ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2022; 8(12): 250-255
www.allresearchjournal.com
Received: 28-09-2022
Accepted: 06-11-2022

Rezhat Abbas
Postgraduate Scholar, Department of Oral Pathology & Microbiology. Govt. Dental College & Hospital, Srinagar, Jammu and Kashmir, India

Owais Gowhar
Tutor, Department of Oral Pathology & Microbiology. Govt. Dental College & Hospital, Srinagar, Jammu and Kashmir, India

Mohammad Shafi Dar
Assistant Professor, Department of Oral Pathology & Microbiology. Govt. Dental College & Hospital, Srinagar, Jammu and Kashmir, India

Suheel Hamid Latoo
Professor & Head, Department of Oral Pathology & Microbiology, Govt. Dental College & Hospital, Srinagar, Jammu and Kashmir, India

Corresponding Author:
Rezhat Abbas
Postgraduate Scholar, Department of Oral Pathology & Microbiology. Govt. Dental College & Hospital, Srinagar, Jammu and Kashmir, India

Evaluation & comparison of micronuclei in smokers with & without diabetes

Rezhat Abbas, Owais Gowhar, Mohammad Shafi Dar and Suheel Hamid Latoo

DOI: https://doi.org/10.22271/allresearch.2022.v8.i12d.10409

Abstract
Background: Micronuclei are chromatin masses that arise from chromosomal fragments or whole chromosome that lack behind at the anaphase stage of cell division. Their presence in cells means the number of chromosomal aberrations arising during mitosis. Cigarette smoke contains over 4000 chemical carcinogens, with 200 known carcinogens that show significant genotoxic effects in human cells. Chronic high levels of glucose during Diabetes Mellitus are thought to increase oxidative stress and the formation of free radicals that in turn damage cells. Reactive oxygen species chemically attack cellular components altering metabolism, inflammatory mediators, and antioxidant defense mechanisms, overall favoring the pathogenesis of the disease and the persistence of genetic damage. Scoring of micronuclei is considered a DNA damage biomarker for chromosomal damage and instability.
Aim: Considering this, we decided to evaluate & compare the frequency of micronuclei in oral epithelial cells in smokers & in diabetics.
Materials & Methods: A total number of eligible 150 individuals were included in the study; 50 smokers without diabetes, 50 smokers with diabetes & 50 age and sex matched non smoker healthy individuals group as controls. Oral epithelial cells were taken from buccal smears and subjected to PAP stain. Mean micronuclei were taken from all the subjects.
Results: Least number of micronuclei was found in non smoker, non diabetic group & highest numbers were found in smoker, Diabetic group.
Conclusion: Micronuclei assay is non-invasive technique that offers a very simple method for obtaining information on status of the epithelial cells, particularly DNA damage, proliferative potential of basal cells and cell death.

Keywords: micronuclei, smoking, diabetes mellitus

Introduction
Micronuclei are chromatin masses that arise from chromosomal fragments or whole chromosome that lack behind at the anaphase stage of cell division. Their presence in cells means the number of chromosomal aberrations arising during mitosis. They can originate from chromosome breakage due to unrepaired or misrepaired DNA lesions or chromosome malsegregation due to mitotic malfunctioning [1].
Cigarette smoke contains over 4000 chemical carcinogens, with 200 known carcinogens that show significant genotoxic effects in human cells. It is assumed that nicotine-induced DNA damage to be as a consequence of oxidative stress [2].
Diabetes mellitus is an endocrine metabolic disorder characterized by an abnormal elevated concentration of glucose in plasma that when not treated can lead to ketoacidosis and chronic degenerative diseases of the heart, kidneys, eyes, and nerves. Chronic high levels of glucose during DM are thought to increase oxidative stress and the formation of free radicals that in turn damage cells. Reactive oxygen species chemically attack cellular components altering metabolism, inflammatory mediators, and antioxidant defense mechanisms, overall favoring the pathogenesis of the disease and the persistence of genetic damage. Scoring of micronuclei is considered a DNA damage biomarker for chromosomal damage and instability [3].
Considering this, we decided to evaluate & compare the frequency of micronuclei in oral epithelial cells in smokers & in diabetics.

