Eastern Journal of Agricultural and Biological Sciences (EJABS)
ISSN: 2753-3247
Website: https://qabasjournals.com/index.php/ejabs

Evaluation of tobacco smoking genotoxicity in buccal epithelial cells of young student smokers

Firas Rahi Alhachami

Department of Biology, College of Education for Pure Science, Wasit University, Wasit, Iraq
Corresponding Author: Firas Rahi Alhachami; E-mail: *fhandhal@uowasit.edu.iq*

ARTICLE INFO

Received: April 03, 2024
Accepted: April 26, 2024
Volume: *4*
Issue: 1

KEYWORDS

Cytomeassay, Smoking, Genotoxicity, Exfoliated buccal cells, Micronuclei, Nuclear anomalies

ABSTRACT

Smoking increases the risk of mouth, throat, larynx, and esophagus cancer. Cigarette smoking is responsible for more than 480,000 deaths per year in the United States. Oral mucosa epithelium is considered the first barrier and direct contact with the carcinogens in cigarettes. Therefore, the study designed to evaluate the nuclear abnormalities in the lining of the mouth, which is an early indicator of cancerous developments. Totally, 30 smokers and 30 non-smokers students from Wasit University, Iraq participated in this study, whose ages ranged from 20-25 years. Exfoliated buccal mucosa cells obtained from these students were analyzed applying the micronucleus cytome assay to determine micronucleated cell and other nuclear anomalies. Nuclear anomalies were compared among smokers and non-smokers and correlated with consumption of cigarette per day and duration of smoking in years. The results indicated a clear increase in the number of micronuclei and other nuclear anomalies in smokers as compared to non-smokers. Further analysis indicated that the strongest difference was seen for karyolitic cells with an about 5-folds increase in smokers as compared to non-smokers. Further, it is found associations between the frequencies of micronuclei and karyolysis with the number of cigarette consumed per day and duration of smoking. The micronuclei and other nuclear anomalies reflected genetic damage and cytotoxicity, associated with tobacco and cigarette consumption. Further, these data reveal a risk for cancer development.

1. Introduction

Smoking is the world's largest killer of people. Tobacco consumption commonly leads to heart disease, gum disease, and lung disease and is a significant risk factor for heart attacks and cancer (particularly of the lung, larynx, and pancreas). In the United States, it contributes to more than 480,000 deaths annually, this is nearly one in five deaths (Mokdad et al., 2004). The most lethal of all malignancies is lung cancer, and smoking tobacco has long been known to contribute to the development of this disease (Fisher et al., 1989) About 90% of lung cancer deaths are attributable to smoking, and more women die from lung cancer each year than from breast cancer (Xu, 2017). Annually, more than 274,000 people develop oral, larynx, pharynx, and lung cancers worldwide. Epidemiologic studies has been established the relationship between smoking and the development of these cancers (Proia et al., 2006).

A cigarette contains numerous carcinogens, such as aromatics amines, nitrosamines, polycyclic aromatics, hydrocarbons, heavy metals and poisonous gases (Crispino et al., 2007). The nicotine of cigarettes is considered a highly toxic substance, in the case of light exposure to nicotine, one to two hours pass between the onsets of symptoms. In the case of a significant exposure, symptoms may last for up to 18 to 24 hours. After a serious exposure, death can happen in less an hour (Maessen et al., 2020). For every eight smokers that are killed by active smoking; one nonsmoker is killed by secondhand smoke (the smoke that is released into the air when individuals smoke tobacco cigarettes). Secondhand smoke becomes 2–4 times more toxic when ages after entering the air (Schick and Glantz, 2006). Carcinogens present in the smoke of tobacco cigarettes have an important role incausing cellular abnormalities, releasing free radicals, binding blood fat, change the DNA and grow the cell of cancer (Valavanidis et al., 2009; Andriulli et al., 2010; Moktar et al. 2011).

