IJOEM
International Journal of Occupational and Environmental Medicine

Original Article



To review this article online, scan this QR code with your Smartphone

# Genomic Instability in Exfoliated Buccal Cells among Cement Warehouse Workers

Lalu Krishna[1,2], Ursula Sampson[2], Panthapulaykal Theru Annamala[3], Kumudam M Unni[4], Bhaskarapillai Binukumar[5], Alex George[4], Ranjith Sreedharan[1]

## Abstract

**Background:** Workers in cement warehouses of Kerala are enduring long-standing exposure to cement dust, which is considered genotoxic.

**Objective:** To evaluate the extent of genotoxicity and cytotoxicity caused due to exposure of cement dust among those working in cement warehouses.

**Methods:** The study included 82 cement warehouse workers and 82 age-matched individuals with no exposure to cement dust. Exfoliated buccal micronucleus cytome assay (BMCyt) was performed to analyze the genotoxic and cytotoxic effects caused by inhalation of cement dust.

**Results:** The frequency of various genotoxic and cytotoxic end markers (micronucleated cells [2-fold increase, $p<0.001$], nuclear buds [4-fold increase, $p<0.001$], binucleated cells [4-fold increase, $p<0.001$], karyorrhectic cells [2-fold increase, $p<0.001$], pyknotic cells [3-fold increase, $p<0.001$], and karyolytic cells [2-fold increase, $p<0.001$]) were higher in the exposed workers compared with unexposed group. Increase of these parameters represented an increased level of chromosomal damage, nuclear disintegration and increased cell death among exposed group compared with unexposed group.

**Conclusion:** Continuous exposure to cement dust results in increased frequency of nuclear aberrations and cellular apoptosis. This may lead to defects in genome maintenance, accelerated ageing, increased chance of oral cancer and neurodegenerative disorders in those occupationally exposed to cement dust.

**Keywords:** Occupational exposure; Mutagenicity tests; Chromosome aberrations; Apoptosis; Oral mucosal absorption; Micronuclei, chromosome-defective; Micronucleus tests; Biomarkers; DNA damage

[1]Department of Anatomy, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India
[2]Department of Biochemistry, Meenakshi Medical College Hospital and Research Institute, Meenakshi academy of higher education, Tamil Nadu, India
[3]Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India
[4]Jubilee Centre for Medical Research, Jubilee Mission Medical College, Thrissur, Kerala, India
[5]Department of Biostatistics, National Institute of Mental Health and Neuro Sciences, Bengaluru, India

## Introduction

Portland cement is one of the most commonly used building materials across the world. The essential compounds of cement include calcium oxide, silicon dioxide, aluminium oxide, manganese, iron oxide, lead, chromium, cadmium, arsenic, and zinc.[1] Supplementary raw materials such as silica sand, iron oxide, and bauxite containing hydrated aluminium, may be used in lesser amounts to get the required composition.[2] Employees in cement factories, construction sites,

Correspondence to
Dr. Ranjith Sreedharan, Department of Anatomy, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India
Tel: +91-938-834-4448
E-mail: drranjithanatomy@gmail.com
Received: Jun 24, 2019
Accepted: Sep 27, 2019

**Cite this article as:** Krishna L, Sampson U, Annamala PT, *et al*. Genomic instability in exfoliated buccal cells among cement warehouse workers. *Int J Occup Environ Med* 2020;**11**:33-40. doi: 10.15171/ijoem.2020.1744

warehouses, and asbestos manufacturing factories are exposed to various levels of cement dust. A two-fold increase in the emission of cement dust from these industries have been noticed worldwide in the last few decades due to an upsurge of more cement plants to satisfy the high demand of cement for infrastructure.[2] The aerodynamic diameter of cement particles ranging from 0.05 to 10 μm, which could reach to the level of alveoli in lungs. The main route of entry of this dust is through inhalation (respiratory system) and swallowing (digestive system).[3,4] Cement as a whole, and its components are considered chemical hazards; excess amounts of these elements are potentially harmful to the biotic and abiotic components of the environment.[5] Microelements in cements, *eg*, chromium, are considered carcinogenic in 1980's. A strong association between exposure to cement dust and developing laryngeal carcinoma has been established.[6] An increased relative risk for the malignancies of lip, stomach, lung, and prostate has been recorded among concrete workers.[7] A significant increase in total leukocyte count, chest pain, cough, ophthalmic problems, increased micronuclei levels in lymphocytes (representing increased genotoxicity) have been reported in various studies conducted on people across the world working in cement industries.[8-11] In addition, an increased activity of the antioxidant enzymes and depletion of total antioxidant capacity revealed that exposure to cement dust leads to increased oxidative stress.[12] Neboh, *et al*, reported a decrease in hemoglobin content among cement warehouse workers in Nigeria chronically exposed to cement dust.[13] To the best of our knowledge, no study has so far been conducted among loading and unloading workers of cement warehouses, who are considerably exposed to cement dust.

