Original Article

Evaluation of DNA Damage in Peripheral Blood Leukocytes in Oral Potentially Malignant and Malignant Disorders by Comet Assay

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Abstract

Purpose: Oral squamous cell carcinoma (OSCC) and potentially malignant disorders (PMD) are associated with DNA damage which can be caused by exposure to carcinogens, genotoxins, or increased oxidative stress. Early detection and assessment of the amount of DNA damage using a biomarker such as a comet assay can prove to be extremely beneficial for the patients. The present study evaluated the efficacy of comet assay in assessing DNA damage in peripheral blood leukocytes (PBLs) in oral potentially malignant and malignant disorders. **Materials and Methods:** The study included fifty-five patients each of leukoplakia, oral submucous fibrosis (OSMF), and OSCC along with fifty-five healthy individuals as control. The patients with deleterious oral habits were categorized into smokeless, smoked, and mixed habit groups. DNA damage was evaluated by measuring the mean tail length (μm). **Results:** An increased mean tail length (μm) and higher DNA damage were found in OSCC (22.4335 ± 1.52341), and there was a progressive stepwise increase in mean tail length from control (6.8307 ± 0.84261) to PMD (leukoplakia [13.0022 ± 0.74316]; OSMF [10.6085 ± 0.88140]) to OSCC. Although there was a significant increase in the DNA damage in different habit groups (smokeless [14.9380 ± 5.18516]; smoked [15.4947 ± 4.59589], and mixed [16.3650 ± 5.62407]) compared to controls, there was no significant difference between the habit groups. **Conclusion:** Thus, comet assay technique can be used as a sensitive and reliable indicator for DNA damage evaluation.

Keywords: *Comet assay, DNA damage, oral squamous cell carcinoma, peripheral blood leukocytes*

Introduction

In 2005, the World Health Organization (WHO) recommended that the term "potentially malignant disorders (PMDs)" may be used as not all the lesions and conditions described under this term may transform to cancer.[1,2] The most common PMDs affecting the oral cavity include leukoplakia, erythroplakia, and oral submucous fibrosis (OSMF).[1,2] Almost 30%–80% of the oral malignancies arise from PMDs such as leukoplakia and OSMF. Malignant transformation rates for leukoplakia and OSMF range from 0.13% to 17.5% and 2.3% to 7.6%, respectively.[3] Oral squamous cell carcinoma (OSCC) is the most common malignant disorder of the oral cavity, accounting for over 90% of all malignant neoplasms in this region.[4-6]

OSCC has a multifactorial etiology. The various factors implicated in the etiopathogenesis of OSCC include smoking, chewing betel quid/tobacco, and alcohol intake separately or synergistically, viruses, genetic factors, environmental factors, and gene-environment interactions.[7] The above-mentioned deleterious habits may induce oxidative stress or generation of reactive oxygen species (ROS) which leads to cellular damage, as well as DNA damage.[8,9] The DNA damage may occur in the form of DNA breaks, double-strand breaks (DSB) or single-strand breaks (SSBs), alkali-labile sites (ALS), and micronucleus formation which elevate chromosomal aberrations.[10]

Various methods for evaluation of DNA damage known as genotoxicity assays used in the past include single cell gel electrophoresis (SCGE) or comet assay and micronucleus assay (MN assay). Among these, comet assay (SCGE) is widely accepted as an *in vitro* and *in vivo* genotoxicity test.[9,11]

The comet assay or SCGE is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells.[12-14] The assay was first developed by Ostling and Johansson in 1984 and later modified

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by Singh *et al*. in 1988. It depends on the partial unwinding of the supercoiled DNA in agarose-embedded microscopic slides, which allows the DNA to be drawn out toward the anode under electrophoresis, forming "comet-like" images as seen under fluorescence microscopy. The relative amount of DNA in the comet tail indicates DNA break frequency.[13,15] A cell with DNA damage appears as a "comet", and undamaged cell appears as a "halo." The head of the comet is composed of intact DNA while the tail is composed of damaged DNA (SSBs or DSBs). The comet tail length is directly proportional to the amount of DNA damage.[16] This assay can be used on any eukaryotic cell type that can be obtained as a single cell or nuclear suspension.[13] Many genotoxicity tests evaluate the tissue thought to be the primary site of metabolism lymphocytes are the most commonly used cells in comet assay to assess DNA damage.

