ISSN No:-2456-2165

Evaluation of Nuclear Morphology in Buccal Epithelial Cells of Smokers: A Case Study at Mulungushi University

Musonda Deborah Bwalya and Hyden Simwatachela

May, 2022

.

.

.

.

ISSN No:-2456-2165

ABSTRACT

This study investigates the degree of karyolysis, pyknosis, karyorrhexis, and karyokinesis of the exposed epithelium against a control population, to evaluate the cytological features of the nucleus, micronuclei frequency and other nuclear anomalies in buccal mucosal cells of tobacco users, and to asses if micronuclei frequency can be used to access the genotoxicity in tobacco users. The study is generally focused on creation of a biomarker that can be used to detect early pre cancers in smokers this research was a case study. The study used qualitative and quantitative method to collect data around thirty two smokers (32) and twenty (20) nonsmokers around Mulungushi University. The approach used ethical clearance by asking the individuals who were willing to provide two buccal swabs willingly which where smeared on the slides and fixed in 95% ethanol before the staining of slides was done. After the samples were collected they were later stained using the papanicolaou stain. Descriptive statistics were employed in analyzing data by using frequency distribution of the responses from the respondents in SPSS and linear graphing using the excel sheet.

The results of the study revealed that tobacco has an effect on the buccal epitheliumand therefore this test can be used as a biomarker for detecting mouth epithelial cellsthat are pre-cancerous in smokers. When the binucleation analysis was done it was found that at least two cases where present in smokers and none at all in non-smokers. The number of micronuclei detected in most smokers was way greater than nonsmokers looking at the mean value of both in the results.

This study recommends more sensitization on the effects of smoking to people and to create more awareness programs on pre cancers and cancer symptoms. More and further studies should be done on the examination of mouth chromosomes for smokers against a control population to get dipper understanding on the case study.

ISSN No:-2456-2165

CHAPTER ONE

INTRODUCTION

According to the World Health Organization, tobacco kills more than 8 million people per year, including both smokers and non-smokers who experience second-hand smoke. The Center for Disease Control points out that tobacco is the leading cause of preventable disease, disability, and death in the United States.Tobacco uses increases the likelihood of developing lung cancer, oral cancer, heart disease, and blood clots. Tobacco use also increases the risk of heart attack and stroke and leads to tooth and gum decay and wrinkled skin.Tobacco prevalence is affected by several factors. Prosperity is the first of these. Wealthier countries tend to smoke more. Certain religions, such as Christianity and Judaism, are anti-tobacco. Additionally, in some cultures around the world, smoking is part of the social culture and is almost expected for males. (World population review, 2022)

Oral cancer affects as many as 274,000 people world-wide annually and the frequency of oral cancer around the world is often indicative of the patterns of use of tobacco products. It has been established that there is a dose-response relationship between the amount of tobacco product used and the development of oral cancer. Worldwide, more than a billion people smoke cigarettes on a daily basis. (Proia, Paszkiewicz et al. 2006)

The risk of death from cancer has decreased continuously since 1991, resulting in an overall drop of 32% and approximately 3.5 million cancer deaths averted as of 2019. This success is largely because of reductions in smoking that resulted in downstream declines in lung and other smoking-related cancers. (Yabroff, Wu et al. 2021)

Globally, about 82,000-99,000 young individuals are initiated to the habit of smoking a cigarette each day. The addiction component in cigarette smoking is nicotine. On an average an American cigarette contains 8-9 mg of nicotine whereas average Indian cigarette contains 15 mg. The increased level of this nicotine concentration causes individuals to get addicted (Tobacco survey India, 2010)

Clinically, it is well established that cigarette smoke causes various types of malignancies, like cancer of the oral cavity. (DeMarini DM, 2004).

Oral squamous cell carcinoma is the most common malignant neoplasm of the oral cavity that causes significant morbidity and mortality, since in most instances it is detected at advanced stages (Neville BW et al, 2016).

Tobacco is one of the strongest carcinogens responsible for the development of oral cancer. Cigarette smoking is the main cause of lung cancer worldwide. Tobacco products are estimated to cause approximately 90% of lung cancer cases. Experimental studies in laboratory animals have collectively demonstrated the carcinogenicity of tobacco products. There are over 5,000 identified chemicals and more than 60 known carcinogens in cigarette smoke, with polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK) likely being the most important respiratory carcinogens. The reaction of carcinogens with DNA can cause mutations and, if unrepaired, can lead to the activation of oncogenes or the inactivation of tumor suppressors. Epigenetic changes may also occur from exposure to tobacco carcinogens, leading to a change in gene expression(Peterson LA, 2010).