Though various studies have been undertaken on the effects of exposure to cement dust, few have focused on its effect on buccal epithelial cells. This issue should be considered important as buccal epithelium is the first barrier against toxic agents and micro-organisms.[14] Buccal micronucleus cytome assay (CBMN cyt assay) is a robust method to study the effect of exposure to a genotoxic agent.[15,16] Presence of micronuclei (MN), binucleated cells (BN), nuclear buds (Nbud), and accelerated apoptosis, or necrosis of buccal cells has to be considered in detail to understand the extent of genotoxicity and cytotoxicity caused. Micronuclei are the products of chromosome fragmentation, whole chromosome loss or DNA double strand breakage (DSB), hence, it can be considered an end-point to analyze DNA damage.[17,18] Nuclear aberrations such as pyknosis, karyolysis, and karyorrhexis represent the cytotoxicity. The present study aimed at analyzing the extent of genotoxicity and cytotoxicity in buccal cells caused by chronic exposure to cement dust of workers in cement warehouses of Kerala, India.

## Materials and Methods

### Study Population

The study was conducted among workers occupationally exposed to cement dust in cement warehouses. After obtaining an informed consent, we recruited 82 male workers with minimum of one year exposure to cement. Subjects with habits of smoking, alcohol consumption, paan or tobacco chewing, poor dental health, having known systemic diseases and under medication, and history of recent x-ray exposure were excluded from the study. Study participants were grouped into three groups based on their years of exposure (YOE) to cement dust: 1–10, 11–20, and >20 years. An equal number of people not exposed to cement dust having similar age, sex, socioeconomic status, and social habits were

selected as controls (unexposed). Based on age, both exposed and unexposed groups were classified into three groups: ≤35, 36–45, and >46 years. Information regarding demographics, *eg*, age of participants and the YOE to cement dust was gained through a scheduled interviewer-administered questionnaire. YOE to cement dust of participants was defined based on the total continuous period of exposure to cement dust at cement warehouses. The time duration for the study was four years. A minimum sample size of 82 exposed individuals and 82 unexposed individuals was calculated based on a pilot study. The study was conducted in Department of Anatomy and Jubilee Centre for Medical Research, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India. The study protocol was approved by the Institutional Ethics Committee.

## Specimen Collection

The subjects were asked to rinse their mouth with clean water to remove the unwanted particles and debris. Buccal mucosa was collected by scraping gently from the inner cheek (both right and left sides) using a wooden spatula and transferred to a 15-mL centrifuge tube containing 5 mL normal saline.

## Buccal Micronucleus Cytome Assay

The sample collected was centrifuged at 1200 rpm for 5 min. The supernatant was discarded and the tube was tapped well. The procedure was repeated three times adding 5 mL normal saline until the pellets were clear. The pellets were fixed using 5 mL fixative (glacial acetic acid and methanol in 1:3 v/v) and stored for 24 hrs. The tubes were then centrifuged for 5 min at 1200 rpm before preparing the slides. The cell pellets were dropped on to clean microscope slides and allowed to dry before it was stained with Acridine Orange. The stained slides were viewed under 40× (Axioscope 1, Carl Zeiss microscope) to score and photography the genotoxic (micronuclei [MN], nucleoplasmic bridge [NPB], nuclear bud [NBUD], and binucleated cells [BN]) and cytotoxic (pyknotic cells [PK], karyorrhectic cells [KH], and karyolytic cells [KL]) end-points. A total of 2000 cells per individual were counted to identify the above incidences.

**TAKE-HOME MESSAGE**

- Essential components of Portland cement include silicon dioxide, aluminium oxide, lead, chromium, cadmium, arsenic; they are considered chemical hazards.
- These compounds enter the body via inhalation and ingestion and are preferentially targeted the buccal epithelial cells.
- Early genotoxic events occurred after the exposure to cement dust are considered genotoxic.
- Chronic exposure to cement dust leads to increased levels of DNA damage.