The aim of the present study was to evaluate the efficacy of comet assay in assessing DNA damage in peripheral blood leukocytes (PBLs) in oral potentially malignant and malignant disorders.

Materials and Methods

Ethics

This study was conducted in the Department of Oral Pathology and Microbiology, Maulana Azad Institute of Dental Sciences, New Delhi, India. The study was approved by the Institutional Ethical Committee Board.

Study design

This prospective study was designed to evaluate the efficacy of comet assay in PBLs in assessing DNA damage in oral potentially malignant and malignant disorders.

Patient selection

Clinically and histopathologically confirmed patients with leukoplakia, OSMF, and OSCC in the age range from 18 to 80 years were included in the study. The patients were selected after obtaining written informed consent from each. A total of 220 patients with leukoplakia (Group B; $n = 55$), OSMF (Group C; $n = 55$), and oral squamous cell carcinoma (Group D; $n = 55$) along with healthy age- and sex-matched individuals (control, Group A; $n = 55$) were included in the study.

The patients with habits were categorized into three habit groups:
Smokeless (consuming tobacco, gutkha, pan, or supari), smoked (consuming cigarette or bidi), and mixed (both smoked and smokeless forms).

Patients not willing to participate, suffering from any infectious or contagious disease, with any other white patch such as candidiasis, oral lichen planus and lichenoid reaction and previous history of surgery, radiotherapy or chemotherapy, or any vitamin, or dietary supplement use were not included in the study.

Sample collection

Three milliliters of peripheral blood were withdrawn from each patient and controls under aseptic conditions in sterile tubes containing EDTA anticoagulant. Blood samples were stored at −80ºC till used.

Comet assay

Slides were dipped in 1% normal melting point agarose (NMPA) and allowed to dry at 37°C. Once NMPA solidified, 80 µl of peripheral blood was diluted with 80 µl of (×1) phosphate buffered saline and added to a microcentrifuge tube containing an equal volume (160 µl) of low-melting-point agarose (LMPA, 37°C). This mixture of gel (LMPA) and blood was placed over the previously NMPA-coated slides. A coverslip was placed carefully over the slide so that a uniform layer over the NMPA coat is formed avoiding trapping of air bubbles. The slides were kept over an ice pack to solidify the gel for 10–15 min. Then, the coverslip was carefully removed, and 100 µl of LMPA was added over the gel mixture layer. A fresh coverslip was placed and again kept over an ice pack to solidify the gel for 10–15 min. The coverslip was finally removed, and the slide was dipped into lysis buffer solution and refrigerated for 24 h. After lysis at 4°C, DNA was allowed to unwind under alkaline conditions. The slides were allowed to stay in the cold (4°C) alkaline buffer (pH >13) for 20 min to unwind DNA strands and expose the ALS (alkali unwinding). Electrophoresis was performed for 20 min under alkaline conditions in the refrigerator (4°C) at 280 mA and 24 V (~0.74 V/cm). The slides were gently picked up from the alkaline electrophoresis buffer and placed on a staining tray. Then, the slides were carefully flooded three times with a neutralizing buffer (pH 7.5) for 5 min each. Following neutralization, the slides were stained using staining solution of ethidium bromide. The DNA comets were visualized using a fluorescent microscope. The images were captured, and measurements of tail lengths were done using Image J software (NIH, MD, USA). A total of 100 randomly selected cells per slide were analyzed. The DNA damage was evaluated by calculating the length of the comet tail for each cell, and the mean tail length was assessed.

Statistical analysis

The mean values, standard deviation, and ranges (maximum and minimum) were calculated for each variable. The resulting data were analyzed using SPSS software, version 20 (Armonk, NY: IBM Corp). Data were expressed as a mean ± standard deviation. Differences between different variables were analyzed using parametric Student's *t*-test and analysis of variance (ANOVA). The correlation was calculated

using the Pearson's correlation. A value of $P \leq 0.05$ was considered to be statistically significant.

