

Comparative study of exfoliated oral mucosal cell micronuclei frequency in normal and tobacco users progressing to malignancy.

Dr. Soma Datta*, Dr. Samiran Sanfui**, Dr. Uma Banerjee***.

* Assistant Professor, dept. of pathology, Burdwan Medical College

**PGT, dept. of pathology, Burdwan Medical College

***Professor, dept. of pathology, Medical college, Kolkata.

Corresponding Author:- Dr. Soma Datta

ABSTRACT : INTRODUCTION : Premalignant lesions have higher risk of developing oral carcinomas. Micronucleus characteristically seen in exfoliated epithelial cell; expresses genotyping alteration caused in process of malignancy during precancerous and cancerous conditions. **MATERIALS AND METHODS :** Among 98 subjects, 32 were with malignant lesions and 33 with premalignant lesions. Buccal scrapings taken from the lesions ; scrape smears stained by rapid PAP method ; micronucleus observed under microscope ; micronucleus index calculated and compared between above two groups and normal control groups. **RESULTS :** MN Index found progressively increasing with premalignant and malignant lesions showing statistically significant differences between normal and neoplastic lesions ($p < 0.001$) in males as well as females. Significant association found in predominant smokers with disease ($p = 0.002$) and predominant chewers with disease ($p < 0.001$) **CONCLUSION :** Micronucleus count and micronucleus Index can be used as useful biomarker for screening in oral premalignant and malignant lesions.

Key words – Micronucleus, tobacco, premalignant, malignant.

Date of Submission: 12-04-2018

Date of acceptance: 30-04-2018

I. Introduction

There is an increasing effort worldwide to determine the impact of environmental, biochemical, genetic, and life style factors on genomic stability in human populations. As a result of rapid globalization and changing social attitude, tobacco betel quid chewing habit have been increasing worldwide. Tobacco chewing along with various ingredients like areca nut, catechu, lime, cardamom, permitted spice, flavoring agents have been reported to be cytotoxic, mutagenic and genotoxic. It may be possible to use genotoxicity assays to identify tobacco users to the DNA damaging effect over baseline by micronucleus assay.¹

Micronucleus (MN), microscopically visible, round or oval cytoplasmic chromatin mass in the extra-nuclear vicinity due to aberrant mitosis. It consists of eccentric chromosomes, chromate fragments or whole chromosomes which failed to reach spindle poles during mitosis and has been used as biomarkers for assessment of DNA damages. MN frequency observed in exfoliated cells of oral mucosa are an appropriate index (MNI) to monitor the genotoxicity as they are in direct contact with the carcinogen. Exfoliated epithelial cells have traditionally been used for cancer screening and bio-monitoring of genotoxic effects in humans.^{2,3} MN scoring is simple, requires shorter training, less time consuming and is expected to be more sensitive than chromosomal aberration assay, because of increased statistical power brought out by the fact that number of cells analyzed can easily be increased to thousands when only few hundred cells scored for chromosomal aberrations.^{2,3} Premalignant lesions (PML) include erythroplakia, leukoedema or early leukoplakia, submucous fibrosis. Dysplastic changes include increased and particularly abnormal mitoses, individual cell keratinisation, epithelial pearls with spinous layer; alterations in nuclear cytoplasmic ratio, loss of polarity and disorientation of cells, hyperchromasia, large prominent nucleoli, dyskaryosis or nuclear atypia including giant nuclei, poikilocarynosis or division of nuclei without division of cytoplasm. The chances of leuko-plakia becoming malignant are cited from 1 to 17.5%.^{1,2,3} Cancer arises through a series of somatic alterations in DNA that results in unrestrained cellular proliferation mostly involving actual sequence changes in DNA.³ Oral cancer may arise as a primary lesion; by metastasis from a distant site of origin or by extension from a neighbouring anatomic structure such as the nasal cavity or maxillary sinus. Areas of preference are lips or tongue followed by floor of the mouth, cheek, gingiva or palate. Most oral cancers are squamous cell carcinomas. Tobacco and alcohol use are acknowledged risk factors in oral and oropharyngeal cancer. Tobacco contains potent carcinogens including nitrosamines (nicotine), polycyclic aromatic hydrocarbons, nitrosoproline and polonium.

