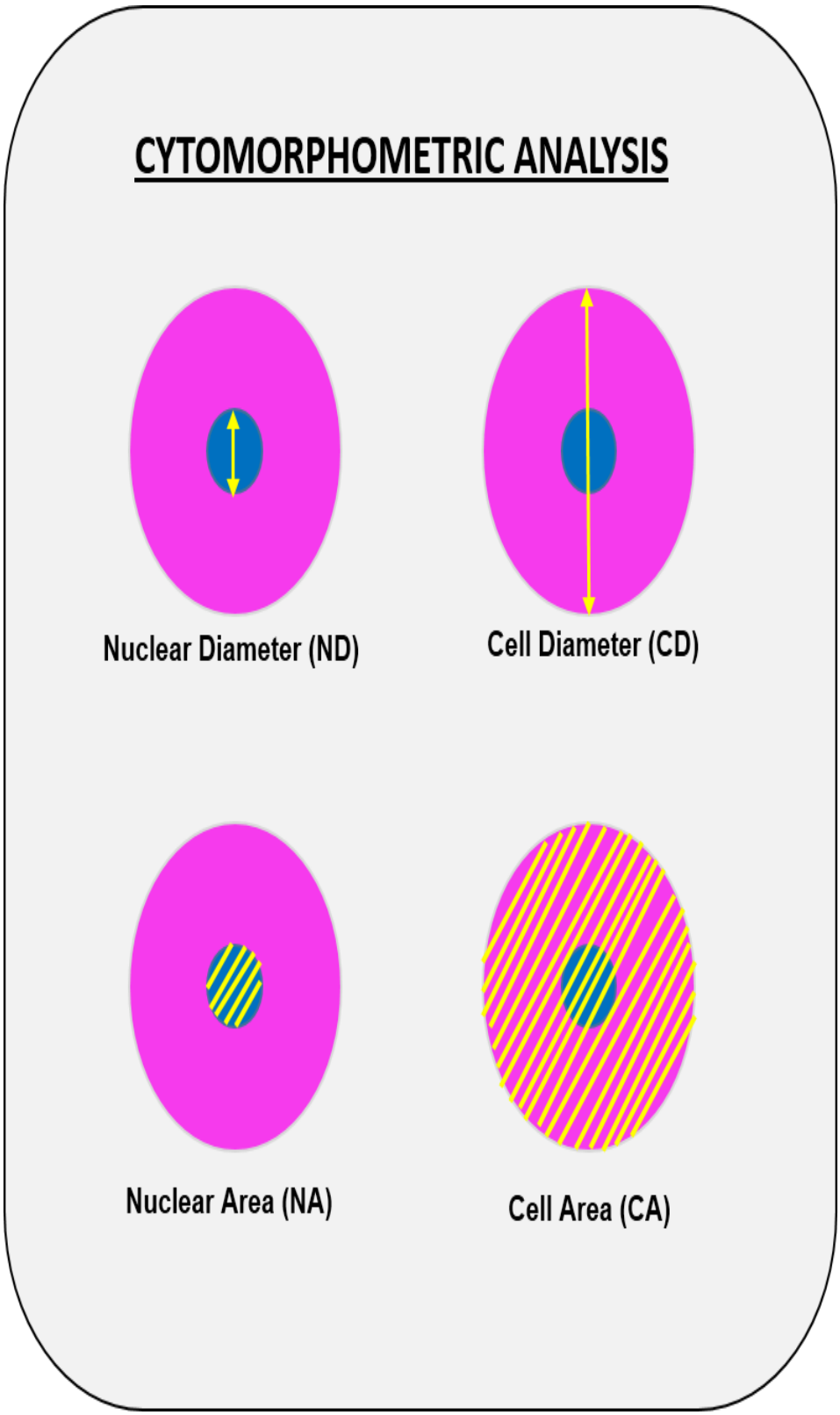

Figure 2: Cytomoprhometrical analysis showing study of various parameters

The micro-nucleus assay and the cytomorphological analysis combined together is a useful and effective way of studying the genotoxic and cytotoxic effects on oral buccal mucosal cells. It is now clear that the oral mucosa is exposed to a variety of factors which are capable of causing mutagenic effects on the epithelial cells. The various studies available in the literature are based on the effects of one individual factor on the oral epithelial cells. The combined effects of the above mentioned factors occurring in tandem could be of a deleterious one in nature. Hence, this study is undertaken to analyze the genotoxic and cytotoxic effects on oral buccal mucosal cells in patients undergoing fixed orthodontic treatment and due to tobacco smoking.