Results

The peripheral blood samples were collected from a total of 220 individuals which included 55 patients each of leukoplakia, OSMF, OSCC, and age- and sex-matched healthy controls. Distribution of patients and controls according to age, gender, and habit was studied [Table 1].

Comet assay was performed on PBLs of all the participants. The DNA damage (mean tail length) in the PBLs in leukoplakia, OSMF, OSCC, and control groups was assessed, and results of each are illustrated in Figures 1-4. The mean tail length (μm) of PBLs in leukoplakia, OSMF, and OSCC was compared with control [Table 2].

Comparison of mean tail length of PBLs in study groups and controls was done using ANOVA test [Table 3].

Comparison of mean tail length of PBLs between different habit groups and control was done using Student's *t*-test [Table 4].

Comparison of mean tail length of PBLs between different habit groups was carried out using ANOVA [Table 5].

Table 1: Demographic data of patients included in the study

Group	No. of patients	Age	Gender (M:F)	Habit
Group A (Control)	55	15-76	41:14	-
Group B (Leukoplakia)	55	20-70	50:5	Mixed-14 Smoked-14 Smokeless-27
Group C (OSMF)	55	18-73	39:16	Mixed-10 Smokeless-45
Group D (OSCC)	55	28-80	44:11	Mixed-16 Smoked-5 Smokeless-34

Table 2 Comparison of mean tail length of PBLs between different study groups

Control	Study groups mean tail length (μm) (mean±standard deviation)		*P*
Group A (6.8307±0.84261)	Group B (13.0022±0.74316)	0.000	*P*<0.05 is considered as statistically significant
	Group C (10.6085±0.88140)	0.000	
	Group D (22.4335±1.52341)	0.000	

On comparing the mean tail length of peripheral blood leucocytes by Student t-test between various study groups, it was found to be highest for OSCC followed by Leukoplakia and OSMF compared to the control. Highly significant difference was obtained between the study groups and control.

Table 3: *P* values obtained by comparison of mean tail length of PBLs between different study groups

	Group B	Group C	Group D
Group B	-	0.000	0.000
Group C	0.000	-	0.000
Group D	0.000	0.000	-

P≤0.05 was considered statistically significant. On comparing the mean tail length of peripheral blood leucocytes between various study groups by one way ANOVA, highly significant difference was obtained between all the study groups

Discussion

Prolonged exposure to carcinogenic agents induces oxidative stress or ROS generation that is genotoxic and cytotoxic to human cells can cause damage leading to genetic alterations. Accumulation of these genetic alterations may initiate the development of premalignant disorders and subsequently OSCC.[8] When the amount of ROS generated in the cells is high, it leads to cellular damage, as well as DNA damage.[8,9] The DNA damage may occur in the form of DNA breaks, DSB or SSBs, ALS, and micronucleus formation which elevate chromosomal aberrations.[10] The ROS also affect the DNA repair mechanisms essential for maintenance of DNA integrity and prevention of cancer.[8] Thus, the progression of OSCC from PMDs is a multistep process.[3,5] The common PMDs such as leukoplakia and OSMF have malignant transformation rates in the range of 0.13%–17.5% and 2.3%–7.6%, respectively.[3]

The comet assay or SCGE has become increasing popular in assessing the DNA damage due to its rapidity, sensitivity, inexpensiveness, and requirement of little biological material.[17-20] Comet assay depends on the partial unwinding of the supercoiled DNA in agarose-embedded microscopic slides, which allows the DNA to be drawn out toward the anode under electrophoresis, forming "comet-like" images as seen under fluorescence microscopy.[13,15] A cell with DNA damage appears as a "comet" and undamaged cell appears as a "halo". The head of the comet is composed of intact DNA while the tail is composed of damaged DNA (SSBs or DSBs). The comet tail length is directly proportional to the amount of DNA damage.[21] The most commonly used cells in human biomonitoring studies are peripheral blood lymphocytes.

