MICRONUCLEUS TEST IN EPITHELIAL CELLS FROM ORAL CAVITY IN KOYA UNIVERSITY STUDENT SMOKERS AND NON-SMOKERS

Received: 1st of July 2020 / Revised: 30th of October 2020
Accepted: 20th of November 2020 / Published: 5th of January 2021

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Keywords : epithelial cells , Cigarettes , micronucleated cell, binucleated cell and condensed chromatin cell
Abstract : This work aimed to investigate the use of epithelial cells from the oral cavity in identifying smoking-related effects in male smokers, normal male, normal female. To establish the relationships between micronucleated cell, binucleated cell, condensed chromatin cell. A total of 59 subjects, corresponding to 11 normal males, 19 normal female, 29 male smokers were registered for this study. The buccal epithelial cell was selected because of the direct exposure of tobacco smoke.
We appraised the incidence of micronucleus formation from 29 male smokers and who had smoked a minimum of 1 pack-year and a maximum of 12. Because of their increased smoke intake, male smokers group showed high buccal micronuclei frequency, significantly $P<0.05$ increased micronucleus frequency was observed in the male smokers group. Micronuclei are cytoplasmic chromatin mass with the appearance of small nuclei that arise from chromosome fragments in the anaphase stage of cell division. Their presence in cells is a reaction of structural and numerical chromosomal aberration arising during mitosis.
In an analysis of the frequency of Binucleated cell in 29 male smokers, 11 normal male statistically non-significant differences were noted. The average frequency of condensed chromatin cell in 11 normal male and 29 male smokers were high $P<0.05$, this is statistically significant and there is a relationship between smoking and increasing in condensed chromatin cell as we mentioned before smoking leads to cytogenetical damage to the human buccal epithelial cell.

INTRODUCTION

Exposure to genotoxic agents occurs through a variety of situations, including pollution of the natural environment, medical procedures (chemotherapy, radiotherapy, etc.) as well as life style factors such as work, diet (Błaszczyk *et al.,* 2014). tobacco smoking, Cigarette smoking is responsible for a substantial number of human health problems (Christobher et al .,2016). One of the major constituents of environmental toxins is tobacco smoke which is responsible for deaths throughout the world . The process of aberrant mitosis gives rise to micronucleus (Ahmad *et al.,* 2015).The oral epithelial cells represent a target site for earlier genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. Buccal mucosa cells are the first barrier which are capable of metabolizing carcinogens to reactive products (Yee *et al.,*2015) .
MNi originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Fenech 2007). Micronuclei originate from chromatin which for different reasons has been lagging in anaphase . In the course of telophase this material is included into one or the other daughter cell where it either can fuse with the main nucleus or form one or several secondary nuclei (Schmid 1976).The micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably(Fenech 2000). Hence micronuclei assay can be used as one of the biomarkers of oral cancer, as it is increased in oral neoplastic conditions. Micronucleus can be identified by various special stains in exfoliative cytology (Suganya *et al .,*2019).
Micronuclei can be identified depending upon following criteria: Cell containing one or more nuclear like substance along with the main nucleus , Each Micronuclei will have the diameter less than 1/3rd of the nucleus , Micronuclei will have oval or circular shape along with membrane , Micronuclei will be located within 3 or 4 nuclear diameters arounda nucleus and will not be in contact with the nucleus and Micronuclei will exhibit similar focal plane, texture and even almostsimilar staining intensity as that of the main nucleus (Vipul *et al.,*2017 and Raj *at al.,* 2019).

MATERIAL AND METHODS

2:1 SUBJECTS :
Subjects (n=59) were the koya university from Kurdistan region of iraq . The foremost inclusion criteria in the present study embrace the analysis Age in year, Body mass index ,Cigarettes per day and Years of smoking. All the controls were physically and mentally normal subjects who had no history of any genetic disorders.

2:2 SAMPLE COLLECTION :
Buccal cell were collected from Smokers and Non-Smokers, Buccal cells were collected from both sides of cheeks by using sterile wooden swab.One swab was used for each cheek and collected the epithelial cell by rotating the wooden swab.

