

EXAMINATION OF THE EFFECT OF SMOKING ON CHEEK CELLS AT A MICROSCOPIC LEVEL

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ABSTRACT

Oral cancer is lifestyle-related cancer, with the consumption of tobacco as a primary factor. The purpose of this study is to determine the impact of smoking on the cheek cells of an individual, using scrapings containing exfoliated buccal cells to correlate the microscopic findings and observations with the period for which each participant smoked and the frequency of their consumption of tobacco. The association between cells showing alterations and indication of buccal mucosa cancer with an abnormally large nucleus to cytoplasm ratio and the time period for which the respective participant has been exposed to smoking is examined. Behaviour intervention to quit smoking may be accelerated if the global public is made aware of risk factors for developing cancer of oral mucosa. Thus, proving the need for increased research on the same.

KEYWORDS :

1. INTRODUCTION

1.1 What is Cancer?

All living organisms are made up of cells.

Under normal circumstances, cells that are too old or damaged are consequently replaced by newly synthesized cells. While the lifespan of a cell is variable, they usually extend to approximately 13 days (excluding skin cells, red blood cells, and liver cells). A normal cell divides till a specific time period in a controlled way. However, due to the interference of genetic changes with this orderly process, normal cells are converted into tumours (an area of inflammation or an invisible biochemical abnormality) cells, thus causing cancer to arise.

Cancer, as stated by the WHO, is 'the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. Cancerous cells lose the property of inhibition and keep dividing.

The most feared property of cancerous cells is metastasis, i.e., invading new sites, thus leading to the formation of tumours (outgrowth)

- Benign tumour- Localized tumours (do not spread and can be removed through Surgery)
- Malignant tumours- Spreads to new sites.

1.2 What is Buccal Mucosa Cancer?

Also known as Cheek cell Cancer, Buccal Mucosa (Inner lining of cheek cell) Cancer is a rare form of oral cancer that can be aggressive and has a high recurrence rate. It occurs when the inner cheek cells grow out of control and form lesions or tumours.

These cancers usually occur in the thin, flat cells called squamous cells that line the buccal mucosa and other parts of the mouth.

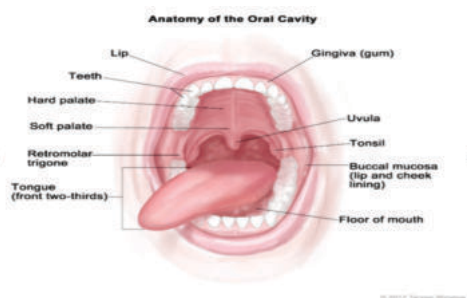


Figure 1 Anatomy of the Oral Cavity

Source: Google image

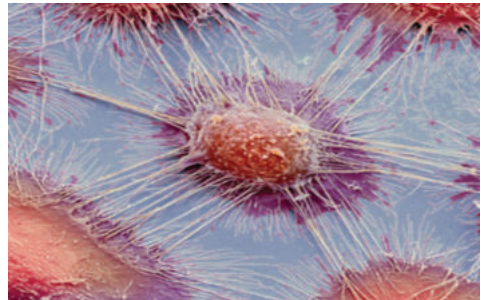


Figure 2 Mouth cancer cells, SEM Magnification x2200

Source: Science photo library

1.3 Risk Factors:

Some tobacco-associated buccal cell changes have been reported to be biomarkers of disease progression; other changes are candidate biomarkers currently being evaluated. These buccal cell changes include genetic alterations (i.e., mutations). It is recognized that bacteria bind and colonize mucosal surfaces. Bacterial adherence (desmosomes) is thought to be the first important step in the pathogenesis of infection. Squamous cell carcinoma of the skin, or SCC, is an invasive epithelial neoplasm with varying degrees of squamous differentiation. Disorganized stratified squamous epithelium forming strands and islands of bizarre epithelial cells presenting severe dysplasia (the presence of cells of an abnormal type within a tissue, which may signify a stage preceding the development of cancer) infiltrating subjacent submucosa is observed. Risk factors include:

- Tobacco use
- Heavy alcohol use
- Human papillomavirus (HPV) infection
- Chronic rubbing from rough teeth, dentures, or fillings
- Medicines (immunosuppressants) that weaken the immune system
- Poor dental and oral hygiene

1.4 Symptoms of Buccal Mucosa Cancer

The tumours are traditionally graded into well, moderately, and poorly differentiated SCC:

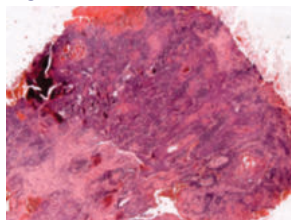
- Well-differentiated carcinoma resembles closely normal squamous epithelium.
- Moderately differentiated carcinoma contains distinct nuclear pleomorphism and mitotic activity, including abnormal mitosis, and there is normally less keratinization.
- In poorly differentiated carcinoma, immature cells predominate, with numerous typical and atypical mitosis and minimal keratinization.

Some symptoms observed by patients are:

- Difficulty in swallowing
- Mouth pain- Jaw pain or swelling
- Ear pain
- Loose teeth
- Presence of lump in the mouth
- Hoarseness
- Presence of patches in the mouth that may be - white, red, or dark
- Difficulty in moving jaw

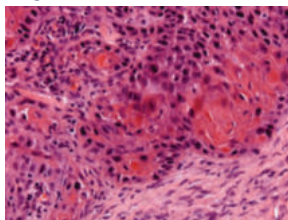
Dyskeratosis, polymorphism, hyperchromatism, atypical mitosis and loss of nucleolus-nucleus and nucleus-cytoplasm ratio are also marked cellular characteristics as shown in Figure 3 and Figure 4 below.

Figure 3



(Magnification X50)
Source: Google image

Figure 4



(Magnification X400)

1.5 Diagnostic methods

The confirmation of the presence of any type of Oral cancer can primarily be done through a biopsy. In the case of oral cancer, the type of biopsies used for detection are:

Incisional biopsy-

is a medical test where a piece of tissue from a lesion or mass is removed. The piece of tissue is then passed on to a pathologist for examination.

Exfoliative cytology-

A suspicious area is gently scraped to collect a sample of cells. These cells are placed on a glass slide and stained with dye so that they can be easily viewed under a microscope. If any cells appear abnormal, a deeper biopsy will be performed.

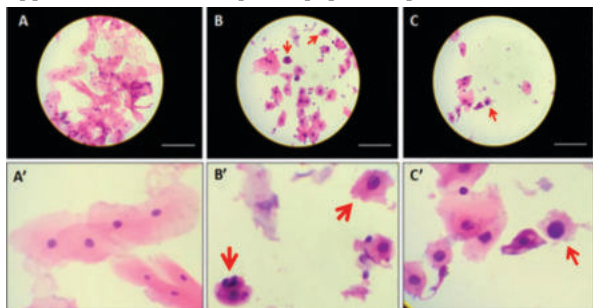


Figure 5 Images of Oral cancer under a microscope
Source: Google image

1.6 Treatment:

Even though there is a tremendous amount of research being conducted in this field currently, if cancer is in the buccal mucosa (the lining of the inside of the cheeks), treatment may include the following:

Surgery (wide local excision) with or without radiation therapy.

Radiation therapy.

A clinical trial of chemotherapy before or after Surgery.

A clinical trial of chemotherapy and radiation therapy.

A clinical trial of hyperfractionated radiation therapy.

2. METHOD OF STUDY

This was a controlled study with six participants between the ages of 40 to 50 years, who were selected after a survey on the history of smoking was done since diversity in terms of the period of smoking is crucial to this study.