The age of patients in the study ranged from 18 to 80 years with the mean age for controls, leukoplakia, OSMF, and OSCC being 36.33 years, 43.54 years, 38.95 years, and 49.05 years, respectively. About 79% of the patients included in the study were males. Other studies had a comparable demographic profile with a predominance of males.[3,7]

Among the patients in the study groups, there was a predominance of smokeless tobacco usage (48.2%) among the study groups, with mixed tobacco (both smokeless and

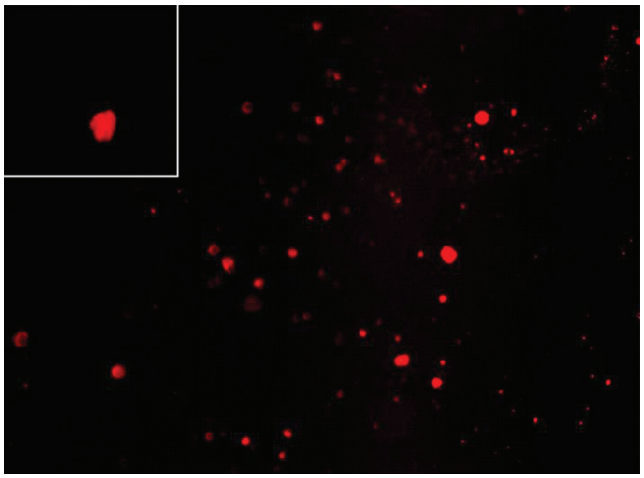

Figure 1: Nonfragmented and undamaged DNA in peripheral blood leukocytes of control

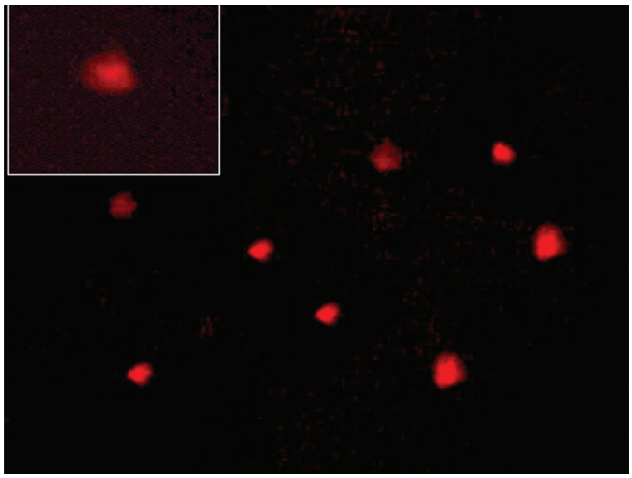

Figure 2: DNA damage in the form of comet in peripheral blood leukocytes of leukoplakia

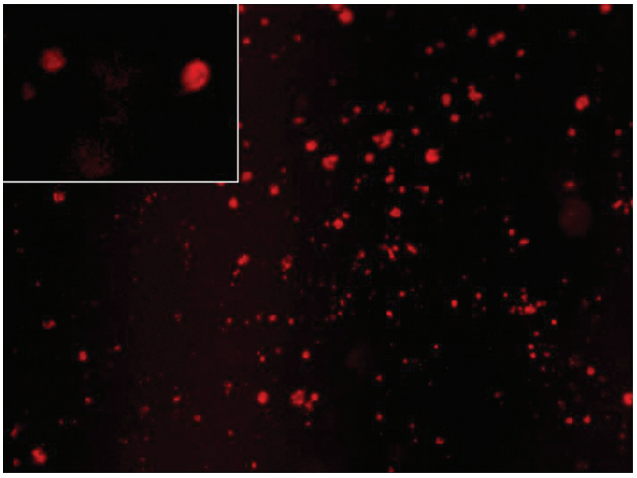

Figure 3: DNA damage in peripheral blood leukocytes of oral submucous fibrosis

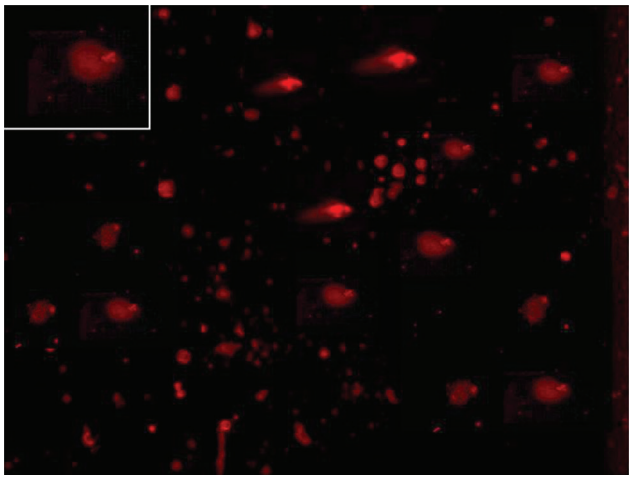

Figure 4: DNA damage in the form of comet in peripheral blood leukocytes of oral squamous cell carcinoma