Materials and Methods

A cross sectional study was conducted on the general population visiting Government Dental College & Hospital Srinagar. All participants were clinically assessed & their detailed smoking history, alcohol consumption, special habits, medical history, with emphasis on diabetes mellitus, occupational history and family history of cancers was taken. A total number of eligible 150 individuals were included in the study; 50 smokers without diabetes, 50 smokers with diabetes & 50 age and sex matched non smoker healthy individuals group as controls. Subjects who had smoked for at least 1 year and reported smoking at least 5 cigarettes per day were considered cigarette smokers. Cumulative lifetime smoking was measured by pack-years smoked. Participants with history of diabetes for more than 5 years were included in study & their plasma glucose levels were checked before sampling. The inclusion criteria were smokers without diabetes within age range of 25-75 years, smokers with diabetes within age range of 25-75 years, age & sex matched Non smoker healthy group. Those that were excluded were Individuals below the age of 25 years, Occupational histories of exposure to chemicals such as organic solvents or paints, History of intake of DNA-damaging Cytostatic drugs, History of ionizing radiation exposure ,Patients with any type of oral lesion (current or recent history) & Medically compromised Patients other than diabetes. A Written informed consent was obtained from each subject before the sampling.

Subjects were asked to rinse their mouth twice with 0.2% chlorhexidine mouthrinse before obtaining the buccal mucosa cells. The exfoliated buccal mucosa cells were scrapped using ice-cream stick or cytobrush and spread over the glass slides to produce smears. The prepared smears were immediately fixed by biofix spray & subjected to rapid-pap staining. After staining, the slide were dried & mounted in DPX.

Microscopic evaluation of stained cells was performed using Light optical microscope with 100X and 400X magnification by two separate observers. For counting the micronuclei, the cells with distinct margin and nuclei were counted. The overlapped cells were not considered for counting.

Results

In microscopic evaluation, the micronuclei were one third of the main nucleus. The micronuclei were round or ovoid with the same color as main nucleus. Figure 01 displays the micronuclei in non smoker, non diabetic individual. Figure 2 & 3 shows the micronuclei in smoker, non-diabetic & smoker, diabetic group respectively. Table 1 summarizes the mean micronuclei count in all the three groups. The mean value of micronuclei in exfoliated cells in group 1, 2 & 3 was 1.43 ± 0.59, 2.01 ± 0.86 & 2.64 ± 0.98 respectively as depicted in table 2,3,& 4.The least number of micronuclei was found to be in group 1 i.e non smoker, non diabetic group with a mean of about 1.43 while as highest counts were found to be in smokers with diabetes with a mean value of 2.64. Comparsion between group 1 & 2 is represented in figure 4 which shows substantial differences in values. Fig 5 shows the difference between group 2 & 3 which is also significant. Comparison between group 1 & 3 is laid down in figure 6. Figure 7 shows the overall intergroup comparisons. It is obvious from the graph that group 3 has the highest counts of micronuclei whereas group having the least count. There was significant difference of micronuclei on comparing group 1 with group 2 & also between group 2 & 3, being stastically significant with p= 0.0002 & 0.0009 respectively. Extremely difference was found between apparently healthy individuals & smokers with diabetes with a p value of 0.0001.