HPV 16 is a known risk factor and independent causative for oral cancer. Histologic features of squamous cell carcinoma consist of sheets and nests of cells originating from squamous epithelium. The nuclei of neoplastic cells are large with presence of individual cell keratinization and epithelial or keratin pearls.^{4,5}

Aims & Objectives : This study is directed to evaluate MN frequency in PML and ML of oral mucosa. The predictive value of MN evaluated as biomarker for oral carcinomas by observing and calculating MNI in oral lesions and risk of genotoxicity evaluated with tobacco usage by comparing MNI in tobacco users presenting with oral PML and ML.

II. Materials And Methods :

A case control study was conducted in pathology department of a tertiary care hospital for a year. 33 patients (25 males ,8 females) in the age group of 18- 60 years with clinically proven PML and 33 patients (27 males , 6 females) in the age group of 18-60 years with clinically proven ML chosen . Informed consent obtained from each patient with detailed history regarding personal habits like chewing tobacco, betel leaves, keeping tobacco quid, pan, drinking alcohol, whisky, local arrack, wine , smoking beedies, cigarettes, keeping churatt in mouth, duration of use and amount they consume each day noted. Family history also enquired with treatment history. The subjects included in the study were between 18-65 years irrespective of gender , using tobacco for more than 5 years; not exposed to radiation, chemotherapy or medication; with clinically proven PML and ML and cooperative ones. The exclusion criteria being subjects beyond range of 18-65 years; treated ones ; patient with injuries, burns; extra-oral lesions and uncooperative patients. Patients asked to rinse the mouth thoroughly before taking the scrapings which were taken from the lesion preferably by using a dry wooden spatula and scraped material smeared on a labelled clean glass slide with a glass spreader. Air dried smears put in plastic jars containing 95% ethyl alcohol used as fixative and then kept in absolute alcohol for about 15 minutes and slide stained by Rapid PAP method using Papanicolaou stain. Stained and mounted smears observed for presence of micronucleus under high power & oil immersion magnification and counted from each cell. Only the clear cells with micronucleus considered for counting. Any unstained cell, cells with lot of artefacts or cells with loss of nuclei and other disrupted cells not taken into consideration. The total number of cells from each slide counted by Zig Zag 19 method using the differential counter machine. About 1000 cells screened from each slide followed by calculation of MNI. The cells were counted at the corner of each slide ; first seen horizontally and then vertically in the upward direction and then in the same manner it is directed downwards and whole slide screened . The number of cells counted by pressing the button in the differential counter and if any micronucleus seen in any of the cell another button is pressed and the counts calculated. MNI calculated as Micronucleus Count / Number of cells counted from each slide .

III. Result :

The study comprised of 68 males (51 cases ,17 controls) and 30 females (14 cases, 16 controls). 26 males and 6 females presented with ML and 25 males, 8 females presented with PML. Smokers showed 27.27% PML ; 34.37% ML and P value of 0.7254 which was statistically insignificant. Tobacco chewers showed 72.72% PML ; 65.62% ML and P value of 0.7254 which also was not significant.

Mean MNI compared in ML and PML and normal females reported as 10.5579, 3.8783, 0.4348 respectively and 6.7410 in total diseased females. Comparing mean MNI in normal with PML and ML presented with highly significant P value <0.001.(Table-1)

Comparing MNI between ML in males and females it was significant with value of 0.006.

All females were tobacco chewers predominantly. (Table-1 &2)

Table : 1: Micronuclear index (MNI) in females -normal, diseased ,with PML & ML

Category	Number	Mean	Standard deviation	Standard Error Mean	Significance (p value)
Normal	16	0.4348	0.35260	0.08815	
Diseased	14	6.7410	3.80958	1.01815	<0.001
PML	8	3.8783	1.88862	0.66773	<0.001
ML	6	10.5579	1.46477	0.59799	<0.001

Comparing mean MN between normal ,those with PML and ML showed a high significant P value of <0.001

(Table-2)

MNI when measured was <2 in 3 PML cases, 2-5 in 3 ML and 17 PML cases,5-10 in 23 ML and 13 PML cases, and >10 in 6 ML cases.

Table :2: Micronuclear index (MNI) in males -normal, diseased ,with PML & ML .