The buccal mucosa in tobacco users especially in those withoral lesions exfoliate with difficulty and show increasedkeratinization, overlapping and degenerative changes.The nuclear anomalies like fragmentation, karyorrhexis,karyolysis, broken egg nuclei, condensed nuclei, andbinucleation are also seen in tobacco users with increasedfrequency compared to controls, but only fragmentation ofnuclei is seen

ISSN No:-2456-2165

significantly higher in tobacco users. MeanMicronuclei was frequency significantly higher in tobaccousers. Thus, micronuclei frequency can be used to accessgenotoxicity and as a screening tool.(Rajabi-Moghaddam, Haji Mirzamohammad et al. 2020)

Cytogenetic biomarkers such as micronuclei (MN) are used for the evaluation of exposure to carcinogens and genotoxic effects in oral epithelial cells. Tobacco is one of the strongest carcinogens responsible for the development of cancer in oral mucosa.(Rajabi-Moghaddam, Haji Mirzamohammad et al. 2020)

Only a few studies report on the effects of cigarette smoke in oral mucosa. (Saba et al., 2017)

A study carried out by Biswas et al. revealed that tobacco users have significantly higher oral cellular abnormalities when compared with healthy control subjects, with chewers of tobacco having worse cellular abnormalities than smokers. Prominent cellular abnormalities observed by these authors include condensed chromatin (CC), pyknosis, karyolysis, and bi nucleation, with the degree of atypia being age-dependent.

Proia et al. earlier reported that diverse buccal cellular changes are associated with smoking and smokeless tobacco and Biswas et al. similarly observed and reported that CC, karyolysis (KL), bi-nucleation, and pyknosis were the nuclear anomalies most frequently associated with tobacco smoking and chewing.

Because of all these, it could be deduced that tobacco use induces varying nuclear changes in the oral mucosal cells of their consumers.

Tobacco in any consumable form is genotoxic. Smoking and smokeless tobacco, when consumed together, synergistically causes higher genetic damage. Different tobacco habits have different deleterious effects on oral mucosa, and these effects are more pronounced when the patients have combined habits.(Kokila, Prasad et al. 2021)

Increased nuclear changes were observed in the buccal epithelial cells of tobacco users, with snuff inhalation slightly impacting more severe effects than cigarette smoking. (Ifedioranma, Ajuluchukwungokere et al. 2019). Both Ifedioranma et al and Kokila et al concluded that buccalsmear cytology may constitute an effective screening method for precancerous lesions amongst tobacco users.

A report by Rajabi-Moghaddam et al, suggests that waterpipe smoking may exert cytotoxic and genotoxic effects on buccal cells and that waterpipe smoking seems to have more damaging effects than cigarette smoking.

Thus, the aim of the present study was to evaluate genotoxic effects in the form of MN in cigarette smokers, and non-smokers to compare the DNA damage between these groups by examination of nuclear morphology.

A. STATEMENT PROBLEM

Oral cancer is a major public health problem, and there is an increasing trend for oral cancer to affect young men and women. Public awareness is poor, and many patients present with late-stage disease, contributing to high mortality. Oral cancer is often preceded by a clinical premalignant phase accessible to visual inspection, and thus there are opportunities for earlier detection and to reduce morbidity and mortality. Smoking has been documented to have genotoxicity effect on cells aiding to the development of the cancerous lesion, so this study seeks to examinethe effects of smoking on the nuclear morphology of the buccal epithelium as a means for early detection in the predisposed population.

ISSN No:-2456-2165

B. RATIONALE OF STUDY

Smoking has been documented as a genotoxic agent on the buccal epithelium, cervical epithelium, as well as the gastro-intestinal tract, with the presentation of increased oral carcinomas which are usually presented at a late stage, it is imperative that a non-invasive method for early detection is realized by using the buccal exfoliated epithelium to determine possible carcinogenesis at an early stage to curb the high morbidity and mortality. This study seeks to utilize the buccal epithelium for analysis of its nuclear morphology to determine the effects of smoking.

C. SIGNIFICANCE OF THE STUDY

The study will correlate smoking and buccal epithelium nuclear morphology, this is significant because potential oral pre-malignant lesions can be detected early and help in management of such patients. It will also add to the scientific community knowledge and evidence-based data that may help in policy formulation in mitigation oral cancers. The current gap in establishing a significance of oral screening using buccal swab and a reduction in mortality paves way for such contributions to be made and established that correlation.

D. HYPOTHESIS

Smoking is a risk factor in buccal epitheliumnuclear morphology abnormalities.

E. OBJECTIVES

a) GENERAL OBJECTIVE

To establish a correlation between smoking and nuclear morphologic changes of the buccal epithelium and the onset of oral cancer

b) SPECIFIC OBJECTIVES

- Determine the degree of karyolysis,pyknosis, karyorrhexis, and karyokinesisof the exposed buccal epithelium against a control population.
- To evaluate the cytological features, Micronuclei frequency and other nuclear anomalies in buccalmucosal cells of tobacco users.
- To assess if micronuclei frequency can be used to access thegenotoxicity in tobacco users, and thus use this as a biomarker in cancer screening.