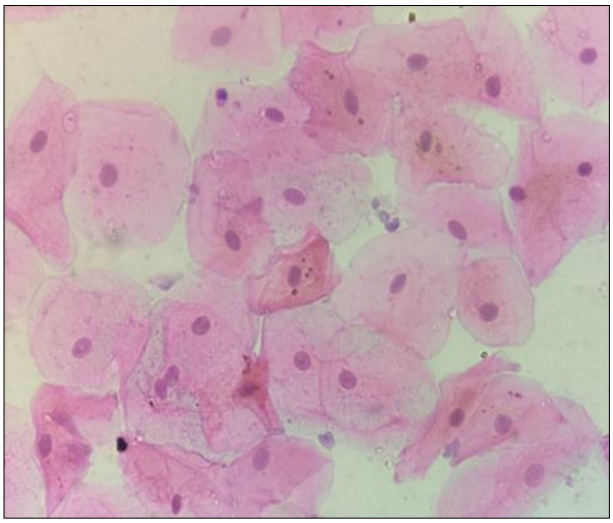

Fig 1: Micronuclei in non-smoker, non-diabetic individual

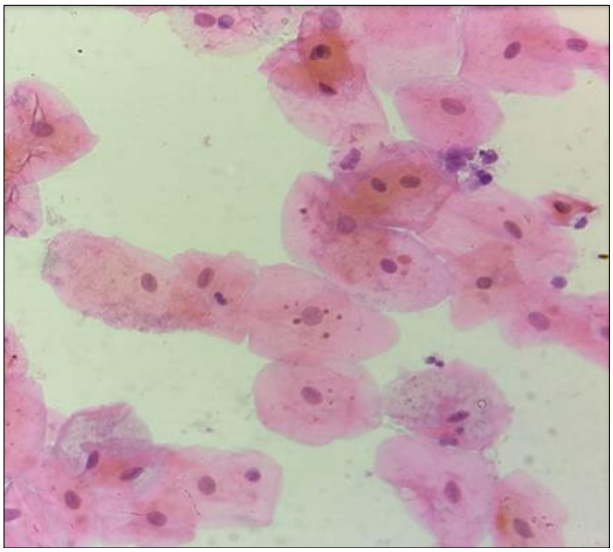

Fig 2: Micronuclei in smoker, non-diabetic individual.

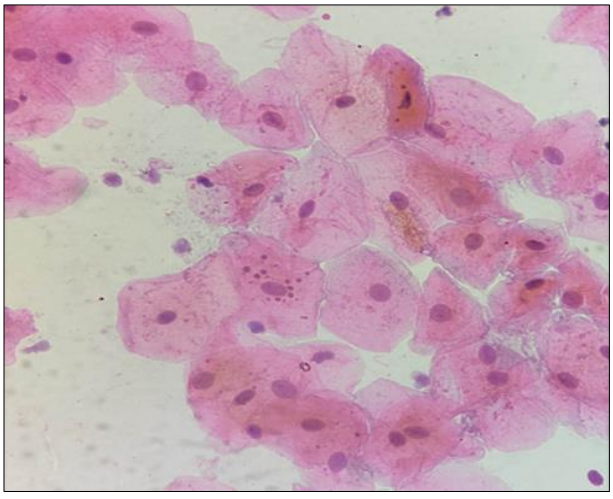

Fig 3: Micronuclei in smoker & Diabetic Group

Table 1: Results

Non-smoker, non-diabetic Control (n= 50)		Group 1: Smokers without diabetes (n= 50)		Group 2: Smokers with diabetes (n= 50)	
Patients	**Mean number of MN in each cell**	**Patients**	**Mean number of MN in each cell**	**Patients**	**Mean number of MN in each cell**
1.	0.5	1.	0.5	1.	2
2.	1	2.	1.5	2.	3
3.	1	3.	1.5	3.	4
4.	1	4.	1	4.	3
5.	1.5	5.	1.5	5.	2
6.	2.5	6.	2.5	6.	2
7.	2.5	7.	3.5	7.	3
8.	2	8.	2.5	8.	1
9.	1	9.	1	9.	3
10.	1	10.	1	10.	2
11.	1.5	11.	2.5	11.	3
12.	1	12.	1.5	12.	3
13.	1.5	13.	2.5	13.	1.5
14.	0.5	14.	1.5	14.	2.5
15.	1	15.	1.5	15.	2.5
16.	1.5	16.	2.5	16.	3.5
17.	1	17.	1.5	17.	4
18.	1.5	18.	1.5	18.	4
19.	0.5	19.	0.5	19.	3.5
20.	2	20.	2.5	20.	4
21.	0.5	21.	2.5	21.	3
22.	1	22.	3.5	22.	2
23.	2	23.	2.5	23.	2
24.	1	24.	3.5	24.	3
25.	2	25.	3.5	25.	4
26.	2	26.	2.5	26.	3
27.	2	27.	2.5	27.	5
28.	1	28.	1.5	28.	4
29.	1.5	29.	1.5	29.	3.5
30.	0.5	30.	0.5	30.	2.5
31.	2.5	31.	2.5	31.	1
32.	1.5	32.	1.5	32.	2
33.	1.5	33.	1.5	33.	3
34.	1.5	34.	3.5	34.	4
35.	2	35.	2	35.	3
36.	1	36.	1	36.	3.5
37.	2	37.	2	37.	2.5
38.	2	38.	2	38.	0.5
39.	1	39.	1	39.	2.6
40.	0.5	40.	3	40.	2
41.	1	41.	1	41.	2
42.	1.5	42.	1.5	42.	1
43.	2.5	43.	2.5	43.	2
44.	2	44.	3	44.	2
45.	2.5	45.	3	45.	3
46.	1.5	46.	3.5	46.	2.5
47.	2	47.	2	47.	3
48.	1.5	48.	2.5	48.	2
49.	1	49.	2	49.	1
50.	1	50.	1	50.	1