Table 4: Comparison of mean tail length of PBLs between different habit groups and control

Control	Habit groups mean tail length (μm) (mean±standard deviation)		*P*
Group A (6.8307±0.84261)	Smokeless (14.9380±5.18516)	0.000	*P*<0.05 is considered as statistically significant
	Smoked (15.4947±4.59589)	0.000	
	Mixed (16.3650±5.62407)	0.000	

On comparing the mean tail lengths of peripheral blood leucocytes by Student t-test between various habit groups, the tail lengths were significantly higher in the habit groups compared to control.

smoked), and smoked form accounting for 18.2% and 8.6% of the patients, respectively.

It was found that the mean tail length (μm) was significantly increased in OSCC (22.4335 ± 1.52341), leukoplakia (13.0022 ± 0.74316), and OSMF (10.6085 ± 0.88140) compared to controls (6.8307 ± 0.84261 μm). The increased comet tail lengths in all study groups compared to controls depicts the presence of DNA damage in the PBLs of these patients. DNA damage can occur in the form of SSBs, ALS, and cross-linking.[22] The generation of ROS and exposure to genotoxins causes DNA breaks and reduced DNA repair capacity. These genotoxins attack different sites on the DNA leading to the accumulation of DNA damage.[23] During oxidative stress, the damage is characterized by the presence of oxidized purines or pyrimidines. All these significantly contribute to increase in DNA damage which increases the risk of cancer.[18,24]

The amount of DNA damage was greatest in OSCC patients as this group had the maximum mean tail length followed by leukoplakia and OSMF. There was a significant stepwise increase in DNA damage in the PBLs from control to precancer patients and from precancer to oral cancer patients. The DNA repair systems protect the integrity of the genome so that any deficiency in DNA

Table 5: *P* values obtained by comparison of mean tail length of PBLs between different habit groups

	Smokeless	Smoked	Mixed
Smokeless	-	0.662	0.150
Smoked	0.662	-	0.560
Mixed	0.150	0.560	-

$P \leq 0.05$ was considered statistically significant. ANOVA was performed to compare the mean tail length of peripheral blood leucocytes between various habit groups. No statistically significant difference was found between the tail lengths in various habit groups

repair leads to increased DNA damage and development of cancer.[25]

Similar to our results, Mukherjee *et al*. found that the mean (± standard deviation [SD]) tail length of OSCC (24.95 ± 5.09 μm) and leukoplakia (12.96 ± 2.68 μm) was significantly greater than in controls (8.54 ± 2.55 μm, $P < 0.05$). In leukoplakia, the mean (± SD) tail length was significantly greater as compared to OSMF (11.03 ± 5.92 μm).[7] Our study also showed similar results. Thus, the DNA damage in blood cells as measured by comet assay is significantly greater in oral cancer and leukoplakia as compared to OSMF.

Various other authors in the past have obtained comparable results. These studies have shown that comet tail length was highest in OSCC patients, lesser in leukoplakia, OSMF patients, and lowest in controls.[3,26] Vellappally *et al*. in their study demonstrated that the mean tail length for leukoplakia patients with moderate-to-severe dysplasia (1.25 ± 0.14 μm) was significantly more than controls (0.31 ± 0.10 μm). Thus, the DNA damage in blood cells evaluated by SCGE is greater in leukoplakia than in controls, and deleterious oral habits are associated with greater DNA damage.[27]

Cancer patients have maximum DNA damage as depicted by the greatest mean tail length of the comet in lymphocytes. This has been observed in cancers other than those of the oral cavity.[25,28-31] Udumudi *et al*. in cervical cancer patients and Lou *et al*. in lung cancer patients found significantly higher mean tail moment compared to controls.[32,33] Likewise, the comet assay results in patients with squamous cell carcinoma of the head and neck were similar.[34]

On comparing the mean tail length of PBLs in different habit groups with the control group of no habit, highly significant results were obtained in this study.