TOBACCO AND ITS ILL-EFFECTS:

Nicotina or plant of tobacco which is mentioned as a holy herb in history, is a most preeminent cause for approximately 5.4 million deaths per year (WHO data, 2015). It is the most easily avertible cause of untimely morbidity and mortality (Rockville, 1982). The name Nicotine came from the name of Jean Nicot, the French ambassador to Lisbon, who advocated its use to treat rodent ulcer in year 1560's (Monardes N, 1596).

FORMS OF TOBACCO

Tobacco is currently used in number of ways which include smoked tobacco and smoke-less tobacco. Smoked tobacco include bidi, chillum, chutta, cigarettes, dhumti, hookah and hookli. Smoke-less tobacco include khaini, Manipuri tobacco, mawa, masher, paan, snuff, zarda, gutka, pan masala and gudakhu are generally used in India (Chadda R and Sengupta S, 2003; Singh A and Ladusingh L, 2014).

SMOKED TOBACCO

Bidi contains 0.2-0.3 grams of tobacco flakes hand rolled in a temburni or tendu leaves and tied with a thread. It contains nicotine content approximately around 1.7-3 mg and tar around 45-50 mg. Chillum is straight, conical clay pipe which contains coarsely cut tobacco with glowing charcoal kept on its top (O'Connor RJ, 2012). A cigar has been described asa smoked form of tobacco which is prepared as a roll of tobacco leaf or any other material and is

filled with dried tobacco, and smoked without the use of a filter and contains roughly around 100-400 mg of nicotine in a cigar. This approximates to about 17 grams of tobacco (Viegas CAA, 2008). Cigarettes is described as a roll of tobacco which has been cured in sun or artificial heat and is covered by paper which contains 1-1.4 mg of nicotine and 19-27 mg of tar. It has filter to trap tar which is around 12 mm in length and is present in 51% of total available brands in India. Dhumti is a conical cheroots where rolled leaf tobacco is used inside a leaf of jack fruit tree or banana plant. This form of tobacco is used by women in reverse smoking. Hookah, also known as water pipe or hubble bubble is a way of smoking tobacco where smoke is drawn through water present in base of it which cools and filter the smoke. Hookli is a clay pipe which is used in Gujarat for smoking tobacco. This pipe is short of size around 7-10 cms with a mouth piece and a bowl (Chadda R and Sengupta S, 2003).

SMOKE-LESS TOBACCO

Khaini is a powdered sun dried tobacco and slaked lime paste mixture used with or without arecanut and is popular in several states of North India. The components of khaini are mixed using the thumb and palm to make the mixture alkaline in nature and is placed in the bicuspid region of the lower vestibule. Mainpuri tobacco, which is popular in villages of Uttar Pradesh, is a combination of tobacco mixed with slaked lime, areca nut that has been finely cut, camphor and cloves. It is associated with high prevalence of leukoplakia and oral cancer. Mawa is a form of smoke-less tobacco that contains thin shavings of areca nut mixed with tobacco and slaked lime in the form of small balls that has been packed in pouch made up of cellophane sheet. Before consumption, packet is rubbed vigorously to mix the ingredients and is chewed to make it soft, after which it is placed in mandibular groove. Misheri/Masheri is prepared by roasting tobacco on a metallic plate until it turns homogeneously blackish in colour. Then it is consumed in combination with or without catechu. Previously, its application was in cleaning of teeth. Betel Quid is another form of consumption of tobacco and is described as a type of smoke-less tobacco. It contains areca nut and lime in betel leaf with tobacco and other flavouring agents (aniseed, catechu, cardamom, cinnamon, coconut, cloves, sugar etc.). Snuff contains finely powdered air/fine cured