Category	Number	Mean	Standard deviation	Standard Error Mean	Significance (p value)
Normal	17	0.3166	0.30611	0.7424	
Diseased	51	6.2762	2.73035	0.41162	<0.001
PML	25	4.3507	2.06674	0.48714	<0.001
ML	26	7.6093	2.32381	0.45574	<0.001

When independent paired t- test done between predominant smokers with PML and ML ; it was found to be statistically significant (p = 0.002).When independent paired t- test done between predominant chewers with PML and ML; it was found to be statistically significant (p<0.001) (Table -3)

Table : 3 : Paired t-test amongst smokers and chewers with premalignant and malignant lesions.

Category	Number	Mean	Standard deviation	Standard error mean	Significance (p value)
Smokers with PML	9	3.2986	1.7951	0.5984	0.002
Smokers with ML	11	7.4189	2.8749	0.8668	
Chewers with PML	24	4.5256	1.99438	0.3967	<0.001
Chewers with ML	21	8.5515	2.1932	0.4786	

IV. Discussion :

The present study done to observe and calculate MNI in oral lesions and also to compare it with PML and ML ; thus used as a bio marker for screening in such patients .Majer et al stated that MN seen in blood lymphocytes and urinary bladder epithelial cells used for measuring the clastogenic effect .⁶

Moore et al scraped buccal cells by wood tongue depressor⁷; EL Ahmer et al with a cotton swab⁸; Besarati Nia et al with short bristle cytobrush⁹ ; Marchand LL et al used brush¹⁰ ; Obwald et al with swish and spit method, which showed both buccal and polymorphonuclear leukocytes with more viability of the latter¹¹.

Armen Nersesyan et al in a study stated that micronuclei frequencies found in heavy smokers and non smokers varied with different staining procedures. MN frequencies scored with Giemsa were 5 fold higher in smokers than in non smokers; with May Grunwald-Giemsa it was 4.5 fold higher. On the contrary,no significant effects observed with DNA specific stains.With acridine orange the MN frequencies were 90% higher in smokers and with DAPI and Feulgen, the differences were 30% and 120% respectively. Feulgen stained slides evaluated under fluorescence showed 89% increase¹².

The levels of MN were increased in a study reported by Kumar V et al who followed a fluorescent acridine orange staining method and fluorescence microscope analysis, increasing the specificity to identify DNA containing structures which is a time consuming method requiring costlier chemicals and equipment.¹³ Devendra H Palve used Rapid PAP technique instead of fluorescent dyes in analysing micronuclei which was simple, less time consuming and economical.¹⁴Mala Kamboj used acridine orange and feulgen stains for micronucleus and found the increasing frequency of micronucleated cells in comparison to controls in leukoplakia and squamous cell carcinoma and stated that fluorescent staining found to be more sensitive than the conventional one for micronucleus detection.¹⁵

Casarelli documented a significant increase in MN of PML (n=47) and for ML (n=21); and the MN frequency did not vary with sex or age of patients but with anatomic site of the lesions.¹⁶ Saleha.B.vuyyuri used buccal cells to determine the MN frequency in glass workers exposed to arsenic and found increased frequencies of micronuclei in the buccal cells and increased levels of DNA damage in leukocytes in comparison to controls (p<0.001).¹⁷ The percentage of MN frequency in precancerous lesions was 3.2+0.873 and one fold increase of micronucleus seen by Chatterjee et al¹⁸.

Waranum Buajebe showed significant elevation in frequency of exfoliated micronucleated cells in oral lichen planus as compared to normal individuals (p<0.01).¹⁹ Pastor compared MN frequencies in buccal epithelial cells of farmers exposed to pesticides and those not which revealed no statistically significant differences with p>0.05 between these two groups.²⁰ Celik studied MN in buccal mucosal cells of 60 painters comprising 30 smokers and 30 non-smokers compared to healthy controls, where microscopic observation of 3000 cells per individual done and found statistically significant increase in frequency of MN in buccal epithelial cells of the exposed groups (painters) when compared with control group (p<0.05).²² Joshi found significantly higher MN in chewers and with submucous fibrosis compared to non-chewers (p<0.0001); with significantly higher analysis and nuclear buds in oral submucous fibrosis compared to chewers and non-chewers.(p<0.0001).²³ MN in study by Mariza on exfoliated oral mucosal cells in chronic denture stomatitis patients and healthy controls showed no statistically significant differences (p>0.0571).²⁴