## Statistical Analysis

Data were analyzed with SPSS® for Windows® ver 22.0 (IBM SPSS, Armonk, NY, USA). Quantitative variables were summarised using mean and SD for normally distributed data; median (IQR) otherwise. One-sample Kolmogorov-Smirnov test was used to test the normality of the distribution of the variables. Continuous variables were compared between two groups with *Student's t* test for independent samples and Mann-Whitney U test. One-way ANOVA or Kruskal-Wallis tests were used for comparing three or more groups. *Post hoc* analysis was carried out to identify pairwise significance.

**Table 1:** Genotoxicity and cytotoxicity in buccal cells among exposed and unexposed groups. Values are median (IQR). The frequency of all studied variables differ significantly (p<0.001) between exposed and unexposed groups.

| Nuclear anomalies | Exposed (n=82) | Unexposed (n=82) |
|---|---|---|
| Buccal MN | 15 (13.3) | 7.33 (8) |
| Buccal BN | 12 (11) | 3.00 (2) |
| Buccal Nbud | 13 (10) | 3.00 (2) |
| Karyorrhexis | 15 (4.3) | 9 (5) |
| Pyknosis | 15 (6) | 5 (2) |
| Karyolysis | 18 (7) | 10 (3.5) |

Buccal MN: buccal micronuclei, Buccal BN: buccal binucleated cells, Nbud: nuclear bud

## Results

### General Demographics of the Groups

The median age of exposed group was 44 (IQR 12) years; it was 44.5 (13) in unexposed group. The median YOE to cement dust among the exposed group was 14 (10) years. The mean BMI was 24.6 (SD 3.21) and 25.91 (2.62) kg/m$^2$ in the exposed and unexposed groups, respectively.

**Table 2:** Genotoxicity caused by exposure to cement dust on buccal cells stratified by years of exposure. Values are either mean (SD) or median [IQR]. The frequency of all studied variables differ significantly (p<0.001) among the three groups.

| Nuclear anomalies | 1–10 yrs (n=27) | 11–20 yrs (n=42) | >20 yrs (n=13) |
|---|---|---|---|
| Buccal MN | 10 [4] | 18 [9] | 27 [4] |
| Buccal BN | 3 [2] | 14 [4] | 14 [4.5] |
| Buccal Nbuds | 5.0 (2.24) | 14.29 (2.68) | 17.85 (2.41) |
| Karyorrhexis | 12.22 (2.06) | 15.74 (2.35) | 19.54 (3.04) |
| Pyknosis | 11 [3] | 16 [5] | 20 [6.5] |
| Karyolysis | 14 [4] | 19 [5] | 24 [5.5] |

Buccal MN: buccal micronuclei, Buccal BN: buccal binucleated cells, Nbud: nuclear bud

### Genotoxic and Cytotoxic Biomarkers Measured in Buccal Epithelial Cells among Exposed and Unexposed Groups

The frequency of MN was significantly (p<0.001) higher in the exposed compared with unexposed group (Table 1). There was also a significant (p<0.001) difference in the frequency of BN cells and Nbuds between the study groups (Table 1). In both groups, the frequency of MN was higher than that of BN cells and Nbuds. The frequency of cytotoxic end-markers in the exposed group was higher than that in unexposed group (Table 1).

### Correlation of Genotoxicity and Cytotoxicity with Years of Exposure:

YOE significantly affected all genotoxicity end-markers (Table 2). A significant (p<0.001) increase in the number of MN and Nbuds was noticed as the duration of exposure increased. The formation of BN cells (damage in the cytokinesis during mitosis) in the 11–20 YOE group and >20 YOE group was much more than that observed in 1–10 YOE group (p<0.001); however the difference was not significant between 11–20 YOE and >20 YOE groups. The genotoxic levels, represented with the formation of BN cells, were more during the initial stages of exposure to cement dust and became constant in later years. YOE also significantly affected cytotoxicity end-markers (Table 2); the frequency of nuclear anomalies increased with the YOE.

### Correlation of Genotoxicity and Cytotoxicity with Age in Exposed and Unexposed groups

The frequency of genotoxic and cytotoxic end-markers significantly changed by increasing age in the exposed group (Table 3). The frequency of nuclear anomalies also increased by age in unexposed group (Table 4).