ISSN No:-2456-2165

CHAPTER 2

LITERATURE REVIEW

Tobacco is chemical carcinogen, having genotoxic effects.Buccal cells are the first barrier for the inhalation oringestion route and are capable of metabolizing proximalcarcinogen to reactive products, resulting in genomic instabilityand in late stage reflected grossly as submucous fibrosis,leukoplakia, erythroplakia and finally in squamous cellcarcinoma. Microscopic changes are said to occur earlierin buccal mucosa which include micronuclei, and othernuclear anomalies like karyorrhexis, karyolysis, pyknosis,binucleation, fragmentation and broken egg nuclei. (Standring et al, 2006)

Oral exfoliative cytology is a simple and noninvasive diagnostic technique that can be used for early detection of potentially malignant lesions. (Khot, Deshmane et al. 2015)

Micronucleus is a microscopically visible round or oval cytoplasmic chromatin mass in the extra nuclearvicinity, originatedfrom aberrant mitosis, which consists of eccentric chromosomes that have failed to reach spindle poles during mitosis and are used as biomarkers for assessment of DNA damage. Micronuclei (MN) are characteristically seen in exfoliated cells of the buccal mucosa and urinary bladder wall in precancerous and cancerous conditions. (Kamath, Anigol et al. 2014)

Micronuclei are small, extra-nuclear bodies which have separated from the main fragment, generated during cellular division by late chromosomal fragments because of their association with chromosomal aberrations. They have generated interest as a cytological feature of genotoxicity. The MN test is a quantitative measure of the genotoxic action of carcinogens and mutagens.(Holland, Bolognesi et al. 2008).

The presence of micronuclei (MN) in mammalian cells is related to several mutagenetic stresses. MN are formed as a result of chromosome damage and can be readily identified in exfoliated epithelial cells. MN is chromatin particles derived from acentric chromosomal fragments, which are not incorporated into the daughter nucleus after mitosis. It can be visualized by chromatin stains. A variety of factors influences the formation of MN in cells such as age, sex, genetic constitution, physical and chemical agents, and adverse habits such as tobacco, areca nut chewing, smoking, and alcohol consumption. Micronucleation has important implications in the genomic plasticity of tumor cells. (Sabharwal, Verma et al. 2015)

Clinically, cigarette smoke results in oral mucosa dysplasia, leukoplakia, and finally carcinoma.(Speight, Khurram et al. 2018). On the molecular level, it is known that cigarette smoke causes carcinogenesis by inducing severe damage to both somatic DNA and mitochondrial DNA.(Tan, Goerlitz et al. 2008)

In vitro studies showed that these changes result in various posttranslational modifications, for example, upregulation of MMP-2, AP-1, and VEGF. (Pal, Melling et al. 2013)

A recent study by Foki et al showed supporting data on the early effects of cigarette smoke exposure on oral keratinocytes. It revealed an upregulation of ITGA-2, MMP-1, and a downregulation of TEK in oral keratinocytes. To confirm the results on the posttranslational level, they performed immunohistochemistry of oral mucosa, oral leukoplakia, and oralsquamous cell carcinoma. Most interestingly,ITGA-2 and MMP-1 were significantlyoverexpressed incancerous tissue. Taken together, these changes seem toinfluence various transformation states of oral mucosaand thus contribute substantially to early oral carcinogenesis after cigarette smoke exposure. (Foki, Gangl et al. 2020)

ISSN No:-2456-2165

Recent work has demonstrated a role for epigeneticchanges, especially changes in DNA methylation (DNAme), during the earliest stages of carcinogenesis.(Jones, Teschendorff et al. 2013)Another study shows evidence that tobacco constituents influence the expression of miRNA within oral fibroblasts promoting a phenotype that increases oral cancer migration and sheds new light on the mechanisms underlying oral cancer pathogenesis. (Pal, Melling et al. 2013)

Cao et al indicated that the function and phenotype of fibroblasts can be altered by various tobacco products, thereby changing the metabolism of fibroblasts, the secretion of growth factors and the construction of extracellular matrix to self-regulate their expansion, regulate inflammation, immunity. Under the milieu of such circumstances, the adjacent epithelial cells can therefore acquire advantageous growth, migratory, survival even cancerous properties from the released metabolite and growth-promoting factors. (Cao, Ao et al. 2021)

Coppe et al. first reported that Soluble Factors Secreted by Tobacco-Exposed Fibroblasts promote proliferation and interstitial invasion in nonmalignant keratinocyte cell lines but not in normal human primary oral or skin keratinocytes, which indicated that preneoplastic changes in epithelial cells are necessary to sensitize them to stimulation by tobacco-altered fibroblasts. (Coppe, Boysen et al. 2008)

Among all these carcinogens, tobacco-specific nitrosamines such as NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNN (N'-nitrosonornicotine), polycyclic aromatic hydrocarbons and aromatic amines are considered to play the most important role in malignant transformation. DNA damage and adduct formation are thought to be the major mechanism by which these compounds cause mutations and drive the carcinogenic transformation of the epithelial cells. (Domingo-Vidal, Whitaker-Menezes et al. 2019)