2:3 PROCEDURE :
1-Ask the students to wash their mouth with sterile water
2-Collect the buccal cell by gentle scrapping of wooden swab on their cheek.
3-Spread the swab that contain the collected sample on a clean slideStain the slide by 1% methyl blue then allow the slide to dry for(15 – 20) minute.
4-After drying the slide examine it under the microscope at 40 or 100 X
5-Count 100 cell on the slide under the microscope and detect the presence of micronuclus , Binuclus and condensed nucleus out of 100 cells

2:4 STATISTICAL ANALYSIS :
The statistical significance of the differences in the frequencies-genotypes between groups was calculated. Mean, Standard error of the mean and p-value were calculated to assess the difference between the male smokers and non-smokers and also between normal male and female the level of significance was calculated by t test calculator.

RESULTS :

Table 3: 1 Distribution of groups based on normal males and females with male smokers

Groups	Number	Percentage
Normal male	11	18.64%
Normal female	19	32.20%
Male smoker	29	49.15%
Total	59	100%
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Table 3:2 General Characteristics of the smoker group and nonsmoker groups

Variable	Male smoker	Mean ± SEM	Normal male	Mean ± SEM	Normal female	Mean ± SEM
Age in year	18-25	21.0± 0.30	18-24	21± 0.77	17-21	19.89±0.27
Body mass index	15.9-32.3	21.21±0.66	20.7-26.7	24.02± 1.03	12-34.6	21.37± 1.01
Cigarettes per day	10-90	24.10±1.82				
Years of smoking	1-12	5±0.54				

Table 3 :3Mean frequency of micronuclei , binucleated cells , Condensed chromatin cells and SEM in buccal epithelium cells of male smokers and normal males

Types of cell	Mean ± SEM of male smoker	Mean ± SEM of normal male	p-value

Micronucleated cells	2.82±0.26	0.45±0.28	0.0001
Binucleated cells	1.89±0.19	1.3±0.20	0.0849
Condensed chromatin cells	0.86±0.19	0.18±0.12	0.0397

Table 4:4 Mean frequency of micronuclei, binucleated cells, Condensed chromatin cells and SEM in buccal epithelium cells of normal males and females

Types of cell	Mean ± SEM of normal male	Mean ± SEM of normal female	p-value
Micronucleated cells	0.45±0.28	0.34±0.14	0.6977
Binucleated cells	1.3±0.20	0.52±0.15	0.0040
Condensed chromatin cells	0.18±0.12	0.11±0.07	0.6327