tobacco leaves. It may be in dry or moist form. Dry snuff is carried in small metallic containers and is placed over the tooth and gingival tissue with the help of a twig. It is mostly used by women in the state of Gujarat. Zarda is a form of smoke-less tobacco which is formed by boiling tobacco in water along with lime and spices such that the moisture content is entirely lost due to evaporation. Residual tobacco is then dried and coloured with dyes. Gutka, a commercial form of smoke-less tobacco is manufactured by mixing crushed betel nut, tobacco, and sweet or savoury flavourings. Gudakhu, more common among Bihari women, is a powdered tobacco, molasses and other ingredients which is primarily used for tooth cleansing (Singh A and Ladusingh L, 2014).

CONSTITUENTS OF TOBACCO WHEN SMOKED

Nicotine- It is a dibasic amine compound, which contains pyridine and pyrrolidine rings. It is a clear liquid in pure form and has a particular odour. It becomes brownish on exposure to air. On usage, it is absorbed through the oral mucosa, lungs, skin or gut. The absorption increases in alkaline medium due to increased concentration of uncharged lipophilic nicotine which allows it to permeate through all biological membranes. This compound is responsible for causing addiction in smokers as it trigger the release of numerous neurotransmitters especially dopamine which is associated with feeling of pleasure. It is metabolised in liver after ingestion in two phases. In first phase, microsomal oxidation of nicotine take place resulting in production of metabolites like cotinine and nornicotine, demethyl cotinine, trans-3-hydroxy-cotinine and d-(3-pyridyl)-g-methylaminobutyric acid. Next phase consist of metabolites like glucuronidation followed by excretion through urine, faeces, bile, saliva, sweat etc. 5-10 percent of unchanged nicotine is also excreted by renal route, however it is reabsorbed from bladder if urine pH is alkaline. Various in vivo studies has shown that nitrosation of nicotine can result into formation of known carcinogenic metabolites like N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Inflammation in oral cavity predisposes nicotine to nitrosation (Mishra A, 2015).

Tar- It is the particulate matter inhaled with lighted cigarette. It consists of large varieties of organic and inorganic compounds. It appears as a sticky brown substance in its condensed form which can stain smokers' finger and teeth to yellow brown colour. Studies has shown that one of the major tumour initiator is benzopyrene which is found in tar itself (Talhout R, 2011).

Carbon monoxide- It is an odourless and tasteless gas which has 200 times more affinity towards haemoglobin than oxygen and hence, it interfered with the oxygen transportation in the body. Oxygen level is seen to reduced to 15% in smokers in comparison with healthy non-smokers. Along with nicotine, it acts as a predisposing factor for coronary diseases. It also restricts the oxygen to the foetus in cases of smoker pregnant females resulting in low weight babies at the time of birth (Mishra A, 2015).

Nitrogen oxides- It has been hypothesized for initiating lung damage causing emphysema (Talhout R, 2011)

CARCINOGENS IN SMOKE-LESS AND SMOKED TOBACCO

The smoke which emerges from the mouth piece of the cigarettes consist of an aerosol containing about 10^{10} particles/ml and 4800 compounds. Glass fibre filter can be used experimentally to separate vapour phase component from particulate phase components. Nitrogen, oxygen and carbon dioxide are the major contributor of vapour phase, in addition to other potentially carcinogenic substances like nitrogen oxides, isoprene, butadiene, benzene, styrene, formaldehyde, acetaldehyde, acrolein, and furan. Particulate phase contribute to 3500 different components among which including polycyclic aromatic hydrocarbons (PAH), N-nitrosamines, aromatic amines, and metals. The IARC [International-Agency for Research on Cancer] has listed around 60 carcinogens to be present in cigarette smoke. These all belongs to different categories of chemicals including PAH (Polycyclic aromatic hydrocarbon), aza-arenes, N nitrosamines, aromatic amines, heterocyclic amines, aldehydes, volatile hydrocarbons, nitro compounds, miscellaneous organic compounds, and metals and other inorganic compounds (Hoffmann D, 2001).