Some scholars point out that autophagy is a significant mechanism in the interactions between fibroblasts and tumor cells. A previous study has shown that the development of cancer cells may highly depend on the autophagy process in tumor stromal. (Katheder, Khezri et al. 2017)

In this study, we seek to evaluate degree of karyolysis, pyknosis, karyorrhexis, and karyokinesis of the exposed buccal epithelium against a control population, and the micronuclei frequency as a determinant of genotoxicity in smokers, which may increase the sensitivity of exfoliativecytology for early diagnosis since these are precise,objective, and reproducible

ISSN No:-2456-2165

CHAPTER 3

MATERIALS AND METHODS

A. Study Design

The subjects will be asked to rinse their mouth thoroughly before taking the swabs in order to remove food particles, debris and oral bacteria from the oral cavity. Using a sterile dry swab, the swab will be taken from the right or left buccal mucosa and spread over a clean frosted glass slide in circular manner from central of slide to periphery. The smears will be wet fixed, in ethyl alcohol for 30 minutes and stained with Papanicolaou stain. Slides will be examined under light microscope for cellularity and oil for nuclear morphology. Overall cytological features will be noted.

100cells will be screened from each slide in zig-zag method to avoid repetition of count. Total Micronuclei (MN), Total Number of Micronucleated cells (MNt) and mean micronucleifrequency (Mean MN= Total MN/MNt) per 100 screenedcells will be counted.The criterion which was developed by Tolbert et al, 1992 will beused for counting the micronuclei. Parameters for the cellsto be scored are:

- Intact cytoplasm and relatively flat cell position on theslide,
- Little or no overlap with adjacent cells,
- Little or no debris, and
- Nucleus normal and intact, nuclear perimeter smoothand distinct.

To designate an extra nuclear body as a MN, the followingcriteria given by Tolbert *et al*.2,9 will be used:

- Rounded smooth perimeter suggestive of a membrane
- Less than a third the diameter of the associated nucleus,but large enough to discernshape and color
- Staining intensity similar to that of the nucleus
- Texture similar to that of nucleus
- Same focal plane as nucleus
- Absence of overlap with or bridge to the nucleus.Nuclear Anomalies (NA) other than MN like CondensedNuclei, Karyorrhexis, Karyolysis, Binucelation, Brokenegg nuclei and fragmented nuclei were also counted in bothstudy groups

B. Study Site

The sample collection will be conducted in Kabwe, Central Province. The staining and examination of the slides will be done at the University Teaching Hospitals, Adult Hospital, and Pathology Laboratory.

C. Study Frame/Population

a) Inclusion Criteria
The study will include 30 participants who are active smokers regardless of method smoking and 20 non-smokers as controls. Both males and females within the age range of 18 to 35 years old.

b) Exclusion Criteria
Participants who take alcohol will be excluded as this maybe a confounding factor in the results interpretation. Participants outside the inclusion age range will be excluded.

c) Sample Size
The sample size for the population of interest is 30 and 20 control population.

d) Experimental Approach
The slides with the swab smear will be stained using the Papanicolaou stain and examined for cytomorphologic changes, and nuclear anomalies as outlined in the methodology.

D. Ethics Considerations

All participants will be included on the study on a voluntary basis

ISSN No:-2456-2165

CHAPTER 4

RESULTS

EVALUATION OF NUCLEAR MORPHOLOGY IN BUCCAL EPITHELIAL CELLS OF SMOKERS

SN TEST SLIDES	Micronuclei per smear	Binucleation per smear
Q1	61	Absent
Q2	73	Absent
Q3	7	Absent
Q4	82	Absent
Q5	8	Absent
Q6	69	Absent
Q7	79	Absent
Q8	51	Absent
Q9	9	Absent
Q10	25	Absent
Q11	30	Absent
Q12	87	Absent
Q13	32	Absent
Q14	40	Present
Q15	51	Absent
Q16	185	Present
Q17	229	Absent
Q18	150	Absent
Q19	176	Absent
Q20	256	Absent
Q21	56	Absent
Q22	21	Absent
Q23	35	Absent
Q24	53	Absent
Q25	76	Absent
Q26	No slide available	NA
Q27	11	Absent
Q28	16	Absent
Q29	26	Absent
Q30	81	Absent
Q31	5	Absent
Q32	7	Absent

ISSN No:-2456-2165

EVALUATION OF NUCLEAR MORPHOLOGY IN BUCCAL EPITHELIAL CELLS OF NON SMOKERS

CONTROL SLIDES	**Micronuclei Per smear**	**Binucleation per smear**
C1	1	Absent
C2	7	Absent
C3	6	Absent
C4	2	Absent
C5	5	Absent
C6	11	Absent
C7	2	Absent
C8	5	Absent
C9	3	Absent
C10	1	Absent
C11	7	Absent
C12	11	Absent
C13	No slide available	NA
C14	5	Absent
C15	No slide available	NA
C16	3	Absent
C17	8	Absent
C18	6	Absent
C19	10	Absent

