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Effect of Smoking Habit on the Frequency of Micronuclei in in Exfoliated Oral Epithelial Cells and Comparative Image Analysis

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Abstract: ***Cigarette smoking is the largest preventable risk factor for disease and death in developed countries. The history of smoking dates back to long periods of time, dating back to the late fifteenth century AD, but its transmission to our Islamic world occurred in the early twentieth century with the coming of colonialism to Islamic countries. The important factors that predict the start of smoking in western societies are the presence of friends who smoke, the presence of parents who smoke, low social level, and the tendency to have mental health problems and impulsivity, while the important factors that predict the transition to regular smoking are the presence of friends who smoke and weak academic orientation. Recently, the use of biomarkers as tools to evaluate Geno toxicity, known as biomarkers are biological parameters that provide information about the physiological or pathological state of an individual or population. There were two sets of participants: nonsmokers and smokers. The results showed that the mean and variation of the total percentage of nuclear abnormalities were, respectively, for smokers and controls. [10.35 ± 4.14], [65.08 ± 17.48*]. The means of the two groups differed significantly, according to an independent sample t-test. Both the smokers' and the controls' results were statistically significant.***

Keyword: Smoking, Micronucleus Assay, Cytomorphometric.

1. INTRODUCTION

One important modifiable risk factor for cancer and disorders of the organ systems that are common in the US and other countries is cigarette smoking. Tobacco usage can be defined as a behavioral process that causes consumers to become emotionally and physically addicted. Nicotine, the primary component of tobacco, is extremely addictive and can be abused. Tobacco in a sustainable manner. There are two types of tobacco products: combustible and

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non-combustible. The national prevalence rate of use of tobacco products currently in the United States is 21.3% among adults over the age of 18 [1]. Smoking cigarettes has become the tobacco use of choice among many young people and adults around the world. The smoke from cigarettes is meant to be breathed deeply into the lungs, where it releases large concentrations of nicotine into the brain in a matter of 10 to 20 seconds. This sudden increase in nicotine levels For this reason, smoking cigarettes is the most common and effective way to get dependent on tobacco [2]. While adult cigarette smoking is the most frequent tobacco use in the US, the number of adults who smoke has decreased recently. In 2005, 20.9% of adults over the age of 18 were cigarette smokers; by 2015, that number has dropped to 15.1% according to the National Health Survey. [3]. World Health Organization reports indicate a decrease in the global smoking rate among adults over the age of 15 years from 23.5% in 2007 to 20.7% in 2015, which reflects a decrease in the smoking rate by 2.8%, despite a decrease in the prevalence of smoking at the global level. The world, but the number of people who smoke around the world remained at 1.1 billion people in the period from 2007 to 2015 due to population growth [4]. Numerous negative effects of cigarette smoking on the body include the development of cancer and chronic disorders. People who are subjected to passive smoking as well as smokers experience negative health impacts. The length of time spent smoking cigarettes determines its impact on health. The process via which exposure to free radicals from tobacco smoke's constituents results in negative health effects from smoking cigarettes. This raises the risk of oxidative stress, inflammation, and DNA damage. Cancer is the first of many illnesses that smoking causes. About 30% of cancer-related fatalities are currently attributable to smoking, making it the leading preventable cause of cancer-related deaths [6]. Human DNA is connected to the carcinogenic compounds in cigarette smoke, which causes damage to DNA and genetic mutations [5]. Since cigarette smoking and cardiovascular disease are causally related, cardiovascular disease is the second disease. One of the primary causes of smoking-related cardiovascular disease is endothelial impairment. insulin resistance, blood circulation, thrombotic consequences, inflammation, changed fat metabolism, elevated demand for oxygen and blood in the heart, and decreased oxygen and blood supply in the heart [6]. Although respiratory disorders are included in the third disease category, smoking cigarettes is the primary cause. In the US, of chronic obstructive pulmonary illness [7, 8]. While the last effect or disease includes reproductive effects, as the mother’s smoking of cigarettes [tobacco] creates a variety of reproductive problems; the carbon monoxide in cigarette smoke attaches itself to hemoglobin, depriving the developing child of oxygen and ultimately resulting in low birth weight. [9]. The micronuclei test uses multi-colored red blood cells and works to detect agents [drugs] that can cause such structural abnormalities, as many cancer-causing substances can be detected by this test [10]. Since micronucleus tests must be conducted on actively dividing cells, bone marrow stem cells and the red blood cells they produce through cell divisions are ideal candidates because these cells have a constant turnover, it is rapid, and there is no true nucleus. Micronuclei tests provide important information about the ability of a chemical to interfere with the structure and function of the chromosome. For example, many human cancer-causing agents test positive in micronuclei tests in mammals. In these tests, organisms are treated with a chemical substance, and the resulting frequency of micronuclei is measured. If there is a noticeable increase in the number of cells that contain micronuclei, it