Smoke-less tobacco usage for a longer duration showed increased risk of oral cancer. A significant relation between oral cancer and elevated level of

tobacco-specific N-nitrosamines (TSNAs) has been established. (Brown BG, 2003). Some forms of smoke-less tobacco which are derived from bacterial or enzyme action on nicotine during processing are shown to have increased amount of carcinogen (Savitz DA, 2006). PAH, cadmium, polonium, formaldehyde and lead are the other potentially carcinogenic substances associated with oral cancer (Janbaz KH, 2014).

SYSTEMIC EFFECTS OF TOBACCO

Immediate effects of nicotine and its toxicity- Its immediate effects include transient tachycardia, hypertension, burning sensation of mouth and throat with gastrointestinal symptoms like nausea, vomiting and diarrhoea. It increases level of catecholamine resulting in hyper-glycemia and plasma free fatty acids level. It also causes tachypnoea resulting in hypothermia and hyper-coagulable state due to increased blood viscosity (Dani JA, 1996; Vellappally S, 2007). Nicotine is one of the most potent poison which can effect central and peripheral nervous system. Lethal dose of nicotine is 30-60 mg and 10 mg for adults and children respectively. Severe poisoning can result into tremors, cyanosis, dyspnoea, convulsions followed by collapse and coma. Death can result in severe cases due to failure of respiratory system secondary to respiratory muscles paralysis (CDC, 2014)

Nicotine and Green tobacco sickness- It is an acute form of nicotine toxicity in which symptoms like headache, nausea, vomiting, appetite loss, fatigue and arrhythmias can occur lasting from 12-24 hours. It occurs in tobacco industry workers secondary to green tobacco leaves handling, although it has a very low significant mortality rate (McKnight RH and Spiller HA, 2005).

Nicotine and addiction of tobacco- Nicotine is the primary cause for addiction in users of tobacco. US surgeon concluded its addictive action secondary to interaction with nicotinic acetyl choline receptors which stimulated dopaminergic transmission. It is associated with mood uplift and probable augmentation of intellectual functioning. The constant habitual incitement of GABA neurons by nicotine make them less sensitive resulting in loss of their inhibitory function on dopamine. This in turn result into build up of enslavement by generating craving for the nicotine. Studies has supported

heritable dependency on nicotine by its effect on CYP2A6 gene which can be transmitted from the mother or grand-mother through epigenetic mechanism (Mansvelder HD and McGehee DS 2002; Leslie FM, 2013).

Nicotine and metabolism- It causes release of catecholamine and stimulates autonomic nervous system. It stimulates α- adrenoceptors resulting in reduction of body weight secondary to lipolysis. It promotes glycogenesis with reduction in fasting blood glucose level. It affects insulin resistance making the person prone to diabetes (Bruin JE, 2007).

Nicotine and cardiovascular system- Nicotine affects haemodynamic system through its sympathomimetic mechanism. It alters structure and functioning of vascular smooth muscle and endothelial cells by promoting release of fibroblast growth factor and inhibition of transforming growth factor- β. These all results into atherosclerotic plaque formation causing tachycardia and hypertension ultimately resulting into coronary vascular diseases followed by acute myocardial ischemia (Villablanca AC, 1998).

Nicotine and respiratory system- The effects of nicotine occur through direct exposure to lungs and indirectly through central nervous system mechanism. It decreases elastin in lung's parenchyma and increase alveolar volume causing emphysema. It also promotes bronchoconstriction by triggering vagal reflex and parasympathetic ganglia. Its effect on central nervous system results into severe respiratory disorders secondary to bronchoconstriction and apnoea (Mishra A, 2015).