Guttikonda *et al*. found similar results wherein the mean tail length was highest in tobacco habituated patients with OSCC (25.375 μm) followed by tobacco habituation but with clinically normal mucosa (2.5833 μm), and it was least in healthy individuals (2.18 μm).[35] Many other investigators have investigated the effect of habit on mean tail length and have obtained comparable results.[11,36]

Tobacco smoking and smokeless tobacco are important etiologic factors leading to oral cancer. These products are composed of carcinogens such as polyaromatic hydrocarbons nitrosamines and aromatic amines. These carcinogenic agents after deactivation in the liver are converted into electrophilic intermediates which in turn react with DNA to form covalently bound adducts. The formation of DNA adducts and the resulting mutations are responsible for oncogene activation and inactivation of tumor suppressor genes, leading to cancer. Few authors have reported the presence of these DNA adducts in smokers.[23,37,38]

On the contrary, Hoffmann and Speit showed no significant difference in DNA damage between smokers and nonsmokers. It was concluded that cigarette smoking had no effect on the amount of DNA damage in peripheral blood cells.[39] Further, Betti *et al*. proved that there was no correlation between comet tail length in smokers and the number of cigarette smoked per day.[16] No correlation between the length of the comet and the number of cigarettes or the frequency of smoking was detected by Frenzilli *et al*.[40] and Mohankumar *et al*.[38] These findings can be explained by assuming that the single DNA strand breaks can be induced in leukocytes also by free radicals generated due to the inflammation normally present in smokers. This reaction is independent of the amount of cigarette smoked and is related to individual susceptibility. The DNA SSBs induced by agents such as hydrogen peroxide are quickly repaired.[16,38]

Conclusion

The present study attempted to evaluate the efficacy of comet assay in assessing the DNA damage in PBLs in oral potentially malignant and malignant disorders. An increased tail length and higher DNA damage were associated with OSCC, and a progressive stepwise increase in tail length was observed from control to PMD to OSCC. Our study has shown promising results, and hence, comet assay of PBLs can be used effectively in early detection of oral PMDs and malignant disorders.

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Conflicts of interest

There are no conflicts of interest.

References

1. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007;36:575-80.
2. Yardimci G, Kutlubay Z, Engin B, Tuzun Y. Precancerous lesions of oral mucosa. World J Clin Cases 2014;2:866-72.
3. Katarkar A, Mukherjee S, Khan MH, Ray JG, Chaudhuri K. 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.