Since 1937, micronuclei (MN) have emerged as one of the most effective biomarkers for genotoxic exposure (Kamath et al. 2014). The small nucleus that forms whenever chromosome pieces or entire chromosomes are not included in the primary daughter nuclei during cell division is referred to as a micronucleus. It is a symptom of chromosomal instability and genotoxic events. Cancerous cells frequently have micronuclei, which could be signs of genetic damage events that raise the chance of developing developmental or degenerative diseases (Kamath et al., 2014). As a result of poorly repaired or unrepaired DNA breaks, chromosome nondisjunction, or lagged acentric chromosome or chromatid fragments, micronuclei occur during anaphase (Pratheepa et al., 2008).

Beside the micronuclei, there are other nuclear abnormalities that were considered as additional biomarkers for genotoxic effects of numerous compounds, such as binucleated cells (BN), nuclear bud (NBudes), pyknosis (PN), karyorrhexis (KR), karyolysis (KL). Binuleated cells, which often have two major nuclei that are comparable in size and appearance, are most frequently seen in cancer cells. Nuclear buds are tiny, micronucleus-like structures that are connected to the nucleus by a nucleoplasmic ring. The cell nucleus shrinks during pyknosis. Karyorrhexis is the catastrophic disintegration of a dying cell's nucleus, resulting in an asymmetrical distribution of chromatin throughout the cytoplasm. When the nucleus is completely devoid of nucleus chromatin, which likely denotes an advanced state of cellular death, karyolysis is diagnosed (Castaneda et al., 2016; Sabah, 2021).
This study aims to evaluate the genotoxic effect of smoking by evaluating DNA damage, nuclear abnormalities and cell death in the oral mucosa, which is considered as biomarker for early oral carcinomas.

2. Methodology

Ethical Approval
The study was approved by Wasit General Directorate of Education's Committee on Research Ethics (registration no. 3941 of December 17, 2021) and Wasit University (register no. 245 of December 11, 2022). All of the chosen students freely agreed to participate after receiving thorough information about the study.

Study group
This study included 30 smokers, all of them were males and students at Wasit University, Iraq, aged between 20-25 years, and all of them smoked more than 10 cigarettes per day for ≥ 1 year.Among smokers, 14 smoked more than 10 cigarettes, 11 smoked more than 20 cigarettes, while only 5 smoked more than 30 cigarettes per day.Another 30 non-smokers students were recruited as control sample. Exclusion criteria were the exciting of visible lesion on the oral mucosa, chronic illnesses, history of cancer, diabetes, and medical treatment or exposure to X-rays during the last 3 months. This study was carried out in the laboratories of the Pathological Analysis Department in the College of Science after the approval of the Scientific Council of Science College, university of wasit and the Student Committee of wasit, Wasit Governorate, Iraq.

Sample collection
Exfoliated buccal cells were collected from both smokers and non-smokers in 20 Januaryas described by Thomas et al. (2009) with slight modification. Briefly, participants were asked to rinse the mouth twice with distal water before sample collection. The exfoliated buccal mucosa cells were scrapped gently using cylindrical cytological brush from both cheeks. The head of the brush was then dipped into tubes containing 3 mL of phosphate buffered saline solution (PBS) (Sigma-Aldrich-USA), centrifuged (800 rpm) for 5 min and remove the supernatant. The pellet was re-suspended and dropped onto microscope slides,fixed in 3:1 methanol/acetic acid (Merck, Germany) for 15 min. Then, Coded slides were stained with Giemsa dye (Hercules, CA, USA) for 30 min at room temperatureand examined under a light microscope with a magnification of 400×.

Buccal micronucleus cytome (BMCyt) assay
A total of 1000 cells per subjects from the control and exposed groups were examined following the method of Buajeeb et al. (2007). Micronuclei were scored as a parameter of DNA damage (mutagenicity). Karyorrhexis (nuclear disintegration) and karyolysis (nuclear dissolution) were classified as apoptosis. For cytotoxicity, the following nuclear abnormalities were considered: binucleated cells, nuclear buds and pyknosis, the frequencies were calculated as the number of classified cells in 1000 differentiated cells and reported as percentage (%). The correlation of the number of cigarettes consumed per day and duration of smoking versus the frequencies of micronuclei and karyolysis were performed.