Nicotine and gastrointestinal system- Studies has shown a significant relation of occurrence of diseases like GERD (Gastro Esophageal Reflux Disorder)/ PUD (Peptic Ulcer Disease) with habit of smoking. The proposed pathogenesis behind these disease is increased gastric acid, pepsinogen and vasopressin secretion. Smooth muscle relaxation via nitric oxide is thought to be responsible for decreased colon tonicity, gastric motility and lowered oesophageal sphincteric pressure which ultimately result into GERD. It also increases chances of treatment resistant Helicobacter pylori infection (Li LF, 2014).

Nicotine and immune system- Tobacco causes damage of antigen receptor mediated signal response in lymphoid organs leads to immunosuppression. It also hampers T-cell production by arresting cell cycle. Impairment of macrophage response make the smokers prone for diseases like tuberculosis. Decreased epithelisation and cell adhesion leads to hampered wound healing causing increased chances of secondary opportunistic infections (Bagaitkar J, 2008).

Nicotine and ocular system- Various animal and clinical models has proven that nicotine triggers pathological and retinal neovascularization which contribute to age related maculopathy. Synergistic effects of nicotine with glucose metabolism tends to increase risk for diabetes mellitus which might accelerate cataract formation (Tirgan N, 2012).

Nicotine and renal system- Renal system get effected as a result of introduction of COX-2 isoform. It results into glomerulonephritis manifested clinically as albuminuria, decreased glomerular filtration rate and impaired mechanism to control systemic hypertension. It is also associated with renal artery stenosis and hence are strongly related with increased fatality among the patients with end-stage kidney disease (Halimi JM, 1998).

Nicotine and reproductive system- Nitrous oxide (NO) released from postganglionic nerve fibres of parasympathetic system and endothelial cells are responsible to cause penile vasodilation and corpus cavernosum relaxation to attain penile erection in males. Nicotine inhibits NO production resulting in erectile dysfunction diseases (Dean RC, and Lue TF (2005). It also affect testosterone level by hampering production of a protein called StAR (Steriodogenic Acute Regulatory) protein, necessary for synthesis of testosterone (Oyeyipo IP, 2013). Nicotine hampers production of androgens in female by inhibiting metabolites like 21 hydoxylase resulting in recurrent anovulation and irregular menstrual cycle (Mishra A, 2015).

ORAL EFFECTS OF TOBACCO USAGE

Staining- Excessive tobacco use in any form smoke-less or smoked tobacco, will result stains over the teeth, oral mucosa, prosthesis or restoration

resulting in compromised esthetics. Smoking causes much harsh staining than drinking caffeine rich beverages (Ness L, 1977).

Olfaction and gustation- Studies has proven that smoking adversely effects taste and smell perception. Apart from that smoking is the most common cause for halitosis (Vennemann MM, 2008).

Dental caries- Many studies support correlation of smoking with etio-pathogenesis of dental caries. Altered buffering action of saliva and microbial shift towards cariogenic microorganism seen in smokers are the two most important argument which support higher incidence of dental caries in smokers (Heintze ULF, 1984; Vellappally S, 2007).

Wound healing- Smoking increases plasma concentration of adrenaline and non-adrenaline which in turn causes vasoconstriction at the place where vasodilatation is required. Impaired functioning of neutrophils along with vasoconstriction further delays the process of wound healing (Vellappally S, 2007).

Periodontal diseases- There is growing scientific data which accounts for significant correlation between smoking and diseases of the periodontium. These periodontal diseases include gingivitis, periodontitis and acute necrotizing ulcerative gingivitis[ANUG]. However, exact correlation is not understood, hence authors have kept smoking as a risk factor, not an exact etiology. Studies revealed deposition of more supra-gingival plaque in smokers as compared to non-smokers because of poor oral hygiene. Some other studies has reported higher number of B. forsythus in smokers than non-smokers (Zambon JJ,1996; Preber H, 1992). However, none of the results of these studies are accepted worldwide. Hence, impairment of immunity in smokers in form of disturbed immunoglobulin and cytokine level with hampered lymphocyte functioning both qualitatively and quantitatively is hypothesized as increased prevalence and severity of periodontal disease (Bostrom, 1998).