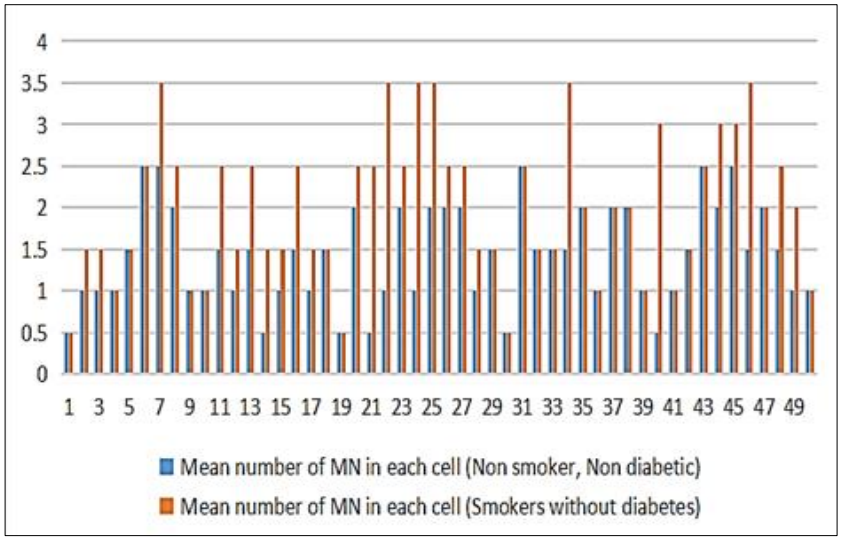

Fig 4: Comparison of micronuclei assay between non smoker, non diabetic & smokers without diabetes

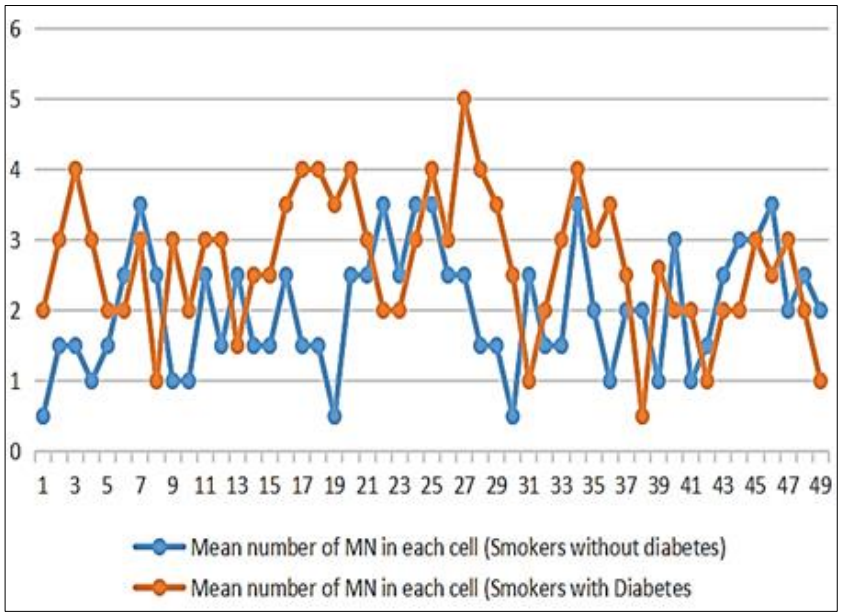

Fig 5: Comparison of micronuclei assay between smokers without diabetes & smokers with diabetes