Statistical analysis
All the results obtained from the biological examinations were statistically analyzed using the SPSS version 23. Data on genetic damage between the control and smoker groups were analyzed using Poisson regression analysis. Frequencies of micronuclei and karyolysis among smokers were correlated with number of cigarettes per day and duration of smoking using linear regression analysis. Micronuclei and other nuclear abnormalities counts were expressed as mean ± standard deviation (SD) and reported as a percentage (%). A P value of ≤ 0.05 was considered to be statistically significant (Gharban et al., 2023).

3. Results and Discussion

Characteristic of study groups
This study included 30 smokers and 30 nonsmokers who were both 22.9 years old. All individuals were of male gender,to avoid the influence of sex on the results. Furthermore, the studied area lacks female smokers of the target age due to the

religious and societal traditions and customs in the Arab regions.Smokers were classified according to the number of cigarettes consumed per day and the duration of smoking (Table 1).

Table 1 Classification of smokers according to the number of cigarettes consumed per day and duration of smoking

Duration (years)	Size of sample	No. of cigarettes / day		
		≥ 10	≥ 20	≥ 30
1	7	2	2	3
2	13	7	4	2
3	7	4	3	0
4	2	1	1	0
≥ 5	1	0	1	0

Frequencies of micronuclei and other nuclear anomalies

The frequencies of micronuclei and other nuclear anomalies in smokers buccal epithelial cells were evaluated and compared with frequencies among non-smokers students. In Fig. 1, representative images of micronucleated cells and other nuclear anomalies are displayed.

Only 0.0 to 0.9% of cells in the general population have micronuclei. Any deviation from this range results in chromosomal abnormalities due to a genotoxic effect (Naderi et al., 2012). In this study, the results indicated a clear increase in the number of abnormal cells in smokers. MN, BN, KR and PN cells were higher in smokers with no significant difference (1.17, 1.26, 3.71, and 1.58 %, respectively). While Nbuds and KL were significantly higher (1.04, 5.04 %, respectively) as shown in table 2. An excellent biomarker for assessing the effect of carcinogens in smoke seems to be the genotoxicity assay. It has been observed that the number of chromosomebreaks increases when exposed to carcinogenic substances, and it has been demonstrated that these breaks are a precursor to the development of cancer (Bonassi et al., 2000; Yerlagudda et al., 2012).

The largest difference was observed for KL with an about 5-folds increase in smokers as compared to non-smokers.Apoptosis is thought to serve as a surveillance system and eliminate cells with genetic damage (Palve and Tupkari, 2008). Because these occur during the pre-keratinization phase, greater percentage frequencies of karyolysis may be used as a sign of necrotic cells and genotoxic damage (Pindborg et al., 1980; Tolbert et al., 1991).

Numerous studies have found that using tobacco products, including cigarettes, causes more DNA damage in the buccal mucosa (Joshi et al., 2011).The formation of micronuclei and other nuclear abnormalitie occurs as a result of misrepaired or unrepaired chromosome breakages (Yadav and Saini, 2015). A recent results found that micronuclei and other nuclear anomalies frequencies in oral exfoliated cell are higher in squamous cell carcinoma patients compared to control.

Similar research was conducted by Pereira da Silva et al. (2015) to assess the impact of smoking on all the previously mentioned criteria, and it revealed a substantial increase in the frequency of micronuclei but not an increase in karyorrhexis, karyolysis, or pyknosis. In contrast, Yadav A. S. et al. discovered that smokers had considerably greater rates of karyorrhexis, karyolysis, and pyknosis than non-smokers did (Yadav and Saini, 2015). These variations may result from a variety in sample sizes and staining methods(Yadav and Saini 2015). To find more conclusive results, standardization of the used methodologies is necessary.