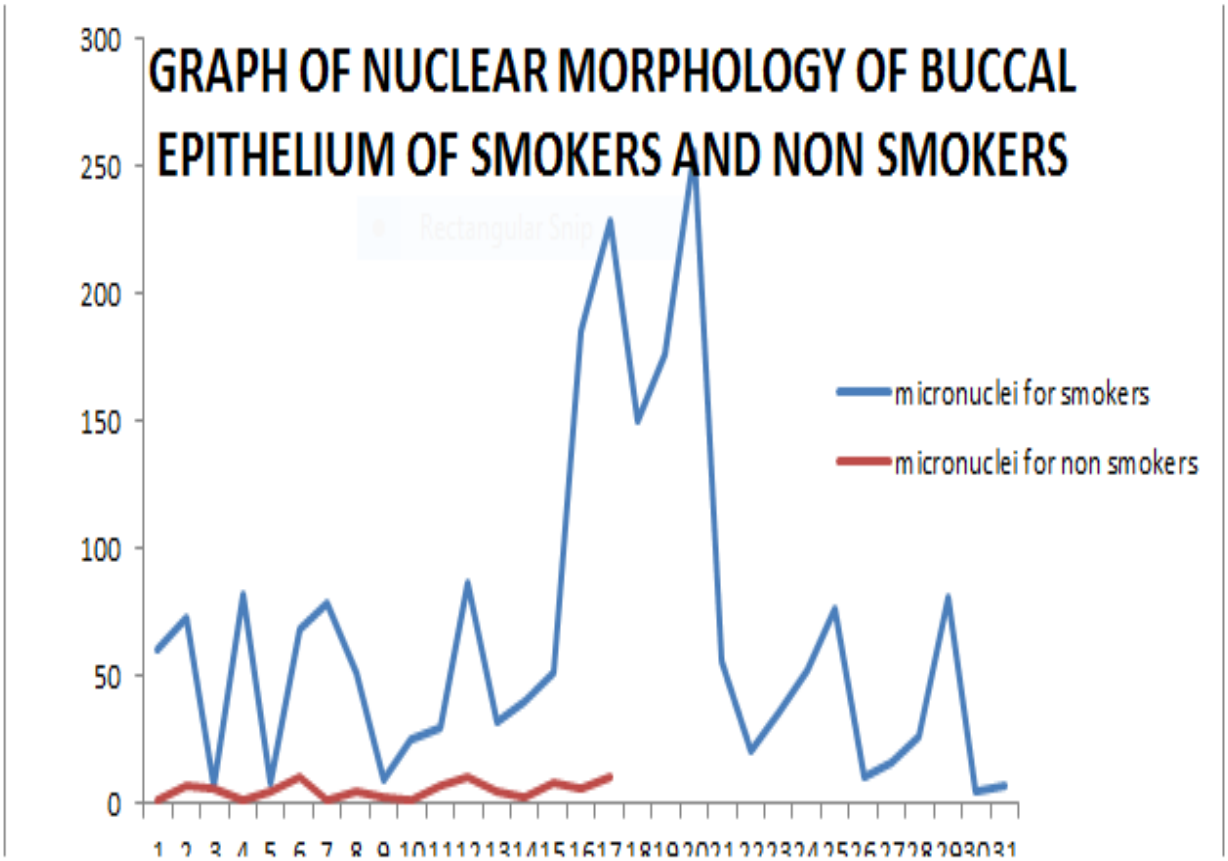
ISSN No:-2456-2165

DURATION OF SMOKING AND HOW MANY CIGARRETE PER DAY

SAMPLE NUMBER	HOW MANY CIGARRETE PER DAY	DURATION OF SMOKING (YEARs)
1	5	4
2	2	1
3	50	7
4	20	15
5	2	3
6	3	5
7	8	4
8	5	3
9	12	5
10	3	5
11	6	2
12	60	2
13	2	10
14	1	1
15	11	4
16	5	3
17	5	3
18	4	6
19	3	1
20	2	4
21	1	4
22	8	7
23	10	4
24	40	Unknown
25	6	7
26	5	5
27	6	3
28	3	1
29	10	3
30	5	4
31	8	5

ISSN No:-2456-2165

DATA ANALYSIS



Graph 1: Nuclear Morphology of Buccal Epithelium of Smokers and non Smokers

ISSN No:-2456-2165

Statistics

Number of Cigarette per day

N	Valid	31
	Missing	2
Mean		9.87
Mode		5
Std. Deviation		14.177
Variance		200.983
Minimum		1
Maximum		60

Statistics

Duration of smoking (years)