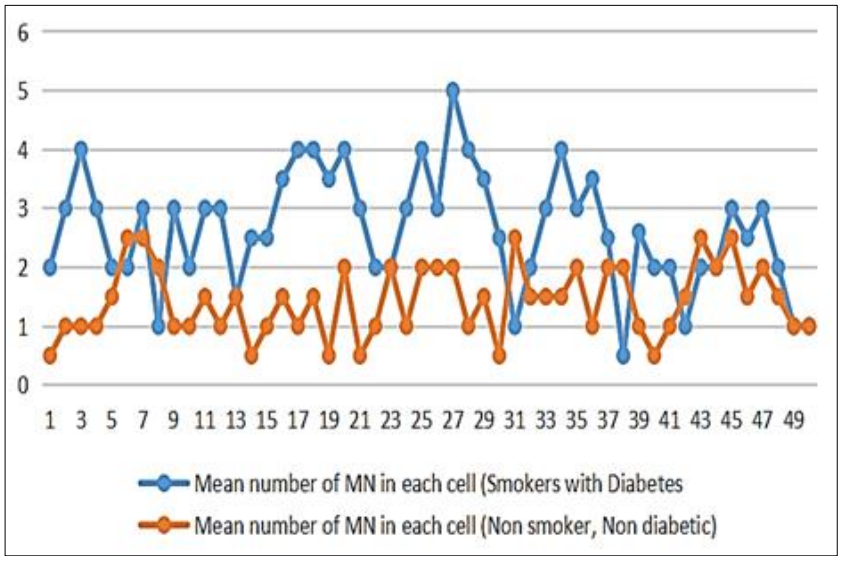

Fig 6: Comparison of micronuclei assay between non smoker, non diabetic & smokers with diabetes

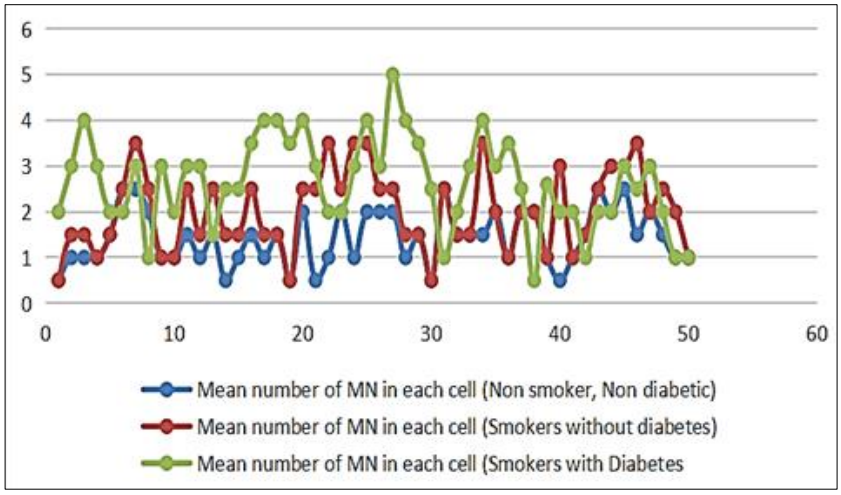

Fig 7: Comparison of micronuclei assay amongst control group, smokers without diabetes & smokers with diabetes

Table 2: Comparison of micronuclei assay between control group & smokers without diabetes

Statistics	Group 1: Non-smoker, non-diabetic Control (n= 50)	Group 2: Smokers without diabetes (n= 50)
Mean±SD	1.43±0.59	2.01±0.86
Standard error of mean	0.84	0.1225
Standard error of difference	0.147	
T, Df	-3.93, 98	
P value	0.0002	

Table 3: Comparison of micronuclei assay between smokers without diabetes & smokers with diabetes

Statistics	Group 2: Smokers without diabetes (n= 50)	Group 3: Smokers with diabetes (n= 50)
Mean±SD	2.01±0.86	2.64±0.98
Standard error of mean	0.1225	0.1392
Standard error of difference	0.1844	
T, Df	-3.416, 98	
P value	0.0009	

Table 4: Comparison of micronuclei assay between control group & smokers with diabetes

Statics	Group 1: Non-smoker, non-diabetic Control (n= 50)	Group 3: Smokers with diabetes (n= 50)
Mean±SD	1.43±0.59	2.64±0.98
Standard error of mean	0.84	0.1392
Standard error of difference	0.1618	
T, Df	-7.47, 98	
P value	0.0001	