Mahimakar showed an increased MN levels in oral leukoplakia patients compared to normal healthy subjects ($p=0.01$).²⁵ Kausar found significant increase in MN frequency in sadagura chewers (0.48%, $p<0.01$); in smokers (0.46%, $p<0.01$); in betel quid with sadagura chewers (0.91%, $p<0.001$) and smokers chewing betel quid with sadagura (0.53%, $p<0.001$) when compared to unexposed control group (0.07%).²⁶ Martins showed statistically significant difference in MN frequency in oral mucosal cells of gas petrol attendants and non exposed ones

($p<0.05$).²⁸ Nersesyan.A. found significantly increased MN frequency but not of other nuclear abnormalities in exfoliated buccal cells of polycystic ovarian syndrome patients ($p<0.05$) and DNA leucocytes found significantly damaged compared to healthy females.^{21,27} Bortoli found significantly higher MN frequency in Brazilian workers exposed to pesticides in soyabean fields (3.55 \pm 2.13) compared to non-exposed ones (1.78 \pm 1.23). MN frequency is statistically similar in the present study ($p=0.776$).²⁹

Konopacka also found buccal cell MN count more in the malignant condition compared to pre-malignant and normal condition.³⁰ Haveric evaluated MN frequency in peripheral blood and buccal exfoliated cells of young smokers and non smokers and found significantly higher MN frequency in peripheral blood lymphocytes in smokers ($p<0.05$). No significant correlations found for age, duration and intensity of smoking with MN frequency in lymphocytes with significantly higher frequency in smokers ($p<0.0578$).³¹

In the present study, MN count was more in ML compared to PML. Stich et al found an elevated MN frequency in smokers and khaini tobacco chewers.³² Angelieri found no statistically significant differences in MN frequency in oral mucosal cells in smokers and non-smokers exposed to dental X-rays using two anatomic sites ($p>0.05$).³³

Evaluation of MN in buccal cells and peripheral blood lymphocytes showed no significant difference compared to smokers and non smokers shown by Yildirim ($p>0.05$).²⁷ Present study also showed no such statistical difference seen with smoking and tobacco when compared with $p>0.05$. Pratheepa Sivasankari et al found two folds higher MNI in ML compared to PML. The comparison of mean MNI between ML and PML cases was significant with $p<0.05$.³⁴ The present study show statistically significant mean MNI between ML and PML cases with $p<0.001$.

Mean MN count when compared between PML and controls was significant with $p<0.01$ in buccal mucosal cells showed by Suhas.S.³⁵ Analysis of MN in buccal cells taken from children with chronic tonsillitis was compared to control group where mean frequencies in children with chronic tonsillitis was found to be 5.29 \pm 1.67 and that with the control group was 1.58 \pm 0.33 the difference was found to be statistically significant with $p<0.00183$. Mean percentage of MN cells was significantly higher in non-smokers or non-users ($p<0.01$). Mean percentage of MN cells was 1.86 \pm 0.26 in users and 1.99 \pm 0.30 in smokers.

There was no difference between the mean percentage of MN cells in these two groups in a study by Yusuf Ozkul et al in 1997.³⁶ Mean frequencies of MN in buccal mucosa and blood lymphocyte of chromium platers by smoking were significantly higher than those of controls ($p<0.01$). However Hyeong – Ryeol showed no statistically significant ($p>0.05$) difference of mean MN frequencies between smokers and non smokers.³⁷

V. Conclusion :

MN count and index alteration observed in PML and ML in oral cavity with significant difference. This index can be used as a biomarker or as screening test in PML and ML. Oral tobacco is genotoxic to cell producing MN in high significant number in PML and ML cases than normal control.

Acknowledgement :

We thank our principal of Burdwan Medical college for allowing us to carry out this study and other faculty members for their utmost cooperation in carrying out this study work.

References :

- [1]. Schatzkin A, Freedman LS, Shiffman MH, Dawsey SM. Validation of intermediate end points in cancer research. J Natl Cancer Inst. 1990;82:1746-52.
- [2]. Patel PB, Trivedi PJ, Brahmabhatt MM, Shukla SN, Shah PM, Bakshi SR, et al. Micronuclei are chromosomal aberration in healthy tobacco chewers and controls: a study from Gujrat; India. 2009;17:7.
- [3]. Palaskar S, Jindal C. Evaluation of micronuclei using papanicolaou and May Gronwald-Giemsa stain in individuals with different tobacco habits: a comparative study. J Clin Diagn Res 2011;4:3607-13.
- [4]. John D Bancroft, Marilyn Gamble. Theory and Practice of Histological Techniques. 6th Edition. Elsevier, Churchill Livingstone. 2008;123-26.
- [5]. Bernard Rosner. Fundamentals of Biostatistics. 5th Edition. Duxbury. 2000; 80-240.
- [6]. Majer BJ, Laky B, Knasmuller S, Kassie. Use of micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring at elevated risk of genetic damage and in chemoprevention trials. Mutat Res 2001;489:147-72.

