WORK PLAN AND METHODOLOGY:

Source of Data:
Young adult patients between the age group 18-30 of both genders undergoing fixed orthodontic treatment in the Department of Orthodontics who meet all of the selection criteria will be included in the study.

Study type:
This will be a cross sectional study conducted for a period of one year between January 2015 till December 2015.

Sample size calculation:
Since the number of patients undergoing orthodontic treatment who are smokers is quite low. The convenient sampling technique will be used and the individuals who meet the inclusion and exclusion criteria during the study period will be included in the study. The patients participating in the study will be informed about the study protocol and a written informed consent (in local language – Nepali or English) will be taken. An ethical clearance for the study will be taken from Nepal Health Research Council (NHRC).

Study sample:
Group A: Orthodontic patients who are smokers.
Group B: Orthodontic patients who are non smokers.
Group C: Smokers.
Group D: Non smokers.

Inclusion criteria:
- Patients undergoing fixed orthodontic treatment will be included in this study (group A & B).
- Group C & D will include individuals who are not undergoing any form of orthodontic treatment.
- Group B & C will form the positive control whereas Group D will form the negative control.

Exclusion criteria:
- Any other deleterious habits (tobacco chewing, alcohol consumption).
- Patients with systemic diseases.
- Patients with malignant and premalignant lesions.
• Patients under medications (antibiotics/steroids).
• Use of alcohol containing mouthwash.
• Use of partial or complete removable dentures.

Sample collection:
The oral buccal mucosal cells will be collected after taking informed consent from the participant. The participant will be instructed to rinse his/her mouth with water after which the buccal mucosal cells will be collected using a sterile wooden spatula. A smear will be prepared on a clean microscopic slide and staining will be done with Rapid PAP stain (BIOLABS, India) as per the instructions of the manufacturer.

Analysis of sample:
The slides will be analyzed under a binocular light microscope (Olympus CH 20i) for adequacy of the smear. Micronuclear count will be done at 400x magnification according to the criteria laid down by Tolbert et. al.; which are as follows:

A. Parameters for cell inclusion in the cells to be scored:
   i) Cells with intact cytoplasm and relatively flat position on the slide.
   ii) Minimum or no overlap of cells with each other.
   iii) Absence of debris on the slide.
   iv) Normal intact nucleus with smooth and distinct perimeter.

B. Parameters for identifying micronucleus:
   i) Perimeter should be round and smooth suggestive of a membrane.
   ii) Less than a third the diameter of associated nucleus, but large enough to discern shape and colour.
   iii) Intensity of staining comparable to that of the adjacent nucleus.
   iv) Texture should be similar to that of nucleus.
   v) Should be in the same focal plane as nucleus.
   vi) There should be absence of bridge between micronucleus and nucleus.

A total of 1000 exfoliated cells will be counted per slide in a step ladder pattern starting from the upper left hand corner or the slide and the micronuclear count among these will be determined and documented. Cytomorphological analysis will be done for 50 random cells in a similar fashion at 400x magnification. Photographs of these cells will be taken using a digital camera and transferred to a computer for image analysis. The parameters will be
analyzed using ImageJ 1.48V software (Wayne Rasband National Institute of Health, USA) and will include nuclear diameter, cell diameter, nuclear diameter/cell diameter ratio, nuclear area, cell area, nuclear area/cell area ratio.

**Statistical analysis:**

Statistical analysis will be performed using SPSS software version 11.5. One Way ANOVA will be performed to compare the micronuclear count and cytomorphological changes in the various study groups.