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can be concluded that the chemical causes structural or numerical chromosomal damage [11,12]. Because bone marrow stem cells and the red blood cells they generate through cell divisions are constantly dividing, they make perfect candidates for micronucleus assays, which need active cell division. Because red blood cells lack a proper nucleus and because it happens quickly, micronuclei are readily apparent under a microscope. [13]. When a chromosome, or a portion of a chromosome, is not integrated into one of the daughter nuclei during cell division, micronuclei are created. It typically indicates chromosomal instability and genotoxic events. Micronuclei are frequently observed in cancer cells and may be indicative of genetic damage events that raise the possibility of degenerative or developmental diseases[14]. During anaphase, lagging acentric chromosomes, chromatid fragments from incorrectly or not at all repaired DNA breaks, or chromosomal non-disjunction, combine to generate micronuclei. The hypomethylation of repeated sections in pericentromeric DNA, abnormalities in the assembly or kinetochore protein structure, spindle apparatus malfunction, or dysfunctional anaphase checkpoint genes can all contribute to this erroneous chromosomal segregation. [15] By encouraging chromosomal fragmentation, a disastrous mutational event, micronuclei can exacerbate genomic instability. [16] Numerous micronuclei assays have been created to check for these structures and find out how frequently they appear in cells under stressful or chemically exposed environments.

2. RELATED WORDS

According to the results of the study [17], the presence of micronuclei suggests a genotoxic exposure because chromosomal breakage or aberrant segregation causes eukaryote cells to produce micronuclei. These chromosome laggards or pieces become nuclei but are prevented from entering the post-mitotic daughter cells. Consequently, these micronucleated cells (MNCs) exhibit either chromosomal breakage (clastogenicity) or a breakdown in the spindle fiber process (tubragenicity) during cell division. The induction of micronuclei in oral mucosal cells appears to be caused by the genotoxic chemicals found in tobacco.

3. MATERIALS AND METHODS

Sample Collection

Collected samples from random people—smokers and non-smokers. The number of samples I collected was 180, with 100 samples from people who smoke and 80 samples from people who are not smokers. The samples were collected during a period that began in September 2023 and ended in December 2023.

Micronucleus Assay in Exfoliated Buccal Cells

Samples were collected from. The test was conducted according to the method described by Gopal and Padma (2018) as follows: After instructing the individuals to rinse their mouths with water, the cells were scraped from the lining of each cheek using a wooden spatula that had been previously wet. The cells were then placed on a sterile microscope slide. They were air-dried and fixed with methyl alcohol. It was colored with May-Grunwald stain and then

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with Giemsa stain. The frequency of micronuclei was recorded as 2000 cells per individual in each case to determine the percentage of MN.

4. RESULTS AND DISCUSSION

Measuring the Number of Micronuclei in Smokers' Oral Buccal Mucosa Epithelial Cells:

Oral mucosa samples from 180 samples—100 smokers and 80 non-smokers—were divided into two groups for the purposes of the current study and put through an MN assay. The age range of the healthy individuals and sick was 19 to 70. When calculating the correlation between the number of MN cells per 2000 cells and smoking and non-smoking users, a There was a substantial positive connection ($p<0.0001$) with every anomaly. noticed the aberrant cells, as indicated by the table and figure below :

Table 1: Micronucleus anomalies in buccal mucosa cells

Anomalies	Non-smokers M ± S.D	Smokers M ± S.D	P value
Binuceated cell	3.72 ± 2.96	29.21 ± 10.70	<0.0001
Binucleated cell with micronucleus	2.95 ± 2.96	29.67± 9.52	<0.0001
Karyolytic cell	0.00 ± 0.00	2.53 ± 2.56	<0.0001
Cell with broken egg	0.05 ± 0.44	0.73 ± 0.76	<0.0001
Transitional cell	3.73 ± 3.01	3.18 ± 3.29	<0.0001
Percentage of mni	10.35 ± 4.14	65.08 ± 17.48	<0.0001