- [7]. Moore LE. Investigation of Genetic Polymorphisms and Smoking in a bladder cancer case control study in Argentina. *Cancer Lett* 2004; 211:199-207.
- [8]. EL Ahmer OR, Essery SD, Saadia A T . The Effect of cigarette smoke on adherence of respiratory pathogens to buccal epithelial cells. *FEMS. Immunol Med Microbiol* 1999;23:27-36.
- [9]. Besarati Nia, Van Straaten HMW, Godschalk RWL. Immunoperoxidase detection of polycyclic aromatic hydrocarbons DNA adducts in mouth floor and buccal mucosal cells of smokers and non smokers. *Environ Mol Mutagen* 2000;36:127-33.
- [10]. Marchand LL, Lum Jones Saltzman B, Visaya V, Normura AMY, Kolonel LN. Feasibility of collecting buccal cell DNA by mail in a cohort study. *Cancer Epidemiol Biomarkers Prev* 2001;10:701-3.
- [11]. Obawald K, Mittas A, Glie M, Pool Zobel BL. New arrival of an old biomarker: characterization of buccal cells and determination of genetic damage in the isolated fraction of viable leukocytes. *Mutat Res* 2003; 544:321-9.
- [12]. Armen Nersesyan, Miochail Kundi, Kambis Atefie, Rolfshcutle Helmann, Siegfried Kuasmuller. Effect of staining procedures on the results of Micronucleus Assays with Exfoliated Oral Mucosa Cells. *Cancer Epidemiology and Biomarkers Prev* 2006;15(10):1835-40.
- [13]. Kumar V Rao NN, Nair NS. Micronuclei in Oral Squamous Cell Carcinoma. A marker of genotoxic damage. *Indian J Dent Res* 2000;11: 101-6.
- [14]. Devender H. Palve, Jagadish. V. Tupkari. Clinicopathological correlation of Micronuclei in Oral Squamous Cell carcinoma by exfoliative cytology. *Journal of oral and Maxillofacial pathology* 2008;(12):2-7.
- [15]. Mala Kamboj, Sumita Mahajan. Micronucleus-an up coming Biomarker of genotoxic damage. *Journal of clinical oral investigations* 2007;(11):1432-6981.
- [16]. Casartelli G, Monteghirfo S, De Ferrari M. Staining of Micronuclei in the Squamous Epithelial Cells of Human Oral Mucosa. *Anal Quant Cytol Histol* 1997;(19):475-81.
- [17]. Saleha B Vyurri, Mohammed Ishaq, Danadevi Kuppala, Paramjit Grover, Y R Ahuja. Evaluation of Micronucleus frequencies and DNA damage in glass workers exposed to arsenic. *Environmental and Molecular Mutagenesis* 2006;(5):562-570.
- [18]. Sumana Chatterjee, Tapash Rudra, Sandeep Poddar, Sila Chakrabarti. GST and CYP Polymorphism Related to Tea Drinking and Oral Pathology. *Int J Of Hum Genet* Garewal HS, Ramsey L, Kangars G, Boyle J. Clinical Experience with the Micronucleus Assay. *Journal of Cellular Biochemistry* 2004;53(17):206-12.
- [19]. Waranam Buajeel, Petcharat Kraivaphan, Cholchicha Amornchat, Terd Phong Triratana. Frequency of Micronucleated Exfoliated Cells in Oral Lichen Planus. *Mutat Res* 2007;(2):191-6.
- [20]. Pastor S, Gutierrez S, Creus A, Cebulska Wasilewska A, Marcos R. Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. *Mutat Res* 2001; 495(2):147-56.
- [21]. Nersesyan A, Chobanyan N. Micronuclei and other nuclear anomalies levels in exfoliated buccal cells and DNA damage in leukocytes of patients with polycystic ovarian syndrome. *J Buon* 2010; 15(2):337-9.
- [22]. Celik A, Diler SB, Eke D. Assessment of genetic damage in buccal epithelium cells of painters: Micronucleus nuclear changes and repair index. *DNA cell Biol* 2010; 29(6):277-84.