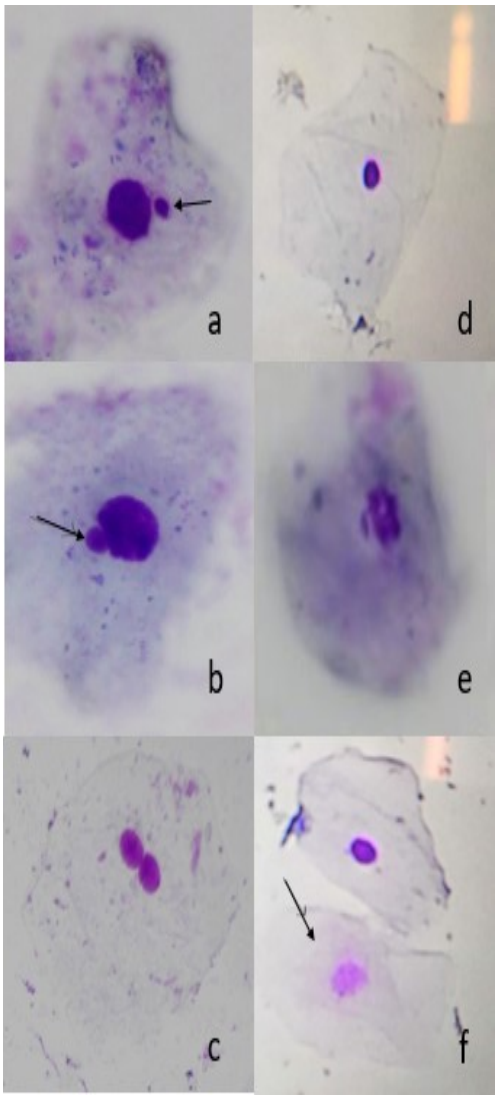

Figure 2 Nuclear anomalies in oral mucosa cells: (a) cell with a micronucleus (arrow), (b) cell with a nuclear bud (arrow), (c) binucleated cell, (d) pyknosis, (e) karyorrhectic, and (f) karyolitic cell (arrow)

Table 2 Micronuclei and other nuclear anomalies (%) (mean ± SD) in the buccal epithelial cells of smokers and control group

Category	Sample size	Micronucleated cells (mean %)	Other nuclear alterations (mean %)				
			Binucleated cells	Nuclear buds	Karyorrhetic cells	Karyolysis	Pynkosis
Non-smokers	30	0.39	0.42	0.14	0.61	0.83	0.45
smokers	30	1.17	1.26	1.04	3.71	5.04	1.58
P value		0.066	0.262	0.006	0.21	0.007	0.794

Correlation of MN and KL with no. of cigarettes per day and duration in years

The data pertaining to MN and KL with respect to no. of cigarette per day and duration of smoking in years reflected linear relationship. The linear coefficient correlation and regression equation is shown in Fig. 2. The frequency of MN and KL correlated positively with no. of cigarette having correlation coefficient R2 = 0.88 and 0.99, respectively. It was also positive with duration of smoking having correlation coefficient R2 = 0.87 and 0.97, respectively.