## Discussion

Early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion are preferentially targeted in the buccal epithelial cells.[16] The oncogene activation and tumor suppressor gene inactivation, which leads to deregulation of cell division and death, are the reasons stated for genetic alterations observed in head and neck cancer.[15] According to the World Health Organization, ,carcinoma of oral cavity is the sixth most common cancer among males in developing countries; it is after lung, prostate, colorectal, stomach, and bladder cancer; 90% of all oral cancers originate from epithelial cells.[15,19] These facts highlight the advantage of the CBMN cyt assay, an *in vivo* examination that explicates the effects of toxic agents acting directly on a target tissue (buccal epithelium). Genotoxic and cytotoxic parameters were analyzed in exfoliated buccal cells and compared between exposed and unexposed subjects to determine the extent of genetic damage involved.

The basal layer of epithelial tissue, where cells undergo mitosis, is the site where micronuclei are formed in response to any toxicity. The epithelial tissue proliferation takes 7–16 days to bring the cells to the surface, where they exfoliate.[20,21] The subjects participated in this study were continuously exposed to cement dust during their daily work. Therefore, the exfoliated cells collected for the analysis were those targeted at the basal layer.

The presence of MN indicates chromosome loss or fragmentation occurring during early nuclear division.[13,14] Exposure to clastogens, genetic defects in cell cycle checkpoint or DNA repair genes, oxidative stress, and absences of major cofactors in DNA metabolism and chromosome segregation machinery induce these events.[22] MN located within the cytoplasm of differentiated cells with uniformly stained nuclei alone was used for scoring.[15] Acridine orange, a DNA-specific stain, was used in this study to stain the exfoliated buccal cells to avoid keratohyalin granules, which can give false-positive results (Fig 1C). The present study found a significant increase in the frequency of MN among exposed groups compared with unexposed group; the frequency increased with YOE. A similar increase in the frequency of MN in buccal cells have also been reported among workers of petrol pump, gutkha and pan

**Table 3:** Genotoxicity and cytotoxicity caused by exposure to cement dust in buccal cells stratified by age. Values are either mean (SD) or median [IQR]. The frequency of all studied variables differ significantly ($p<0.001$) among the three groups.

| Nuclear anomalies | ≤35 yrs (n=18) | 36–45 yrs (n=27) | >46 yrs (n=37) |
|---|---|---|---|
| Buccal MN | 8.00 (2.16) | 14.96 (4.76) | 22.05 (7.54) |
| Buccal BN | 3 [2] | 12.5 [8.5] | 15 [4] |
| Buccal Nbud | 4 [3] | 13.5 [7.5] | 15 [5] |
| Karyorrhexis | 11 [3] | 14 [2] | 18 [3] |
| Pyknosis | 11 [2] | 14 [4.3] | 17 [4.5] |
| Karyolysis | 13 [3] | 18 [5.3] | 21 [5] |

Buccal MN: buccal micronuclei, Buccal BN: buccal binucleated cells, Nbud: nuclear bud

**Table 4:** Genotoxicity and cytotoxicity in buccal cells of unexposed group stratified by age. Values are either mean (SD) or median [IQR]. The frequency of all studied variables differ significantly ($p<0.005$) among the three groups.

| Nuclear anomalies | ≤35 yrs (n=18) | 36–45 yrs (n=27) | >46 yrs (n=37) |
|---|---|---|---|
| Buccal MN | 2 [2] | 6 [3] | 11 [2.5] |
| Buccal BN | 1 [1.3] | 3 [2] | 4 [1] |
| Buccal Nbud | 2 [2] | 3 [2] | 4 [1] |
| Karyorrhexis | 5 [2] | 7 [2] | 4 [1.3] |
| Pyknosisa | 4.50 (1.18) | 5.09 (1.20) | 5.84 (1.62) |
| Karyolysis | 6.50 [3.3] | 10 [2] | 12 [3] |

Buccal MN: buccal micronuclei, Buccal BN: buccal binucleated cells, Nbud: nuclear bud