Discussion

Genomic damage is considered is as prime cause for development of various malignancies. Such genetic damage is produced by toxic agents, radiation & chemicals, micronutrient deficiency, lifestyle factor and genetic factors such as inherited defects in DNA metabolism or repair [4]. To evaluate the genotoxic risks, DNA damage can be assessed by chromosomal aberrations, sister chromatid exchanges and micronuclei. Out of all these, micronucleus test is preferable for screening as it is a non-invasive and cost effective procedure. Literature have shown that Micronuclei test is one of the best indicator which shows mitotic interference, chromosomal mutations and breakages [5]. Micronuclei are biomarker of genotoxic events and chromosomal instability. Presence of these nuclear anomalies could increase the risk of developmental and degenerative diseases along with various types of malignancy. The lifestyle factors like smoking, alcohol consumption, diet, systemic conditions like diabetes are also associated with the genetic damage, in turn with increase frequency of Micronuclei [6]. Epidemiological studies have shown a positive correlation between micronuclei frequency, degenerative disease and development of cancer [7]. The damage of buccal mucosa may trigger inflammatory processes as well as hyperglycemia-induced oxidative stress that may be sufficient to increase the chromosomal damage [8]. Free radical can cause oxidative damage to DNA and thus the chromosomal damage leads to the breakage of DNA strands and exchange of whole DNA duplexes which occurs during mitosis leading to the formation of micronuclei. In mitotic cells, Micronuclei could arise from the dysfunction of the mitotic apparatus along with the chromosome breakage. It is now well established that micronuclei mainly originate from the acentric chromosome fragment, acentric chromatid fragments or whole chromosome that fail to be included in the daughter nuclei at the completion of telophase [9]. Also larger size micronuclei results from the damage to the spindle apparatus of the cell resulting in the exclusion of whole chromosome (aneugenic effect) whereas smaller MN results from structural aberrations causing fragments of chromosomes (clastogenic effect) [10]. Micronucleus assay is useful for screening the populations under the risk of mutagenic agents that may cause oral neoplasms, and also for the identification of pre-clinical steps of carcinogenic process. Casartelli*et al.* observed that there is a gradual increase in MN counts from normal to precancerous lesions to carcinoma, and suggested a link of this biomarker with neoplastic progression [11]. The present study shows that the mean number of micronuclei in buccal mucosa cells of smokers was higher than that of nonsmokers. Konopacka *et al.* have reported that the micronuclei frequency was 1.50% ± 0.47% in smokers and 0.55% ± 0.32% for nonsmokers [12]. Ozkul *et al.* also have reported the mean micronuclei frequency in smokers was 1.99 ± 0.3 [13]. These findings are in agreement with the present study. Wu *et al.* have reported the positive relation between micronuclei frequency and smoking intensity [14]. The micronuclei frequency in buccal cells was higher in heavy smokers. Gopal and Padma (2018) suggest that the degree of damage varies according to the form of tobacco consumption, since chewing tobacco presented a higher amount of MN (8 ± 7.906) [15]. The studies by Gopal and Padma (2018), Metgud and Neelesh (2018) and Dash *et al.* [16, 17] (2018) were the ones which obtained the highest results of MN for smokers, while the studies by Silva *et al.* (2015), Zamani *et al.* (2011)and Naderi *et al.* (2012) [18, 19, 20]

demonstrated significantly lower results and the studies by Chandirasekar *et al*. (2014) and Haveric *et al*. [21, 22] (2010), intermediate results. Nersesyan *et al*. (2011) observed a higher frequency of micronuclei in oral mucosa cells ofmale smokers, but significant differences were measured only in individuals smoking non-filtered cigarettes [23]. Bonassi *et al.* (2011) based on the project HUMNxl results, concluded that the significant increase of micronuclei in buccal cells was associated with heavy smoking exceeding 40 cigarettes per day [24]. A higher frequency of micronuclei was recorded in the group of male smokers from India aged 41 and above, smoking for more than 20 years (Chandirasekar *et al*. 2011) [25].

Conclusion

Our study showed highest number of micronuclei in smokers with diabetes & least in non smoker & non diabetic. Micronuclei assay is an important non invasive technique that offers information about the genetic status of cells including DNA damage.