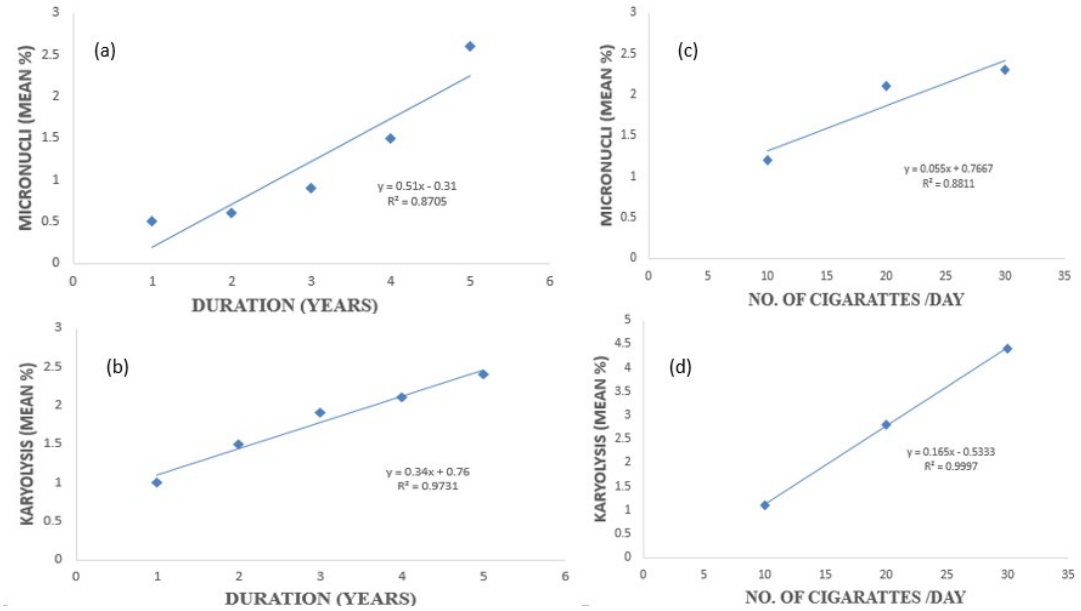

Figure 3 Percentile frequency (mean) of MN (a) and KL (b) with respect to duration of smoking and MN (c) and KL (d) with respect to no. of cigarette per day

Numerous studies have demonstrated that smoking raises the chance of developing any type of cancer depending on how many cigarettes are smoked daily (Alexandrov et al., 2016). Additionally, it takes a long time for cancer to manifest, often between 5 and 20 years (Hussain, 2008).According to research by Hartono et al., smoking 21–30 cigarettes per day in 2007 considerably increased the probability of developing malignant cancer in 2014. The accuracy between smoking 21-30, >30 cigarettes per day in 2007 and having malignant cancer were 93.8%, 95.5%, respectively (Hartono et al., 2019). The similar outcome of cigarette consumption was also revealed by other studies. Smoking 20-29 and 30-39 cigarettes per day increased the incidence of breast cancer (Andersen et al., 2017). Morris et al studyfounds that smoking more than 25 cigarettes a day increased the risk of developing lung cancer (Morris et al., 1997). Additionally, at least 60 compounds included in cigarettes are known carcinogens, including benzoapyrene, a leading cause of lung and liver cancer, and arsenic, a leading cause of skin and liver cancer (Li et al., 2017).

In general, compared to a non-smoker, smoking one cigarette per day dramatically raises the average risk of heart disease by 48%. The chance of dying from lung cancer was about 12 times higher among smokers of one to ten cigarettes per day than it was among non-smokers. The risk of dying from lung cancer is more than 23 times greater in men and around 13 times higher in women for daily smokers (> 20 cig/day) (Bjartveit and Tverdal, 2005). Finally, Praud et al. (2018) noted that the risk of stomach cancer relies on the dose and duration of smoking as well as the drop in risk after quitting (Praud et al., 2018).

4. Conclusion

We evaluated the frequency of micronuclei (MN) and other nuclear anomalies (NA) in the exfoliated buccal cells of 30 students who smoked more than 10 cigarettes per day for almost a year as a biomarker of cytotoxic effects and DNA damage. Comparing smokers to the control groups, it was discovered that smokers have higher levels of MN and NA in buccal mucosa cells. The highest level was displayed by KL, with a significant difference of P = 0.007.Based on the results of the current study, carcinogens and air pollutants produced by smoking can cause cellular death and mutagenesis consequences, as shown by cell damage in oral mucosa cells. To enable a more thorough assessment of the biological harm occurring at the cellular and tissue levels, additional studies are required to ascertain the quantity and quality of air pollutants brought on by smoking.

References

[1] Alexandrov, L. B., Y. S. Ju, K. Haase, P. Van Loo, I. Martincorena, S. Nik-Zainal, Y. Totoki, A. Fujimoto, H. Nakagawa and T. Shibata (2016). "Mutational signatures associated with tobacco smoking in human cancer." Science 354 (6312): 618-622.

[2] Andersen, Z. J., J. T. Jørgensen, R. Grøn, E. V. Brauner and E. Lynge (2017). "Active smoking and risk of breast cancer in a Danish nurse cohort study." BMC cancer 17(1): 1-11.

[3] Andriulli, A., E. Botteri, P. L. Almasio, I. Vantini, G. Uomo and P. Maisonneuve (2010). "Smoking as a cofactor for causation of chronic pancreatitis: a meta-analysis." Pancreas 39(8): 1205-1210.