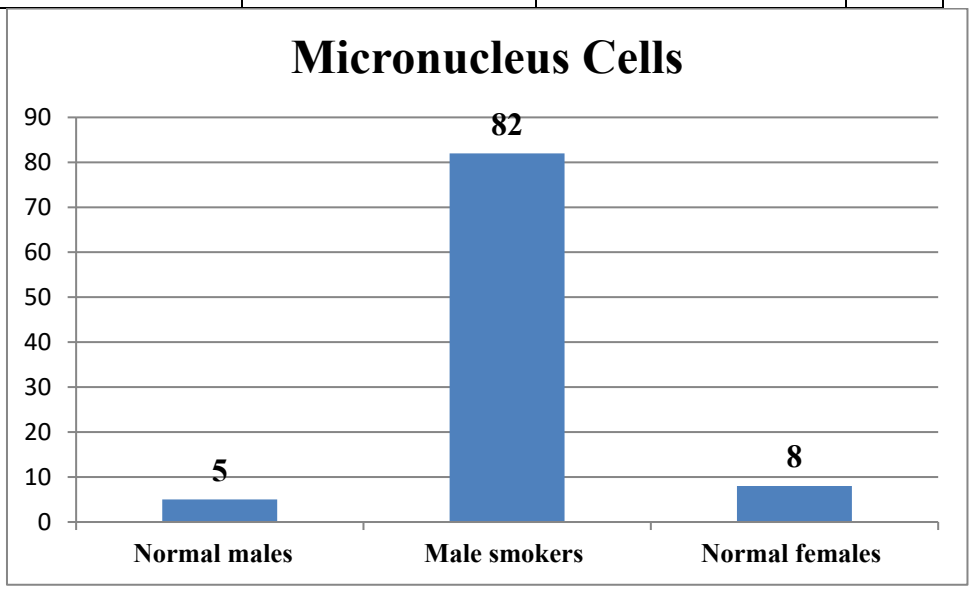

Figure 1:1 total number of micronucleus cells among different groups of students

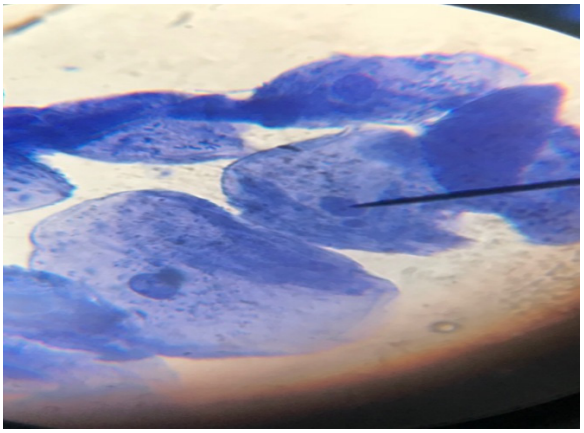

Figure 2:2 micronucleus cells at 100 X of light microscope

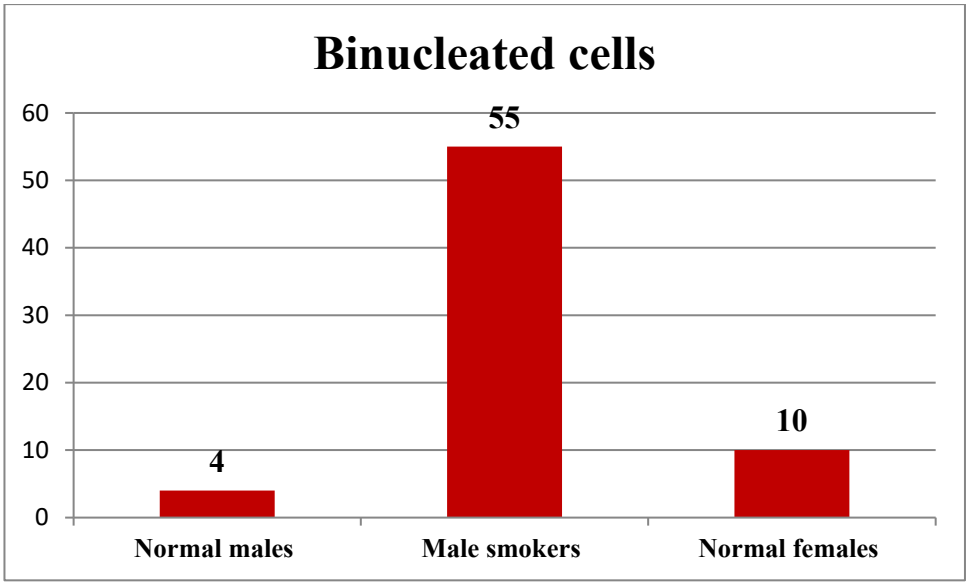

Figure 3:3 total number of binucleated cells among different groups of students

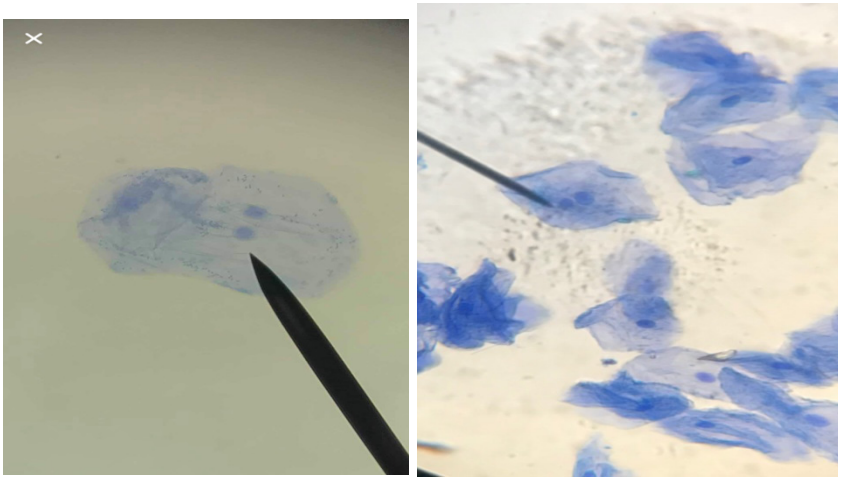

Figure 3:4 binucleated cells at 100 X of light microscope

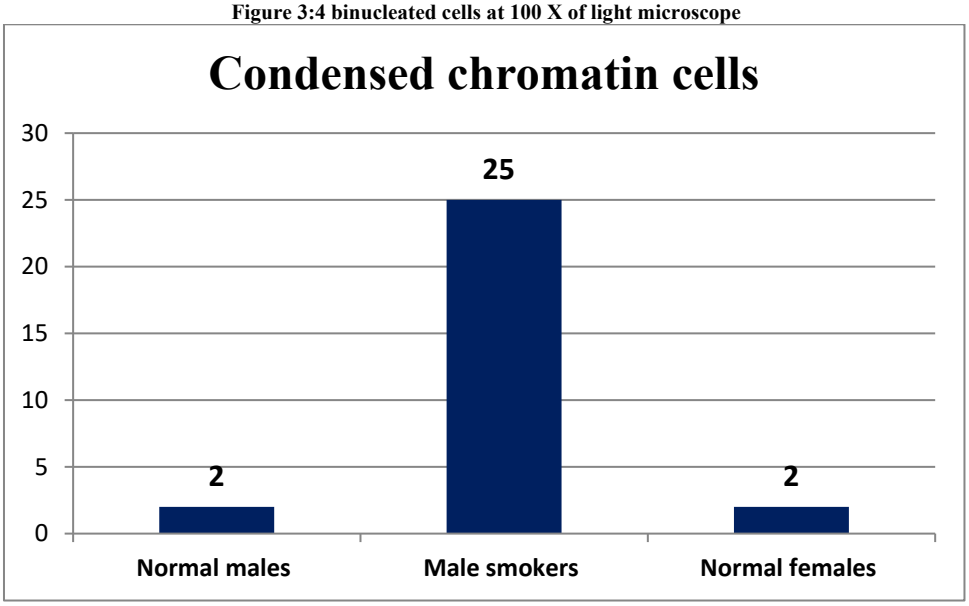

Figure 3:5 total number of Condensed chromatin cells among different groups of students

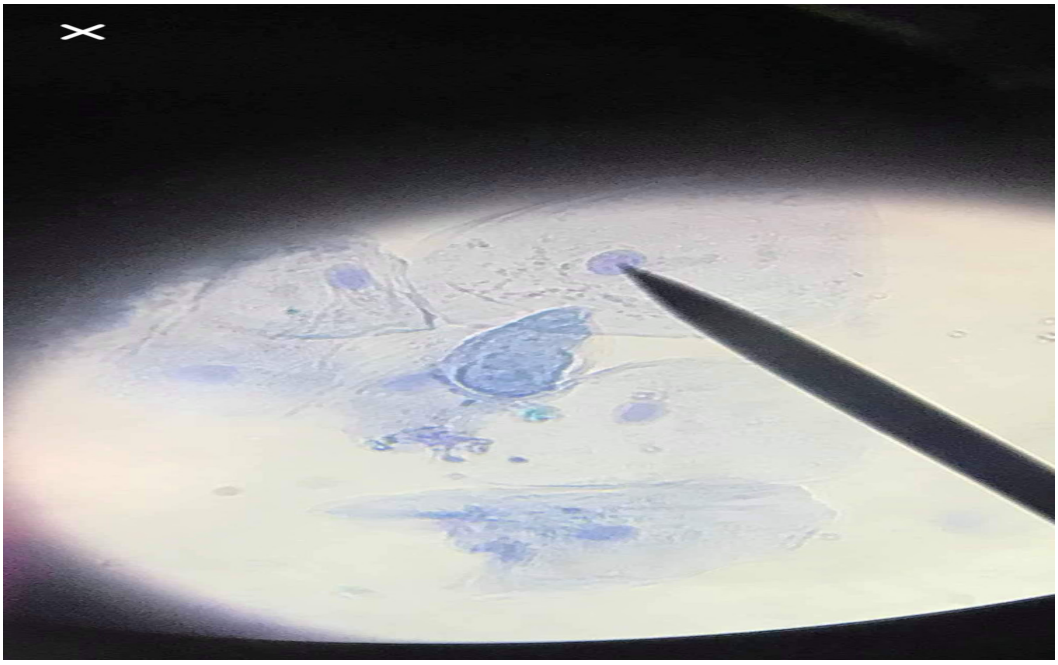

Figure 3:6 Condensed chromatin at 100 X of light microscope

DISCUSSION :

Our study aimed to create relationships in male smokers, normal males and normal females among micronucleated cells, binucleated cells, condensed chromatin cells. A total of 59 participants were registered for this study, corresponding to 11 normal males with 18.64%, and 19 normal females with 32.20% and 29 male smokers with 49.15% (Table 1). Our research aimed to develop the relationships in male smokers, normal males and normal females between micronucleated cells, binucleated cells, condensed chromatin cells. A total of 59 subjects, corresponding to 11 normal males, 18.64%, and 19 normal females, 32.20%, and 29 male smokers, 49.15% (Table 1).