Implants- Number of studies has demonstrated poor prognosis of implant in initial as well as in long term follow up. Also, cessation of smoking has been helpful in obtaining higher implant success rate. Failure rate of implant was

observed 11.3% in smokers as compare to 4.8% in non-smokers. Assessment of maxillary implants separately has shown failure rate of 17.8% in smokers. Another study has shown implant rate failure of 9% of smokers in comparison to 2% of non-smokers before loading in cases of maxilla (De Bruyn H and Collaert B, 1994).

ORAL MUCOSAL DISEASES DUE TO TOBACCO USAGE

Smoker's Melanosis- It is an asymptomatic, non-premalignant and reversible condition which effect the individuals who has habit of heavy smoking. It has a prevalence rate of 30% where attached gingiva is mostly affected. It is more common in coloured races than Caucasians where its prevalence rate is just 10%. The condition is reversible and usually take 1 year or more than that to disappear (Axell T and Hedin CA, 1982).

Tobacco induced keratosis- It is also an asymptomatic and reversible condition which occur secondary to usage of smoke-less tobacco. It induces wrinkling of oral mucosa in the region where quid is placed. It is associated with or without colour changes which are reversible. If colour changes are present, it will be from whitish-yellowish to brown. Gingival recession can also be seen in the same region of quid placement (Behura SS, 2015).

Oral Candidiasis- Various studies has documented the correlation of smoking with candidiasis. Lesions like median rhomboid glossitis or angular chelitis are been associated with the habit of smoking but are not supported by clinical evidences. However, studies has proven improvement of candidal infection after cessation of smoking (Mohd Bakri M, 2010).

Premalignant lesions- A number of potentially premalignant lesions like leukoplakia, erythroplakia or erythro-leukoplakia are been associated with tobacco usage. Studies has shown prevalence of leukoplakia six times more in smokers as compared to non-smokers and cessation of same has resulted into regression of the lesion (Vellappally S, 2007).

Oral cancer- Smoking tobacco is one of the chief predisposing factors for causation of oral cancer followed with alcohol consumption, betel quid usage, compromised oral health and human-papilloma viral infection (Lin WJ, 2011).

Tobacco smoke comprises of thiocyanate, hydrogen cyanide, nicotine and its metabolites. As discussed earlier tobacco constitute potential carcinogens like nitrosamines, polycyclic aromatic hydrocarbons, nitrosodicthanolamine, nitrosoproline and polonium (Vellappally S, 2007).Various epidemiologic studies has concluded a large number of patients suffering from oral cancer had the habit of smoking. Also, high chances of recurrence were seen in patient who continued smoking after treatment (Uplap PA, 2011; Garg KN, 2013). Occurrence of oral cancer shows variations in different regions of the world due to the variation in the form of tobacco usage. In countries of Asia, tobacco is consumed in form of quid which makes oral cancer incidence rate higher in these countries. In non-smokers, tongue is the most common site in comparison to floor of mouth among smokers. Increased use of smoke-less tobacco has attracted the attention of government and non-government authorities due to increased incidence of benign hyperkeratosis, epithelial dysplasia and malignant lesions among youngsters (Silverman, 1998). Various studies has also proven the harmonious effects of drinking and tobacco usage for oral cancer development. This synergism can be explained by the dehydrating effect of alcohol on buccal mucosa(BM) which increases mucosal permeability to different carcinogens (Morse DE, 2007). Carcinogens like N-nitroso compounds, polycyclic aromatic hydrocarbons (PAH), 4-(methylnitrosoamino) -1-(3-pyridyl)-1 butanone (NNK) etc. are capable to do G:T transversions. These mutations keep on accumulating in epithelial cells leading to genomic stability, occurrence of premalignant lesion and in severe cases into invasive carcinoma. Tobacco also has the potential to activate EGFR receptors which in turn will activate cyclin D1, leading to further increase in proliferative activity and mutations. It further make those genetic changes more permanent and make the cells more susceptible to premalignant changes and invasive cancer (Lin WJ, 2011).