[4] Bjartveit, K. and A. Tverdal (2005). "Health consequences of smoking 1–4 cigarettes per day." Tobacco control 14(5): 315-320.

[5] Bonassi, S., L. Hagmar, U. Strömberg, A. H. Montagud, H. Tinnerberg, A. Forni, P. Heikkilä, S. Wanders, P. Wilhardt and I.-L. Hansteen (2000). "Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens." Cancer Research 60(6): 1619-1625.

[6] Buajeeb, W., P. Kraivaphan, C. Amornchat and T. Triratana (2007). Frequency of micronucleated exfoliated cells in oral lichen planus." Mutation Research/Genetic Toxicology and Environmental Mutagenesis 627(2): 191-196.

[7] Castañeda-Yslas, I. J., M. E. Arellano-García, M. A. García-Zarate, B. Ruíz-Ruíz, M. G. Zavala-Cerna and O. Torres-Bugarín (2016). "Biomonitoring with micronuclei test in buccal cells of female farmers and children exposed to pesticides of Maneadero agricultural valley, Baja California, Mexico." Journal of Toxicology 2016.

[8] Crane, M., N. Scott, B. J. O'Hara, S. Aranda, M. Lafontaine, I. Stacey, M. Varlow and D. Currow (2016). "Knowledge of the signs and symptoms and risk factors of lung cancer in Australia: mixed methods study." BMC public health 16(1): 1-12.

[9] Crispino, C. C., K. G. Fernandes, M. Y. Kamogawa, J. A. Nobrega, A. R. A. Nogueira and M. M. Ferreira (2007). "Multivariate classification of cigarettes according to their elemental content determined by inductively coupled plasma optical emission spectrometry." Analytical sciences 23(4): 435-438.

[10] da Silva, V. H. P., R. de Luna Antonio, S. Pompeia and D. A. Ribeiro (2015). "Cytogenetic biomonitoring in buccal mucosa cells from young smokers." Acta Cytologica 59(6): 474-478.

[11] Fischer, S., B. Spiegelhalder and R. Preussmann (1989). "Tobacco-specific nitrosamines in mainstream smoke of West Gennan cigarettes-tar alone is not a sufficient index for the carcinogenic potential of cigarette smoke." Carcinogenesis 10(1): 169-173.

[12] Gharban, H. A., Al-Ghuraibawi, H. N., Al-Rubaye, Z. A., Jahlol, H. A., Al-Zergany, A. A., and Al-Abedi, G. J. (2023). Most Clinically Detected Viral Diseases in Field Animals of Wasit Province, Iraq. Annals of the Romanian Society for Cell Biology, 27(01), 154-168.

[13] Hartono, R. K., S. Abd Hamid and M. Hafizurrachman (2019). "Do the Number of Cigarettes Smokes per Day Contribute to the Incident of Malignant Cancer?" Asian Pacific journal of cancer prevention: APJCP 20(5): 1403.

[14] Hussain, J. N. (2008). "Sensitivity analysis to select the most influential risk factors in a logistic regression model." Journal of Quality and Reliability Engineering 2008.

[15] Joshi, M., Y. Verma, A. Gautam, G. Parmar, B. Lakkad and S. Kumar (2011). "Cytogenetic alterations in buccal mucosa cells of chewers of areca nut and tobacco." Archives of Oral Biology 56(1): 63-67.

[16] Kamath, V. V., P. Anigol and K. Setlur (2014). "Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers." Clinical cancer investigation journal 3(1): 49.

[17] Law, M., J. Morris, H. Watt and N. Wald (1997). "The dose-response relationship between cigarette consumption, biochemical markers and risk of lung cancer." British journal of cancer 75(11): 1690-1693.

[18] Li, W., J. Hu, O. Adebali, S. Adar, Y. Yang, Y.-Y. Chiou and A. Sancar (2017). "Human genome-wide repair map of DNA damage caused by the cigarette smoke carcinogen benzo [a] pyrene." Proceedings of the National Academy of Sciences 114(26): 6752-6757.