N	Valid	30
	Missing	1
Mean		4.87
Median		4.00
Mode		3
Std. Deviation		3.037
Variance		9.223
Minimum		1
Maximum		15

Statistics

Micronuclei of smokers per smear

N	Valid	31
	Missing	0
Mean		67.35
Std. Error of Mean		11.828
Median		51.00
Mode		7[a]
Std. Deviation		65.858
Variance		4337.303
Skewness		1.573
Std. Error of Skewness		.421
Kurtosis		1.926
Std. Error of Kurtosis		.821
Minimum		5
Maximum		256

a. Multiple modes exist. The smallest value is shown

ISSN No:-2456-2165

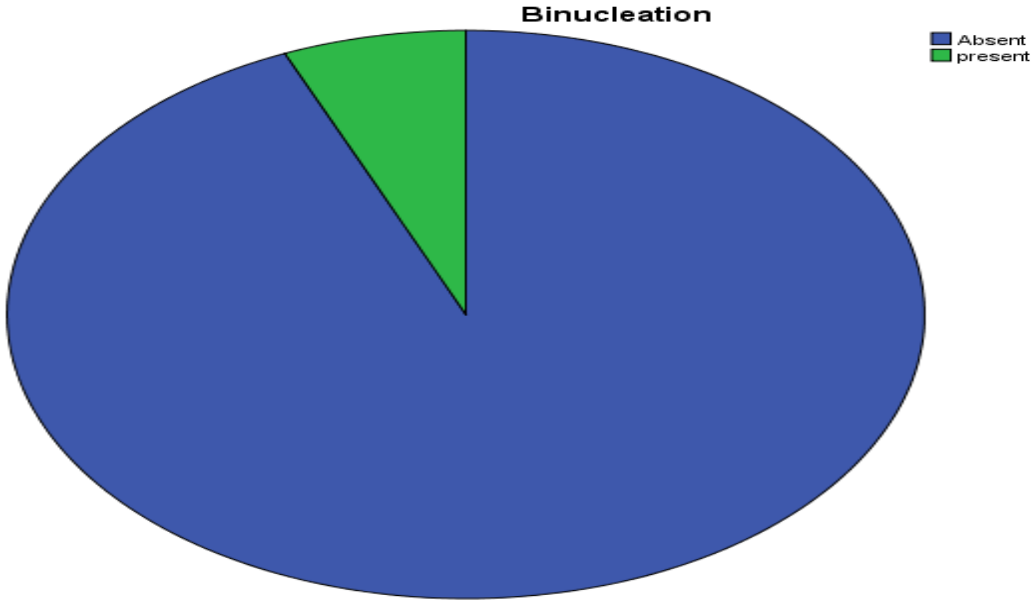
Statistics

Binucleation

N	Valid	31
	Missing	0

Binucleation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	29	93.5	93.5	93.5
	present	2	6.5	6.5	100.0
	Total	31	100.0	100.0	



Statistics

Control micronuclei per slide

N	Valid	17
	Missing	14
Mean		5.47
Std. Error of Mean		.791
Median		5.00
Mode		5
Std. Deviation		3.262
Variance		10.640
Skewness		.330
Std. Error of Skewness		.550
Kurtosis		-.836
Std. Error of Kurtosis		1.063
Minimum		1
Maximum		11

ISSN No:-2456-2165

VARIATION TABLE BETWEEN SMOKERS AND NON SMOKERS

Measure	Smokers	Non Smokers
Number of valid results	31	17
Mean	7	5.47
Maximum	256	11
Minimum	5	1
Skewness	421	330
Kurtosis	1.927	-836

ISSN No:-2456-2165

CHAPTER 5

DISCUSION, CONCLUSION AND RECOMMENDATION

This chapter discusses the findings of the research and compares them with the findings from other research papers and documentations, it also establishes why the findings agree or disagree with other researchers and also discusses if Smoking is a risk factor in buccal epithelium nuclear morphology abnormalities.

The majority of the researchers used to collect data using SPSS software. Aside from that, they manage their data in SPSS by assigning properties to various variables. The data entering interface in SPSS for data analysis appears to be comparable to that of other spreadsheet apps. You can enter data and variables quantitatively and save the files as data files. (Choudhary et al. 2021). The analysis of the results was done using the SPSS to get more accurate and reliable data.