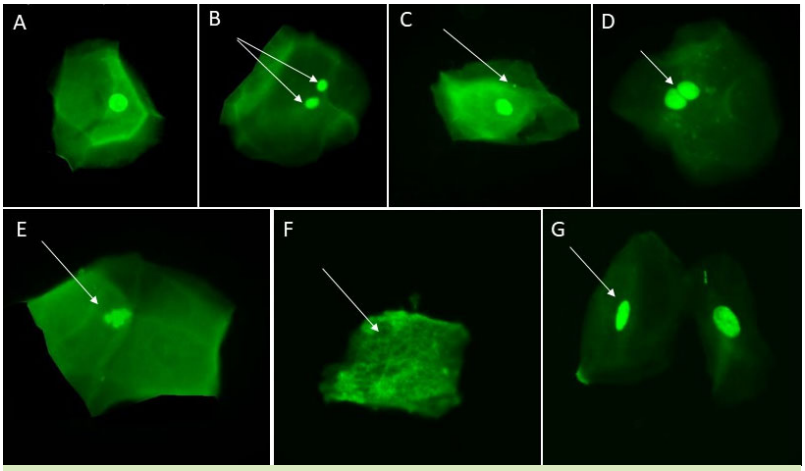


**Figure 1:** Various genotoxic and cytotoxic end-markers in exfoliated buccal cells: A) normal cell, B) binucleated cell, C) micronucleus (MN), D) broken egg (Nbud), E) karyorrhexis, F) karyolysis, and G) pyknosis

masala chewers, beedi smokers, welders, and workers in tannery factory, implying a higher level of chromosomal damage.[23-25] The findings of the present study were in line with their findings in buccal cells in terms of the chromosomal damages. A previous study on cement workers has focussed on MN in peripheral blood lymphocytes.[11] However, study on buccal mucosa must be in the limelight, as it is the first barrier and an easier and non-invasive method.

BN cells are the cells with two main nuclei instead of one; they are morphologically similar to normal cells (Fig 1B). They are probably the signs of failed cytokinesis following the last nuclear division in the basal cell layer.[26] A higher frequency of chromosomal non-dysjunction occurs in BN cells that fail to complete the cytokinesis. This is considered a cytokinesis checkpoint for aneuploid BN.[27] Thomas and Fenech have reported that BN cells might be associated with cell division; Celik, *et al*, considered it an end-marker of genotoxicity.[28,29]

Nbud, also known as "broken eggs," is a marker of nuclear abnormalities such as chromosomal breakage, genetic material loss and excision repair.[30] Nbud is small or large protrusion of chromatin with a diameter ranging from one-sixth to one-third of the main nucleus (Fig 1D). It is attached to the main nucleus by a narrow or wide constriction having similar staining intensity as the main nucleus.[31] In the present study, the frequency of both BN and Nbud significantly increased among exposed workers compared with the unexposed group. Recently, Shaikh, *et al*, has reported similar effects caused by exposure to gasoline among petrol pump workers.[32]

Nuclear changes, other than MN, that represent cellular death, which can enhance the sensitivity of techniques to detect genotoxicity, have been reported by many researchers.[33-35] The factors intimately involved in cell growth and death are affected by toxic agents; an increased cell proliferation indicates carcinogenicity. The repeated exposure to cytotoxic agents can result in chronic cell damage, compensatory cell proliferation, hyperplasia, and eventually tumor growth.[36] Exposure to mutagens can stimulate apoptosis and act as a defensive mechanism by removing genetically damaged tissues. Increased levels of apoptosis can be an indication of genotoxic damage that would be related to the process of malignant alterations.[37] These biomarkers found in BMCyt have been correlated with exposure to genotoxic agents, defects in genome maintenance, accelerated aging, oral carcinoma, and diseases due to neurodegeneration.[38] Therefore, the stages of apoptosis such as karyorrhexis, karyolysis, and pyknosis were considered cytotoxic effects and investigated (Fig 1E, 1F, and 1G). All the three parameters increased significantly in the present study and represent the nuclear disintegration leading to increased cell death, establishing the cytotoxic properties of cement dust. Previously Shaikh, *et al*, and Lorenzoni, *et al*, have reported similar disintegration of DNA leading to cell death among petrol pump workers and those

exposed to formaldehyde.[32,36] The present findings observed in buccal cells also typified the cytotoxic effects of cement dust.

According to the CBMN cyt assay conducted in the present study, mutagenicity increased after exposure to cement dust. This study on the loading and unloading workers exposed to cement dust revealed increased frequencies of genotoxic and cytotoxic end-markers, reflecting the adverse effects of cement dust. Chronic exposure to cement dust led to increased levels of DNA damage and repair inhibition that could be detected in buccal cells.