In (Table 2) we showed general characteristics of smoker-group and non-smoker groups. We estimated many categories for evaluating our work such as Age in the year, Body mass index, Cigarettes per day, years of smoking. The age of male smokers is between(18-25) years, the mean age and standard error of the mean of male smokers are (21.0 ± 0.30). Body mass index of male smokers between (15.9±32.3) the mean body mass index and SEM of male smokers are(21.21 ± 0.66). The range of Cigarettes per day in the male smoker is (10 – 90) Mean and SEM is(24.10 ±1.82). years of smoking in male smokers is(1-12) year, mean and SEM is(5+± 0.54). Age of normal male is between(18-24) years the mean and SEM of normal male age is(21± 0.77 Body mass index of a normal male between(20.6-26.7). the mean and SEM of is (24.02 ± 1.03). The age of the normal female is between(17-21) years, the mean and SEM of normal female age is (19.89±0.27). Body mass index of a normal female between(12- 34.6), the mean and SEM of Body mass index of the normal female is (21.37 ± 1.01).

In our study, the average frequency means and SEM of micronuclei in male smoker buccal cells was 2.82±0.26 but the average frequency mean and SEM of micronuclei in normal male is 0.45±0.28 (Table 3). If the P-value equal or less than 0.05 it is statistically significant. The P-values between the male smoker and normal male results is 0.0001 this is significant and there is a relationship between smoking and increasing micronucleated cell in buccal epithelial cell. Bonassi *et al.* (2011). Micronucleus increased with increasing smoking, Błaszczyk et al., 2014 In cells, the molecular and chromosomal changes lead to the formation of micronuclei according to Fenech *et al.* (2011) male smokers buccal epithelial cell showed a higher frequency of micronuclei than normal males due to the increased pack-years and smoke consumption rate. Figure 1 showed the total number of micronucleus cell among different groups of students. Micronucleus in 29 male smokers highly increased, its 82 in number while micronucleus in 11 normal males is 5 in number also 8 in number in 19 normal females. The result is very near between normal males and females. The average frequency mean and SEM of Binucleated cell in male smokers buccal cell are 1.89±0.19 but the average frequency mean and SEM of Binucleated cell in normal males is 1.3±0.20 (Table 3) The P-value between male smokers and normal male results is 0.0849 which is not important there is no relation between smoking and that Binucleated cell. The total number of binucleated cells in 29 male smokers shown in Figure 2 binucleus is 55 in size, and it is 4 in 11 normal males, and is 10 in 19 normal females. Diler and Celi also documented the lack of statistically significant differences in the binucleus frequency in oral cells of male smokers and the average male and the normal male was also reported by Diler and Celik 2011).

Mean and SEM of condensed chromatin cell in male smoker was 0.86 ±0.19, but in normal males was 0.18± 0.12 the P-value between male smoker and normal male results is 0.0397(table 3) which is significant and there is the relationship between smoking and increasing condensed chromatin cell. Figure 3 shows us the total number of condensed chromatin cells and its 25 in number in 29 male smokers, 2 in 11 normal males and 2 in normal females. Condensed chromatin cell results from same between normal male and normal female but its more in male smokers, that show us the effect of smoking on a buccal cell which caused having more condensed chromatin cell.

In Table 4 we explained mean frequency and SEM in the buccal cell of normal males and females. mean and SEM of the micronucleated cell of the normal male is 0.45 ±0.28 and in a normal female is 0.34 ± 0.14. P-value of a micronucleated cell between normal male and normal female is 0.6977 which is not significant, mean and SEM of the binucleated cell of the normal male is 1.3± 0.2 and normal female is 0.52± 0.15. P-Value of the binucleated cell between normal male and normal female is 0.0040 which is significant and there is a relationship between normal male and normal females in binucleated cell number. Mean and SEM of condensed chromatin cells is 0.18± 0.12 in normal male and 0.11± 0.07 in the normal female the P-Value between normal male and normal female for condensed chromatin is 0.6327 which it is not significant.

CONCLUSION

We concluded, as a final conclusion from the result:

1-The relationship between smoking and growing micronucleated cells is important.

2- The lack of statistically relevant variations in the frequency of binucleated cells in male and regular male oral cells;

3- The relationship between smoking and an increase in condensed chromatin cells is significant.

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