References

1. Kwon M, Leibowitz ML, Lee JH. Small but mighty: the causes and consequences of micronucleus rupture. Exp Mol Med. 2020 Nov;52(11):1777-1786.
2. Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2010. 5, Cancer.
3. Quintero Ojeda JE, Aguilar-Medina M, Olimón-Andalón V, García Jau RA, Ayala Ham A, Romero Quintana JG, Silva-Benítez EL, Sanchez-Schmitz G, Ramos-Payán R. Increased Micronuclei Frequency in Oral and Lingual Epithelium of Treated Diabetes Mellitus Patients. Biomed Res Int. 2018 Jan 9;2018:4898153.
4. Fenech M, Kirsch-Volders M, Natarajan AT *et al.* Molecular mechanisms of Micronucleus, Nucleoplasmic bridge and Nuclear bud formation in mammalian and human cells. Mutagenesis. 2011;26(1):125-132.
5. Pratheepa SN, Kaur S, Reddy KS, Vivekanandam S, Rao RK. Micronucleus Index: An early diagnosis in oral carcinoma. J Anat Soc India. 2008;57(1):8-13
6. Palaskar S, Jindal C. Evaluation of micronuclei using Papanicolaou and May Grunwald–Giemsa stain in individuals with different tobacco habits: A comparative study. J Clin Diag Res. 2010;4:3607-3613
7. Lal A, Ames BN. Association of chromosome damage detected as micronuclei with haematological diseases and Micronutrient Status. Mutagenesis. 2011;26(1):57-62
8. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J. 2012 Feb;12(1):5-18.
9. Luzhna L, Kathiria P, Kovalchuk O. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet. 2013 Jul 11;4:131.
10. Jadhav K, Gupta N, Ahmed MB. Micronuclei: An essential biomarker in oral exfoliated cells for grading of oral squamous cell carcinoma. J Cytol. 2011 Jan;28(1):7-12.
11. Casartelli G, Bonatti S, De Ferrari M, Scala M, Mereu P, Margarino G, *et al.* Micronucleus frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. Anal Quant Cytol Histol. 2000 Dec; 22(6):486-92.
12. Konopacka M. Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells. Neoplasma 2003;50(5):380-382.
13. Ozkul Y, Donmez H, Erenmemisoglu A, Demirtas H, Imamoglu N. Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis 1997;12(4):285-7.
14. Wu PA, Loh CH, Hsieh LL, Liu TY, Chen CJ, Liou SH. Clastogenic effect for cigarette smoking but not areca quid chewing as measured by micronuclei in exfoliated buccal mucosal cells. Mutat Res 2004;562(1-2):27-38.
15. Gopal KS, Padma M. Evaluation of cytogenic damage in the form of micronuclei in oral exfoliated buccal cells in tobacco users. Indian J Dental Res. 2018;29(6):773-780.
16. Dash KC, Nishat R, Kumar H, *et al.* Comparative study of micronucleicount in patients with different tobacco-related habits using exfoliatedbuccal epithelial cells: a tool for assessment of genotoxicity. J Contemp Dent Pract. 2018;19(9):10761081.
17. Metgud R, Neelesh BT. Effect of staining procedures on the results of micronucleus assay in the exfoliated buccal mucosal cells of smokers and nonsmokers: a pilot study. J Cancer Res Ther. 2018;14(2):372-6.
18. Silva VHP, de Luna Antonio R, Pompeia S, *et al.* Cytogenetic biomonitoring in buccal mucosa cells from young smokers. Acta Cytol. 2015;59(6):474-8.
19. Zamani AG, Durakbasi-Dursun HG, Demirel S, *et al.* Evaluation of smoking genotoxicity in 14. Turkish young adults. Indian J Human Genet. 2011;17(1):7-12.
20. Naderi NJ, Farhadi S, Sarshar S. Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian J Pathol Microbiol. 2012;55(4):433-8.
21. Chandirasekar R, Kumar BL, Sasikala K, *et al.* Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. Mutat Res Genet Toxicol Environ Mutagen. 2014;767:21-7.
22. Haveric A, Haveric S, Ibruij S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers. Toxicol Mech Methods. 2010;20(5):260-266.
23. Nersesyan A, Muradyan R, Kundi M, Knasmuller S. Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette type. Mutagenesis. 2011;26(2):295-301.
24. Bonassi S, Coskun E, Ceppi M, Lando C, *et al.* The Human Micro-Nucleus project on eXfoLiated buccal cells (HUMNxl): therole of life style, host factors, occupational exposures, health status, and assay protocol. Mutation Research. 2011;728(3):88-97.
25. Chandirasekar R, Suresh K, Jayakumar R, Venkatesan R, Lakshman Kumar B, Sasikala K. XRCC1 gene variants and possible links with chromosome aberrations and micronucleus inactive and passive smokers. Environmental Toxicology and Pharmacology. 2011;32(2):185-192.