To suggest the risks related to genotoxicity and cytotoxicity, individuals employed at such occupational environments are suggested to undergo constant bio-monitoring. Awareness of workers of the cellular effects of cement dust should also be raised. Efforts should be made to improve the air quality at these occupational areas. Awareness campaigns regarding the exposure and standardized protective devices must be mandated. Proper training in using such devices is necessary. This would benefit the workers with a better working and healthy conditions in such occupational environments.

**Conflict of Interests:** None declared.

**Financial Support:** None.

## Reference

1. Yahaya T, Okpuzor J, Oladele EO. The Bioprotective Efficacy of Hibiscus sabdariffa (Roselle), Moringa oleifera (Moringa) Zingiberofficinale (Ginger) and Telfairiaoccidentalis ('Ugwu') in the Livers and Kidneys of Rattus norvegicus (Albino rats) Exposed to Cement dust. *IOSR J Environ Sci Toxicol Food Technol* 2013;**7**:24-30.
2. Raajasubramanian D, Sundaramoorthy P, Baskaran L, *et al*. Cement dust pollution on growth and yield attributes of groundnut (Arachishypogaea L). *Int Multidiscip Res J* 2011;**1**:31-6.
3. Sgambato A, Iavicoli I, Goracci M, *et al*. Evaluation of in vitro toxic effects of cement dusts: A preliminary study. *Toxicol Ind Health* 2010;**26**:309-17.
4. Krishna L, Pandarikkal JR, Sreedharan R, *et al*. Assessment of respiratory morbidity among loading and unloading workers exposed to cement dust. *Int J Med Res Health Sci* 2019;**6**:2422-7.
5. Gbadebo AM, Bankole OD. Analysis of potentially toxic metals in airborne cement dust around Sagamu, Southwestern Nigeria. *J Appl Sci* 2007;**7**:35-40.
6. Dietz A, Ramroth H, Urban T, *et al*. Exposure to cement dust, related occupational groups and laryngeal cancer risk: results of a population based case-control study. *Int J Cancer* 2004;**108**:907-11.
7. Knutsson A, Damber L, Järvholm B. Cancers in concrete workers: results of a cohort study of 33 668 workers. *Occup Environ Med* 2000;**57**:264-7.
8. Farheen A, Hazari MA, Khatoon F, *et al*. Hematological parameters are acutely effected by cement dust exposure in construction workers. *Ann Med Physiol* 2017;**1**:31-5.
9. Manjula R, Praveena R, Clevin RR, *et al*. Effects of occupational dust exposure on the health status of portland cement factory workers. *Int J Med Public Health* 2013;**3**:192-6.
10. Climate Change and CDM Cell Rajasthan State Pollution Control Board. Human Health Risk duetoCement Dust Exposure Policy-Brief [Internet]. Vijay S Singh:Environment and Forests, Govt ofRajasthan; c2011. Available from *www.indiaenvironmentportal.org.in/files/RSPCB_CEMENT_DUST_PolicyBrief.pdf* (Accessed June 24, 2019).
11. Sellappa S, Prathyumnan S, Balachandar V. DNA damage induction and repair inhibition among building construction workers in South India. *Asian Pac J Cancer* Prev 2010;**11**:875-80.
12. Al Salhen KS. Assessment of oxidative stress, haematological, kidney and liver function parameters of Libyan cement factory workers. *J Am Sci* 2014;**10**:58-65.
13. Neboh EE, Ufelle SA, Achukwu PU, Udet VC. Cement dust exposure affects haematological parameters in cement loaders in Enugu Metropolis, South-East Nigeria. *Journal of Experimental Research* 2015;**3**:1-4.
14. Katarkar A, Mukherjee S, Khan MH, *et al*. Comparative evaluation of genotoxicity by micronucleus assay in the buccal mucosa over comet assay in

peripheral blood in oral precancer and cancer patients. *Mutagenesis* 2014;**29**:325-34.