[19] Maessen, G. C., A. M. Wijnhoven, R. L. Neijzen, M. C. Paulus, D. A. van Heel, B. H. Bomers, L. E. Boersma, B. Konya and M. A. van der Heyden (2020). "Nicotine intoxication by e-cigarette liquids: a study of case reports and pathophysiology." Clinical toxicology 58(1): 1-8.

[20] Mokdad, A. H., J. S. Marks, D. F. Stroup and J. L. Gerberding (2004). "Actual causes of death in the United States, 2000." Jama 291(10): 1238-1245.

[21] Moktar, A., R. Singh, M. V. Vadhanam, S. Ravoori, J. W. Lillard, C. G. Gairola and R. C. Gupta (2011). "Cigarette smoke condensate-induced oxidative DNA damage and its removal in human cervical cancer cells." International journal of oncology 39(4): 941-947.

[22] Naderi, N. J., S. Farhadi and S. Sarshar (2012). "Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years." Indian Journal of Pathology and Microbiology 55(4): 433.

[23] Palve, D. H. and J. V. Tupkari (2008). "Clinico-pathological correlation of micronuclei in oral squamous cell carcinoma by exfoliative cytology." Journal of Oral and Maxillofacial Pathology 12(1): 2.

[24] Pindborg, J., J. Reibel, B. Roed-Petersen and F. Mehta (1980). "Tobacco-induced changes in oral leukoplakic epithelium." Cancer 45(9): 2330-2336.
[25] Pratheepa Sivasankari, N., S. Kaur, K. Reddy and S. Vivekanandam (2008). "Micronucleus index: An early diagnosis in oral carcinoma." J Anat Soc India 57: 8-13.
[26] Praud, D., M. Rota, C. Pelucchi, P. Bertuccio, T. Rosso, C. Galeone, Z.-F. Zhang, K. Matsuo, H. Ito and J. Hu (2018). Cigarette smoking and gastric cancer in the Stomach Cancer Pooling (StoP) Project." European Journal of Cancer Prevention 27(2): 124-133.
[27] Proia, N. K., G. M. Paszkiewicz, M. A. S. Nasca, G. E. Franke and J. L. Pauly (2006). "Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer—a review." Cancer Epidemiology and Prevention Biomarkers 15(6): 1061-1077.
[28] Sabah, J. T. (2021). "Evaluation of genotoxic damage in buccal mucosa cytome assays in Iraqi school children exposed to air pollutants emanating from oil fields." Mutation Research/Genetic Toxicology and Environmental Mutagenesis 863: 503304.
[29] Schick, S. and S. A. Glantz (2006). "Sidestream cigarette smoke toxicity increases with aging and exposure duration." Tobacco control 15(6): 424-429.
[30] Spits, M. and J. Neefjes (2016). "Immunoproteasomes and immunotherapy—a smoking gun for lung cancer?" Journal of thoracic disease 8(7): E558.
[31] Thomas, P., N. Holland, C. Bolognesi, M. Kirsch-Volders, S. Bonassi, E. Zeiger, S. Knasmueller and M. Fenech (2009). "Buccal micronucleus cytome assay." Nature protocols 4(6): 825-837.
[32] Tolbert, P. E., C. M. Shy and J. W. Allen (1991). "Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users." American Journal of Epidemiology 134(8): 840-850.
[33] Valavanidis, A., T. Vlachogianni and K. Fiotakis (2009). "Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles." International journal of environmental research and public health 6(2): 445-462.
[34] Xu, J. (2017). Number of Deaths from 10 Leading Causes,* by Sex-National Vital Statistics System, United States, 2015, CENTERS DISEASE CONTROL 1600 CLIFTON RD, ATLANTA, GA 30333 USA. 66: 413-413.
[35] Yadav, A. S. and M. Saini (2015). "Increased frequency of nuclear anomalies in exfoliated buccal mucosa of cigarette smokers." J Entomol Zool Stud 3(2): 07-10.
[36] Yerlagudda, K., V. V. Kamath and K. Satelur (2012). "Morphological assessment of oral cytological smears before and after application of toluidine blue in smokers and nonsmokers." Int J Oral Maxillofac Pathol 3: 8-14.