The general objective of this study was to establish the correlation between smoking and nuclear morphology in buccal epithelium. Some of the chemicals contained in tobacco smoke cause, initiate or promote cancer. These chemicals cause genetic changes in cells of the mouth cavity which can lead to the development of oral cancer. (Rodgman, Perfetti et al. 2009). During the study most of the samples analyzed where undergoing pyknosis and karyolysis of which all of them had micronuclei after analysis under the microscope. At least two of all of the samples had binucleation and these changes that had occurred in the cells are due to the effect of tobacco on buccal epithelium

Tobacco use increases the risk of oral cancer by exposing the mouth to these carcinogenic chemicals, either during inhalation while smoking or through direct contact while chewing tobacco products. (Hecht SS. 2010). During the study it was observed that some samples had micronuclei as much as 256 in one smear showing that the effect of tobacco on buccal epithelium is extremely massive and this can be used as a biomarker to predict precancerous conditions.Pyknosis involves the shrinkage or condensation of a cell with increased nuclear compactness or density; *karyorrhexis* refers to subsequent nuclear fragmentation. During Cytomorphologic alterations caused by inflammation or physical or chemical trauma usually are nonspecific. Changes are cell destruction, cytolysis, karyorrhexis, and karyolysis. (Harvey et al. 2012). Some cells during the analysis where undergoing karyorrhexis and karyolysis as shown in the some images above, showing the massive changes that occur in the buccal epithelium due to the effects of tobacco.

The MN test is a quantitative measure of the genotoxicity action of carcinogens and mutagens.(Holland, Bolognesi et al. 2008)The maximum number of micronuclei in smokers was found to be 256 and the minimum was found to be 5 of which in nonsmokers the maximum micronuclei was 11 and the minimum was 1, this shows that smoking has an effect on buccal epithelium leading to formation of micronuclei, binucleation and other nucleus cytological changes. Micronucleus is a microscopically visible round or oval cytoplasmic chromatin mass in the extra nuclear vicinity, originated from aberrant mitosis, which consists of eccentric chromosomes that have failed to reach spindle poles during mitosis and are used as biomarkers for assessment of DNA damage. (Kamath, Anigol et al. 2014)

Results that the average number of cigarettes smoked by the respondents was 20 cigarettes and the while some of the students smoke at least five cigarettes per day. On an average an American cigarette contains 8-9 mg of nicotine whereas an average Indian cigarette contains 15 mg. The increased level of this nicotine concentration causes individuals to get addicted (Tobacco survey India, 2010). It was determined that two samples had binucleations and one of the two had 40 micronuclei and has been smoking for one year and the other had 185 micronuclei and has been smoking for three years.Looking at the variations of all the samples of micronuclei,the sample with the highest number of micronuclei had256 in one slideand with no binucleation and has been smoking for two years four cigarrete per day. This leads to a conclusion that

ISSN No:-2456-2165

the content of the cigarette has an effect on the buccal epithelium depending on the content and concentration on the cigarrete.

Micronuclei was as well found in control samples but at least in small amounts ranging from one to eleven and the mode value was found to be five and this is because A variety of factors influences the formation of MN in cells such as age, sex, genetic constitution, physical and chemical agents, and adverse habits such as tobacco, areca nut chewing, smoking, and alcohol consumption. Micronucleation has important implications in the genomic plasticity of tumor cells. (Sabharwal, Verma et al. 2015)

A. CONCLUSION

The cigarette consumption increases micronuclei frequency in buccal epithelium of Mulungushi university male smokers and causes alterations in the nucleus. The frequency of micronuclei is higher in smokers than non-smokers but micronuclei formation is also involved by other factors such us genetic predisposition.

B. RECOMMENDATIONS

- Further studies should be done to evaluate the chromosome morphology of the buccal epithelium of the smokers and get the dipper understanding.
- More studies should be done at Mulungushi university to evaluate the nuclear morphology of the female smokers considering