- [23]. Joshi MS, Verma Y, Gautam AK, Pamar G, Lakkad BC, Kunal S. Cytogenetic alterations in buccal mucosal cells of chewers of areca nut and tobacco. *Arch oral Biol* 2010;(24): 120-29.
- [24]. Marizia A, Matrumoro, Juliane castanho, Roberto Y Kawakami, Daniel A Ribeiro. Cytogenetic damage in exfoliated oral mucosal cells in elderly people suffering denture stomatitis 2010;(27): 183-188.
- [25]. Mahimkar MB, Samant TA, Kannan S, Patil T. Influence of genetic polymorphism on frequency of micronucleated buccal epithelial cells in leukoplakia patients. *Oral onco* 2010; 46(10): 761-6.
- [26]. Kausar A, Giri S, Mazumdar M, Giri A, Roy P, Dhar P. Micronucleus and other nuclear abnormalities among betel quid chewers with/ without sadagura, a unique smokeless tobacco preparation in a population from north east India. *Mutat Res* 2009; 677:72-75.
- [27]. Yildirim I H, Yesilada E, Yolaglu S. Micronucleus frequency in peripheral blood lymphocytes and Exfoliated Buccal Cells of untreated cancer Patients. *Genetika* 2006;42(5):705-10.
- [28]. Martins RA, Gomes GA, Aguiar O, Ribeiro DA. Biomonitoring of oral epithelial cells in petrol station attendants: Comparison between buccal mucosa and lateral border of tongue. *Environ Int* 2009; 35(7): 1062-5.
- [29]. Bortoli GM, Azvedo MB, Silvia LB. Cytogenetic biomonitoring of Brazilian workers exposed to pesticides: Micronucleus analysis in buccal epithelial cells of soybean growers. *Mutat Res* 2009; (30):1-4.
- [30]. Konopacka M. Effect of smoking and ageing on micronucleus frequencies in human exfoliated buccal cells. *Neoplasma* 2003;50(5): 380-2.
- [31]. Haveric A, Haveric S, Ibrulj S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non smokers. *Toxicol Mech Method* 2010; 20(5):260-66.
- [32]. Stich HF, Parida BB, Brunnemann KD. Localized formation of micronuclei in the oral mucosa and tobacco specific nitrosamines in the saliva of reverse smokers, Khaini tobacco chewers and gudakhu users. *Int J Cancer* 1992;8:31-3.
- [33]. Angelieri F, De cassia Goncalves, Moleirinho T, Carlin V, Oshima CT, Ribeiro D. Biomonitoring of epithelial cells in smokers and non smokers submitted to panoramic x ray: Comparison between buccal mucosa and lateral border of tongue. *Clin oral Investigations* 2009; (24):114-120.
- [34]. Pratheepa sivasankari, sohinder kaur, Reddy. K.S, Vivekanandam. Micronucleus Index: An Early Diagnosis in oral carcinoma. *J Anatomical Society of India* 2008;57(1):8-13.
- [35]. Suhas S, Ganapathy KS, Gaytridevi, Ramesh C. Application of Micronucleus test to Exfoliated Epithelial cells from the oral cavity of beedi smokers, a high risk group for oral cancer. *Genetic toxicology and Environmental Mutagenesis* 2004;(561):15- 21.
- [36]. Yusuf Ozkul, Hamiyet Donmez, Aydin Erenemisoglu, Demirtas and Nalan Imanjogh. Induction of Micronuclei by Smokeless Tobacco on Buccal mucosal cells of Habitual users *Mutagenesis* 2000;(12):285-287.
- [37]. Hyeong-Ryeol Yoon, Jang-Rak kim, Dae yong Hong. Micronucleus Test as an Index of Mutagenicity for Workers Exposed to Chromium. *The Korean Journal of Occupational Medicine* 1993; 5(1):45-57.

Dr. Soma Datta "Comparative study of exfoliated oral mucosal cell micronuclei frequency in normal and tobacco users progressing to malignancy." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, vol. 17, no. 4, 2018, pp 23-27.