Only a biopsy can confirm an oral cancer diagnosis. A sample of tissues or cells is required for a biopsy, which must be conducted before treatment begins. The type of biopsy that is typically used for diagnosing oral cancers and will be utilized in this study is Exfoliative cytology (biopsy of scrapings containing exfoliated buccal cells) Diverse methods have been used successfully for harvesting buccal cells. Samples collected by scraping consist almost exclusively of buccal cells. Resources used for conducting this study include:

- Toothpick
- Microscope
- Glass slide
- Filter paper
- Glycerine
- Coverslips
- Methylene blue solution

There are five stages of mouth cancer, starting at zero and going up to four. Since none of the test subjects involved in this study has been diagnosed with buccal mucosa cancer, we mainly inspected the cheek cell specimens for stage 0 mouth cancer, or carcinoma in situ, which describes abnormal cells in the lining of the oral cavity, which have the potential to become cancer. The following procedure was followed:

- We gently scrape the inner lining of the mouth of the first person with a toothpick.
- The cheek cells are transferred from the toothpick to the glass microscope slide through a series of stroking motions.
- We add a drop of methylene blue solution to the cheek cells
- We add one drop of glycerine to the specimen and place a coverslip on top
- The excess solution is removed with the help of a filter paper
- The slide is consequently placed under the microscope and is observed
- The observations are noted, and a picture of the cells under high magnification is taken for later comparisons.
- The exact process is carried out with the cheek cells of all of the participants.

Note- the presence of diversity in terms of the period of smoking among the participants is crucial.

The observations are compared with the period for which each participant has smoked and the frequency of their consumption of tobacco to form a conclusion.

2.1 Limitations of the study:

The *in vivo* and *ex vivo* strategies are intended to identify the precise location of oral cancer and thereby enhance the success of different therapies. These schemes are not without limitations and challenges. For example, screening for premalignant oral tissues by visual examinations with the naked eye, or facilitated with a scope, may be complicated by the presence of nonneoplastic tissue with inflammation or fibrosis.

Ex vivo assays may identify cellular or genetic changes; however, these alterations may not correlate with oral disease and, therefore, cannot be used for tumour detection.

3. Observation:

Figure 6 Brief details of participants of study

Participants	AGE	History of tobacco use
Subject 1	45	Never indulged in any form of tobacco

Subject 2	47	Constant and frequent smoking, multiple times within a single day, since the age of 19
Subject 3	47	Prior smoking history: smoker from the age of 18 till 30 years. Did not frequently smoke- only once or twice a month
Subject 4	43	Has smoked multiple times within a day since the age of 18
Subject 5	41	Never indulged in any form of tobacco
Subject 6	43	Has smoked daily since the age of 19

While subjects 1, 3, and 5, who had little to no exposure to smoking, showcased normal cheek cells with no mutations or signs of buccal mucosa cancer. Subject 3, who had given up smoking 17 years back with a prior smoking history of being an infrequent smoker from the age of 18 to 30 years, showed no indication of buccal cell cancer. Whereas subjects 6, 4, and 2, who had prolonged and frequent smoking habits, and consumed multiple cigarettes daily, showcased a high nucleus to cytoplasm ratio indicating a suspicious cell. This observation is indicated by red arrows in Figure 7. This could indicate buccal mucosa cancer for subjects 6, 4, and 2, and an incisional biopsy needs to be performed to confirm the findings.

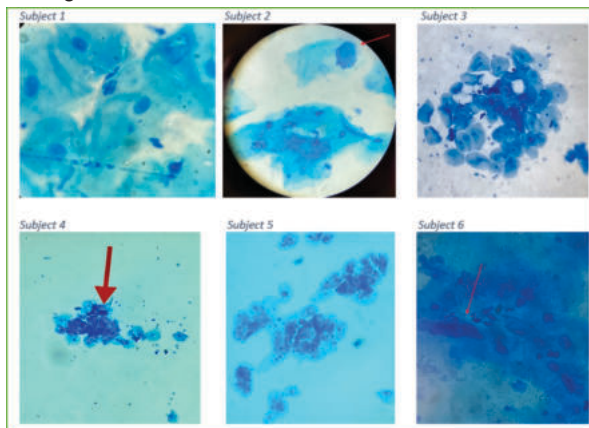


Figure 7 Images of cheek cells under a microscope of study participants

Image source: Ehsaas Madan

4. CONCLUSION:

By comparing the microscopic findings and observations along with the period for which each participant has smoked and the frequency of their consumption of tobacco, we may conclude a positive association between cells showcasing buccal mucosa cancer, which is observed as abnormally large nucleus to cytoplasm ratio and the longer time period for which the respective participant has been exposed to smoking. Hence frequency and period of smoking correlate with buccal mucosa cancer. If cancer is in the buccal mucosa, treatment options may include Surgery (wide local excision) with or without radiation therapy and chemotherapy. Even though research in this area is phenomenal, screening for timely detection and behaviour intervention to quit smoking is critical to the treatment and prevention of buccal mucosa cancer, and hence more research is warranted to educate on the dangers of tobacco.