15. Bonassi S, Biasotti B, Kirsch-Volders M, *et al*. State of the art survey of the buccal micronucleus assay-a first stage in the HUMN(XL) project initiative. *Mutagenesis* 2009;**24**:295-302.
16. Holland N, Bolognesi C, Kirsch-Volders M, *et al*. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project-perspective on current status and knowledge gaps. *Mutat Res* 2008;**659**:93-108.
17. Fenech M, Morley AA. Measurement of micronuclei in Lymphocytes. *Mutat Res* 1985;**147**:29-36.
18. Mehrotra R, Yadav S. Oral squamous cell carcinoma: etiology, pathogenesis and prognostic value of genomic alterations. *Indian J Cancer* 2006;**43**:60-6.
19. AndishehTadbir A, Mehrabani D, Heydari ST. Primary malignant tumors of orofacial origin in Iran. *J Craniofac Surg* 2008;**19**:1538-41.
20. Majer BJ, Laky B, Knasmüller S, Kassie F. Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. *Mutat Res* 2001;**489**:147-72.
21. Sarto F, Tomanin R, Giacomelli L, *et al*. Evaluation of chromosomal aberrations in lymphocytes and micronuclei in lymphocytes, oral mucosa and hair root cells of patients under antiblastic therapy. *Mutat Res* 1990;**228**:157-69.
22. Fenech M. Cytokinesis blocked micronucleus cytome assay. *Nat Protoc* 2000;**2**:1084-104.
23. Sudha S, Kripa SK, Shibily P, *et al*. Biomonitoring of genotoxic effects among shielded manual metal arc welders. *Asian Pac J Cancer Prev* 2011;**12**:1041-4.
24. Sudha S, Shibily P, Shyn J, Kripa SK. Micronucleus Test in Exfoliated Buccal Cells from Chromium Exposed Tannery Workers. *Int J Biosci Biochem Bioinforma* 2011;**1**:58-62.
25. Shaikh A, Chauhan K, Patel D, Chandel D. Evaluation of buccal cell cytotoxicity in gutkha/pan-masala chewers and bidi smokers. *Indian Journal of Applied Research* 2016:6(11). Available from *www.worldwidejournals.com/indian-journal-of-applied-research-(IJAR)/fileview/November_2015_1492508404__125.pdf* (Accessed Jun 20, 2019).
26. Thomas P, Holland N, Bolognesi C, *et al*. Buccal micronucleus cytome assay. *Nat Protoc* 2009;**4**:825-37.
27. Shi Q, King RW. Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines. *Nature* 2005;**437**:1038-42.
28. Thomas P, Fenech M. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. *Mutagenesis* 2008;**23**:57-65.
29. Celik A, Cavas T, Ergene-Gozukara S. Cytogenetic biomonitoring in petrol stations attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis* 2003;**18**:417-21.
30. Knasmueller S, Holland N, Wultsch G, *et al*. Use of nasal cells in micronucleus assays and other genotoxicity studies. *Mutagenesis* 2011;**26**:231-8.
31. Bolognesi C, Knasmueller S, Nersesyan A, *et al*. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay - an update and expanded photogallery. *Mutat Res* 2013;**753**:100-13. doi: 10.1016/j.mrrev.2013.07.002
32. Shaikh A, Barot D, Chandel D. Genotoxic effects of exposure to gasoline fumes on petrol pump workers. *Int J Occup Environ Med* 2018;**9**:1159-79.
33. Carlin V, Matsumoto MA, Saraiva PP, *et al*. Cytogenetic damage induced by mouthrinses formulations in vivo and in vitro. *Clin Oral Investig* 2012;**16**:813-20.
34. Lorenzoni DC, Fracalossi AC, Carlin V, *et al*. Mutagenicity and cytotoxicity in patients submitted to ionizing radiation. *Angle Orthod* 2013;**83**:104-9.
35. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res* 1992;**271**:69-77.
36. Lorenzoni DC, Pinheiro LP, Nascimento HS, *et al*. Could formaldehyde induce mutagenic and cytotoxic effects in buccal epithelial cells during anatomy classes? *Med Oral Patol Oral Cir Bucal* 2017;**22**:58-63.
37. Burmeister B, Schwerdtle T, Poser I. Effects of asbestos on initiation of DNA damage, induction of DNA-strand breaks, P53-expression and apoptosis in primary, SV40-transformed and malignant human mesothelial cells. *Mutat Res* 2004;**558**:81-92.
38. Shimizu N. Molecular mechanisms of the origin of micronuclei from extrachromosomal elements. *Mutagenesis* 2011;**26**:119-23.