REFERENCES

[1.] Biswas SD, Gara B, Banerjee M. Genotoxic effects of tobacco on buccal epithelium: Cell nuclear anomalies as biomarker. Asian Pac J Trop Med and Toxicol2014;3:157-62.
[2.] Cao, Z. Z., et al. (2021). "The role of cancer stromal fibroblasts in mediating the effects of tobacco-induced cancer cell growth." Cancer Cell Int**21**(1): 707.
[3.] Coppe, J.-P., et al. (2008). "A role for fibroblasts in mediating the effects of tobacco-induced epithelial cell growth and invasion." Molecular cancer research : MCR**6**(7): 1085-1098.
[4.] DeMarini DM. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. Mutat Res. 2004;567(2–3):447-474.
[5.] Domingo-Vidal, M., et al. (2019). "Cigarette Smoke Induces Metabolic Reprogramming of the Tumor Stroma in Head and Neck Squamous Cell Carcinoma." Mol Cancer Res**17**(9): 1893-1909. http://mcr.aacrjournals.org/content/molcanres/17/9/1893/F1.large.jpg.
[6.] Foki, E., et al. (2020). "Early effects of cigarette smoke extract on human oral keratinocytes and carcinogenesis in head and neck squamous cell carcinoma." Head Neck**42**(9): 2348-2354.
[7.] Gritz ER. Smoking, the missing drug interaction in clinical trials: ignoring the obvious. Cancer Epidemiol Biomarkers Prev. 2005;14(10):2287-2293.
[8.] Holland, N., et al. (2008). "The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps." Mutat Res**659**(1-2): 93-108.(HUMN; see http://www.humn.org).
[9.] https://worldpopulationreview.com/country-rankings/smoking-rates-by-country
[10.] Ifedioranma, O., et al. (2019). "EVALUATION OF NUCLEAR CHANGES IN THE BUCCAL EPITHELIAL CELLS OF TOBACCO USERS IN NNEWI, SOUTH EAST NIGERIA." Asian Journal of Pharmaceutical and Clinical Research: 269-273.
[11.] Jones, A., et al. (2013). "Role of DNA methylation and epigenetic silencing of HAND2 in endometrial cancer development." PLoS Med**10**(11): e1001551.
[12.] Kamath, V., et al. (2014). "Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers." Clinical Cancer Investigation Journal**3**(1): 49-54.
[13.] Katheder, N. S., et al. (2017). "Microenvironmental autophagy promotes tumour growth." Nature**541**(7637): 417-420.
[14.] Khot, K., et al. (2015). "A cytomorphometric analysis of oral mucosal changes in tobacco users." J Nat Sci Biol Med**6**(Suppl 1): S22-24.
[15.] Kokila, S., et al. (2021). "Evaluation of Micronuclei and Cytomorphometric Changes in Patients with Different Tobacco Related Habits Using Exfoliated Buccal Cells." Asian Pacific journal of cancer prevention : APJCP**22**(6): 1851-1855.
[16.] Motgi AA, Chavan MS, Diwan NN, Chowdhery A, Channe PP, Shete MV. Assessment of cytogenic damage in the form of micronuclei in oral epithelial cells in patients using smokeless and smoked form of tobacco and non-tobacco users and its relevance for oral cancer. J Cancer Res Ther. 2014 Jan-Mar; 10(1):165–70 (2).
[17.] Neville BW, Daam DD, Allen CM, Bouqout JE. Oral and maxillofacial pathology. 4th ed. Missouri: Saunders; 2016. pp. 410–21.
[18.] Oral Cancer. Report of global adult tobacco survey India 2010. Available from: http://www.oralcancerawareness.blogspot.com/2010/10/report-of-global-adult-tobacco-survey.html.
[19.] Pal, A., et al. (2013). "Cigarette smoke condensate promotes pro-tumourigenic stromal-epithelial interactions by suppressing miR-145." J Oral Pathol Med**42**(4): 309-314.
[20.] Peterson LA, Urban AM, Hecht SS. Respiratory toxicology. 8th ed. Missouri: Elsevier; 2010. p. 351.
[21.] Proia, N. K., et al. (2006). "Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer--a review." Cancer Epidemiol Biomarkers Prev**15**(6): 1061-1077.

[22.] Proia NK, Paszkiewicz GM, Nasca MA, Franke GE, Pauly JL. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer a review. Cancer Epidemiol Biomarkers Prev 2006;15:1061-77.
[23.] Rajabi-Moghaddam, M., et al. (2020). "Comparison of Genotoxic Effect in Buccal Exfoliated Cells between Cigarette and Waterpipe Smokers." Acta Cytol**64**(5): 471-476.
[24.] Rajabi-Moghaddam, M., Haji Mirzamohammad, M., Yahyazadeh, E., Gholinia, H., & Abbaszadeh, H. (2020). Comparison of Genotoxic Effect in Buccal Exfoliated Cells between Cigarette and Waterpipe Smokers. Acta Cytologica, 1–6. doi:10.1159/000506893.
[25.] Saba R, Halytskyy O, Saleem N, Oliff IA. Buccal epithelium, cigarette smoking, and lung cancer: review of the literature. Oncology. 2017;93(6):347-353.
[26.] Sabharwal, R., et al. (2015). "Emergence of micronuclei as a genomic biomarker." Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology**36**(4): 212-218.
[27.] Speight, P. M., et al. (2018). "Oral potentially malignant disorders: risk of progression to malignancy." Oral Surg Oral Med Oral Pathol Oral Radiol**125**(6): 612-627.
[28.] Standring S, Ellis H, Healy JC, Jonson D, Williams A, Collins P et al. Grays Anatomy: The anatomical basis of clinical practice. 39th ed. Elsevier, Churchill Livingstone: 2006, p 581-83
[29.] Taioli, E. Gene-environment interaction in tobacco-related cancers. Carcinogenesis 2008; 29: 1467–1474. doi:10.1093/carcin/bgn062.
[30.] Tan, D., et al. (2008). "Associations between cigarette smoking and mitochondrial DNA abnormalities in buccal cells." Carcinogenesis**29**(6): 1170-1177.
[31.] Tolbert, P. E., Shy, C. M., & Allen, J. W. (1992). Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutation Research/Environmental Mutagenesis and Related Subjects, 271(1), 69–77. doi:10.1016/0165-1161(92)90033-i
[32.] Yabroff, K. R., et al. (2021). "Association of the COVID-19 Pandemic With Patterns of Statewide Cancer Services." JNCI: Journal of the National Cancer Institute.