5. REFERENCES

1. "The Biopsy Report: A Patient's Guide." The Oral Cancer Foundation, <https://oralcancerfoundation.org/discovery-diagnosis/biopsy-report-patients-guide/>.
2. "Buccal Mucosa Cancer: Inner Cheek Cancer." Memorial Sloan Kettering Cancer Center, <https://www.mskcc.org/cancer-care/types/mouth/types-mouth/inner-cheek-cancer-buccal-mucosa#:~:text=Inner%20cheek%20cancer%20>.
3. "Buccal Mucosa Cancer: Inner Cheek Cancer." Memorial Sloan Kettering Cancer Center, <https://www.mskcc.org/cancer-care/types/mouth/types-mouth/inner-cheek-cancer-buccal-mucosa>.
4. Cancer Resources from OncoLink | Treatment, Research. "Incisional & Excisional Biopsy." OncoLink, <https://www.oncolink.org/cancer-treatment/>

5. "Cancer." World Health Organization, World Health Organization, <https://www.who.int/news-room/fact-sheets/detail/cancer>.
6. Cebp.aacrjournals.org, <https://cebp.aacrjournals.org/content/15/6/1061.figures-only>.
7. Clark AL; Gillenwater AM; Collier TG; Alizadeh-Naderi R; El-Naggar AK; Richards-Kortum RR; "Confocal Microscopy for Real-Time Detection of Oral Cavity Neoplasia." Clinical Cancer Research : an Official Journal of the American Association for Cancer Research, U.S. National Library of Medicine, <https://pubmed.ncbi.nlm.nih.gov/14581341/>.
8. Dyalram, Donita. "Addressing Squamous Cell Carcinoma of the Buccal Mucosa." Decisions in Dentistry, <https://decisionsindentistry.com/article/addressing-squamous-cell-carcinoma-buccal-mucosa/>.
9. "How to Test, Diagnose and Detect Oral Cancer." Cancer Treatment Centers of America, 31 May 2022, <https://www.cancercenter.com/cancer-types/oral-cancer/diagnosis-and-detection>.
10. "Mouth Cancer Cells, Sem by Science Photo Library." Pixels, <https://pixels.com/featured/6-mouth-cancer-cells-sem-science-photo-library.html>.
11. "Mouth Cancer." Mayo Clinic, Mayo Foundation for Medical Education and Research, 20 Oct. 2020, <https://www.mayoclinic.org/diseases-conditions/mouth-cancer/symptoms-causes/syc-20350997>.
12. "Oral Cancer Screening." Mayo Clinic, Mayo Foundation for Medical Education and Research, 30 Oct. 2021, <https://www.mayoclinic.org/tests-procedures/oral-cancer-screening/about/pac-20394802>.
13. "Oral Cancer: Medlineplus Medical Encyclopedia." MedlinePlus, U.S. National Library of Medicine, <https://medlineplus.gov/ency/article/001035.htm>.
14. Phil Banks. Visual Aids, MRC Laboratory of Molecular Biology. "Experiments Quick Links." Human Cheek - Experiments on Microscopes 4 Schools, <https://www2.mrc-lmb.cam.ac.uk/microscopes4schools/humancheek.php>.
15. "Science Netlinks." Science NetLinks | American Association for the Advancement of Science, <http://sciencenetlinks.com/student-teacher-sheets/cells-your-body/#:~:text=The%20length%20of%20a%20cell's,cells%20live%20about%2018%20months>
16. Skandarajah, Arunan, et al. "Mobile Microscopy as a Screening Tool for Oral Cancer in India: A Pilot Study." PLOS ONE, Public Library of Science, <https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0188440>.
17. "What Is Cancer?" Cancer.Net, 4 Oct. 2019, <https://www.cancer.net/navigating-cancer-care/cancer-basics/what-cancer>.