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, 1994)

Alignment of malpositioned 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. 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, 2014)

Carcinogenesis is a multi-factorial event which involves overlapping of various etiological aspects for the initiation of a malignant process. (Barrett, 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 unpolymerized 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, 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. Poor oral hygiene has found to be associated with the development of oral cancer and hence should be considered as a potential risk factor. (Marques, 2008; Behnoud, 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, 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 proportions of iron, nickel, chromium, cobalt 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, 2012) Various studies have shown the presence of
these ions in the saliva. The accumulation of these ions within the mucosal cells has also been documented. (Amini, 2008; Hafez, 2011; Natarajan, 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 inducing genotoxic and cytotoxic effects on the oral epithelial cells. (Chaturvedi, 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, 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, 2009; Ozturk, 2012; Toy, 2014)

The mutagenic and carcinogenic effects of ionizing raditations are well known, especially at higher doses. These affect the epithelial cells either directly or indirectly through the formation of reactive compounds. (Ogden, 1989) 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, 2012; Arora, 2014; Vidya, 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 parafunctional habits in malignant lesions. The irritation of oral mucosa during orthodontic treatment needs attention in this regard. (Piemonte, 2010; Perry, 2015)

Worldwide, tobacco use causes more than 5 million deaths per year, and current trends show that tobacco use will cause more than 8 million deaths annually by 2030. Smoking causes cancer, heart disease, stroke, lung diseases (including emphysema, bronchitis, and
chronic airway obstruction), and diabetes.(Centers for disease controls and prevention, 2014) Oral cancer is the sixth most common cancer in the world and occurs due to multiple etiologies. Seventy five percent of head and neck squamous cell carcinomas are attributed to tobacco and alcohol use.(Jindal, 2013) Cigarettes contain many carcinogenic substances, mostly DNA-toxic carcinogens. It is well known that these carcinogenic substances cause genetic mutations and chromosomal abnormalities and micronuclei.(Nefic, 2013) Tobacco smoking can cause carcinoma in different parts of the oral cavity, including the lip, tongue, palate, gum, and cheek.(Kamath, 2014) Smoking is thought to be associated with increased keratinization in of the oral mucosa; and even less keratinized areas of the oral cavity are at a risk of deleterious effects of smoking.(Yerlagudda, 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 orthodontic brackets 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 buccal mucosa in patients undergoing fixed orthodontic treatment.

The rationale of oral exfoliative cytology is based on the physiological process of desquamation, wherein the cells are examined that are desquamated or abraded from the surface of the oral mucosa.(Kumaresan, 2014) Miller et al. were the first to study the cytology of the normal oral epithelium. (Miller, 1951) The superficial epithelial cells do contain nuclei, and thus, alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes. This technique is useful for preliminary diagnosis of many oral mucosal diseases but it is not a substitute for the routinely used biopsy to obtain a definitive diagnosis. Lesions caused by reactive changes and inflammatory reactions are non-specific and non-diagnostic cytological findings. A quantitative technique increases the diagnostic ability of exfoliative cytology. It is precise, objective and reproducible.(Kazanowska, 2014)
Micronucleus is a cytoplasmic fragment of DNA reported as a biomarker of mutagenesis. The micronucleus assay is used to detect the subjects at high risk of malignant transformations in their oral epithelial cells. It has been extensively used to evaluate the extent of chromosomal damage in the human population exposed to the genotoxic agents in various occupational settings, in the environment, or as a consequence of the life style. (Jois, 2010) The criteria set by Tolbert et. al. is commonly used for evaluation of micronuclei. (Tolbert, 1991) Various studies have established the association of occurrence of micronuclei in cells exposed to genotoxic agents like tobacco, alcohol etc and also in premalignant lesions and oral squamous cell carcinoma. (Stich, 1982; Salama, 1999; Casartelli, 2000; Halder, 2004)

Cytomorphology is the most widely used method of oral exfoliative cytology, and assesses parameters such as cellular diameter (CD), nuclear diameter (ND), nuclear area (NA), cytoplasmic area (CA), NA/CA ratio, nuclear shape, nuclear membrane continuity, optical density, and nuclear texture. These parameters, especially NA and NA/CA ratio, have been shown to provide meaningful results in the diagnosis of oral lesions. (Kazanowska, 2014) Cytomorphometrical analysis of oral exfoliated cells from normal healthy individuals have been categorized according to age, gender and location of the oral cavity and used to create a baseline data which can be used in the future for comparison with pathologically altered cells. (Cowpe, 1985)

The micronucleus 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 analyse the genotoxic and cytotoxic effects on oral buccal mucosal cells in patients undergoing fixed orthodontic treatment and due to tobacco smoking.