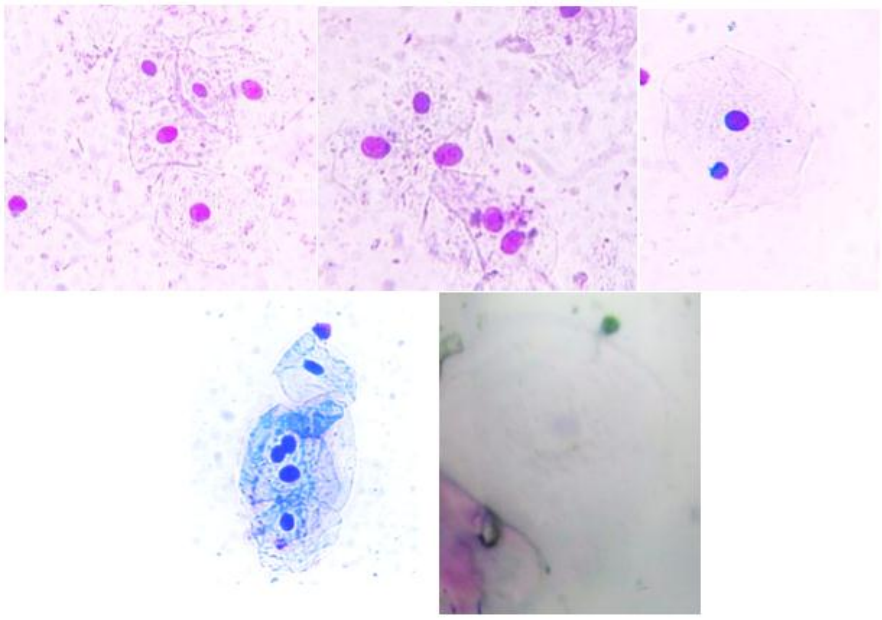

Figure 1: Genetic damage to cellular DNA in the exfoliated Evaluation of oral epithelial cells was conducted: 1. a normal cell 2, 3- Binucleated, 4- Broken eggs, 5- Karyolysis cell.

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For the purpose of identifying the effects of exposure to smoking on inducing micronuclei in epithelial cells scraped from the buccal mucosa cells of the mouth, samples have been collected from smokers and non-smokers as a control group. The samples were prepared and colored, and the results were analyzed using microscopic testing under 40X magnification to identify the frequency of micronuclei in the epithelial cells of the lining of the mouth. An early method of identifying biomarkers of biological effects is the micronuclei test, which is performed on the oral mucosal epithelial cells. When compared to the baseline frequency of micronuclei in the control group, the results indicated a higher frequency of micronuclei in the epithelial cells of the smoker's group. Micronuclei testing of epithelial cells is a cytogenetic technique to measure DNA damage and signs of cell death in the oral epithelium. The epithelial cells of the oral lining represent the first line of defense against ingestion or inhalation. Condensed chromatin, pyknotic cells, and the loss of karyolitic nuclear material—which manifests as a "ghost"—are the signs of decomposition that occur within the cell. Rarely, certain cells may also exhibit two nuclei in the same cytoplasm. These cells may do so as nuclear buds, cracked eggs, or micronuclei (MN) that develop in the cytoplasm next to the nuclei. These indicators can be observed when DNA damage and cell death occur (such as apoptosis and karyolysis), and through this, genotoxicity can be assessed. Micronuclei in sloughed cells to determine genotoxicity is a new and promising technique for studying epithelial carcinogensIt has been discovered that a sensitive way of identifying genetic damage in oral epithelial cells is micronuclei analysis.
Because they are unable to reach the spindle during division, trailing chromosomal fragments or entire chromosomes during mitosis are the primary cause of micronuclei. Oral buccal epithelial cells are a prime target for early genotoxic events resulting from carcinogens and other genotoxic substances that are ingested or inhaled into the body. Exposure to chemicals occurs in industrial areas, which can cause mutations, cancer, and birth defects. Micronuclei are fragments or complete chromosomes that did not reach the spindle filaments during division and are encapsulated in the form of a separate nucleus. The lining of the mouth maintains itself constantly by renewing cells as it produces, new cells in the basal layer by division (17).

Changes in the Cytonuclear of Tobacco Smokers' Oral Epithelium:
When compared to healthy persons, smokers had a higher mean nuclear and cyoplasmic diameters, there was a statistically significant shift in the results.

Table (2): Mean cytoplasmic diameter and nuclear diameter comparation:

Anomalies	Non-smokers M ± S.D	Smokers M ± S.D
Nuclear diameter	139.67 ± 4.68	147.59 ± 6.515
Cytoplasmic	200.62 ± 3.83	216.02 ± 28.552
The nuclear/cytoplasmic ratio	0.76 ± 0.19	0.99 ± 0.28

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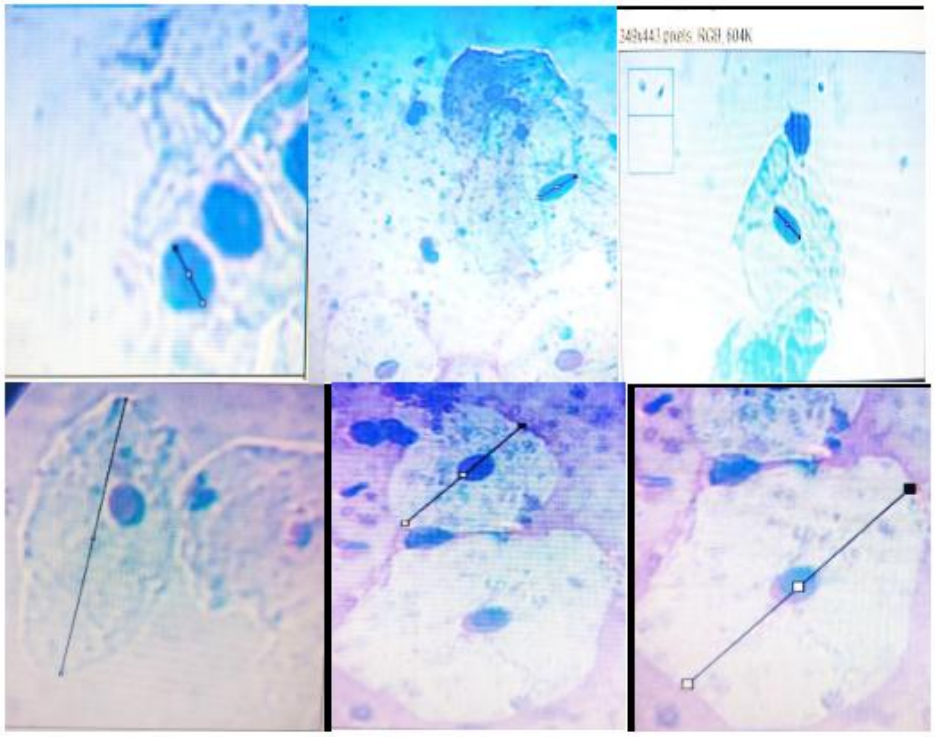
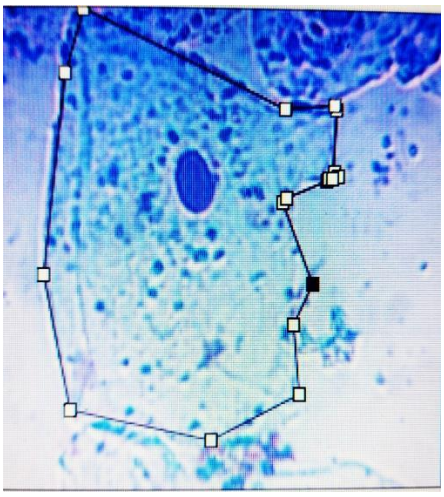


Figure 2: Photomicrograph showing Nuclear diameter and Cytoplasmic diameter
Figure 3: A photomicrograph demonstrating the use of image analysis tools to trace the area of a single cell.



Smoking is the leading preventable cause of disease and death worldwide, affecting multiple organs, and one of the main risk factors for the development of cancer worldwide. Because tobacco use can lead to a number of changes, patients who smoke should be closely watched. Too often, oral cancer is discovered when it is well advanced, which leads to a poor

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prognosis, a high death rate, and expensive treatment expenses .In addition to a rise in problems. As a result, controlling this illness greatly depends on early diagnosis.

Cellular alterations take place at the molecular level during the transition from healthy tissue to precancerous or cancerous tissue, well in advance of any clinical or Histological alterations are also noticeable. In order to reduce the mortality, it may be essential to identify high-risk patients before any possibly malignant lesions appear in the oral mucosa. morbidity, and treatment expenses linked to oral squamous cell carcinoma(18).

Discussion

As was the study's motto, exfoliative cytology may be a useful tool for the detection and monitoring of early alterations and for the establishment of adequate treatment in smokers, taking into account the potential impact of smoking on the incidence of oral cancer and precursor lesions. Because of their accessible location, the lesions are simple to see and gather data for investigation. (19). The major location for collecting smear samples was the buccal mucosa. Since cells extracted from the buccal mucosa are the primary cells for evaluating alterations in the oral mucosa brought on by smoking, this location within the oral cavity was chosen, as reported by Baric et al. (20).

Only people in the age range of 19 to 70 are included in this study. Since smoking for at least ten years was one of the inclusion criteria, the lowest limit of the age group was set at nineteen, while the top limit of the age range was set at seventy to account for potential age-related changes and any underlying systemic disorders. The study's positive findings demonstrated cytology that exfoliates combined with computerized image processing can be utilized as helpful diagnostic tool for detecting pre-malignant abnormalities before any noticeable alterations, including an increase in nuclear diameter and a decrease in cytoplasmic diameter, manifest in the oral mucosa.

5. CONCLUSION

As a result of this study, exfoliative cytology is now a straightforward, effective, noninvasive diagnostic method. method for early detection of malignant alterations in the oral mucosa, allowing for early intervention. One avoidable risk factor for mouth cancer is smoking. Therefore, by informing smokers about changes in their oral mucosa that occur before the presence of any apparent oral.

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