4. Markopoulos AK. Current aspects on oral squamous cell carcinoma. Open Dent J 2012;6:126-30.
5. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: Implications for therapy. J Dent Res 2008;87:14-32.
6. Carvalho AL, Singh B, Spiro RH, Kowalski LP, Shah JP. Cancer of the oral cavity: A comparison between institutions in a developing and a developed nation. Head Neck 2004;26:31-8.
7. Mukherjee S, Ray JG, Chaudhuri K. Evaluation of DNA damage in oral precancerous and squamous cell carcinoma patients by single cell gel electrophoresis. Indian J Dent Res 2011;22:735-6.
8. Jyoti S, Khan S, Naz F, Rahul, Ali F, Siddique YH. Assessment of DNA damage by panmasala, gutkha chewing and smoking in buccal epithelial cells using alkaline single cell gel electrophoresis (SCGE). Egypt J Med Hum Genet 2013;14:391-4.
9. Speit G, Witton-Davies T, Heepchantree W, Trenz K, Hoffmann H. Investigations on the effect of cigarette smoking in the comet assay. Mutat Res 2003;542:33-42.
10. Tsai YS, Lee KW, Huang JL, Liu YS, Juo SH, Kuo WR, *et al.* Arecoline, a major alkaloid of areca nut, inhibits p53, represses DNA repair, and triggers DNA damage response in human epithelial cells. Toxicology 2008;249:230-7.
11. Rojas E, Valverde M, Sordo M, Ostrosky-Wegman P. DNA damage in exfoliated buccal cells of smokers assessed by the single cell gel electrophoresis assay. Mutat Res 1996;370:115-20.
12. Rojas E, Lopez MC, Valverde M. Single cell gel electrophoresis assay: Methodology and applications. J Chromatogr B Biomed Sci Appl 1999;722:225-54.
13. Azqueta A, Collins AR. The essential comet assay: A comprehensive guide to measuring DNA damage and repair. Arch Toxicol 2013;87:949-68.
14. Monteith DK, Vanstone J. Comparison of the microgel electrophoresis assay and other assays for genotoxicity in the detection of DNA damage. Mutat Res 1995;345:97-103.
15. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91.
16. Betti C, Davini T, Giannessi L, Loprieno N, Barale R. Comparative studies by comet test and SCE analysis in human lymphocytes from 200 healthy subjects. Mutat Res 1995;343:201-7.
17. Faust F, Kassie F, Knasmüller S, Boedecker RH, Mann M, Mersch-Sundermann V, *et al.* The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. Mutat Res 2004;566:209-29.
18. Collins AR. Investigating oxidative DNA damage and its repair using the comet assay. Mutat Res 2009;681:24-32.
19. Møller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidemiol Biomarkers Prev 2000;9:1005-15.
20. Nandhakumar S, Parasuraman S, Shanmugam MM, Rao KR, Chand P, Bhat BV, *et al.* Evaluation of DNA damage using single-cell gel electrophoresis (Comet assay). J Pharmacol Pharmacother 2011;2:107-11.
21. Zainol M, Stoute J, Almeida GM, Rapp A, Bowman KJ, Jones GD, *et al.* Introducing a true internal standard for the Comet assay to minimize intra- and inter-experiment variability in measures of DNA damage and repair. Nucleic Acids Res 2009;37:e150.
22. Liao W, McNutt MA, Zhu WG. The comet assay: A sensitive method for detecting DNA damage in individual cells. Methods 2009;48:46-53.
23. Guttikonda VR, Patil R, Kumar G. DNA damage in peripheral blood leukocytes in tobacco users. J Oral Maxillofac Pathol 2014;18:S16-20.
24. Collins AR. Measuring oxidative damage to DNA and its repair with the comet assay. Biochim Biophys Acta 2014;1840:794-800.
25. Iwakawa M, Goto M, Noda S, Sagara M, Yamada S, Yamamoto N, *et al.* DNA repair capacity measured by high throughput alkaline comet assays in EBV-transformed cell lines and peripheral blood cells from cancer patients and healthy volunteers. Mutat Res 2005;588:1-6.
26. Saran R, Tiwari RK, Reddy PP, Ahuja YR. Risk assessment of oral cancer in patients with pre-cancerous states of the oral cavity using micronucleus test and challenge assay. Oral Oncol 2008;44:354-60.
27. Vellappally S, Binmgren MA, Huraib SB, Hashem MI, Patil S, Anil S, *et al.* Assessment of DNA damage in leukoplakia patients with different degrees of dysplasia. J Contemp Dent Pract 2015;16:971-6.
28. Rajeswari N, Ahuja YR, Malini U, Chandrashekar S, Balakrishna N, Rao KV, *et al.* Risk assessment in first degree female relatives of breast cancer patients using the alkaline comet assay. Carcinogenesis 2000;21:557-61.
29. Zhang H, Buchholz TA, Hancock D, Spitz MR, Wu X. Gamma-radiation-induced single cell DNA damage as a measure of susceptibility to lung cancer: A preliminary report. Int J Oncol 2000;17:399-404.
30. Baltaci V, Kayikçioğlu F, Alpas I, Zeyneloğlu H, Haberal A. Sister chromatid exchange rate and alkaline comet assay scores in patients with ovarian cancer. Gynecol Oncol 2002;84:62-6.
31. Vasavi M, Vedicherala B, Vattam KK, Ahuja YR, Hasan Q. Assessment of genetic damage in inflammatory, precancerous, and cancerous pathologies of the esophagus using the comet assay. Genet Test Mol Biomarkers 2010;14:477-82.
32. Udumudi A, Jaiswal M, Rajeswari N, Desai N, Jain S, Balakrishna N, *et al.* Risk assessment in cervical dysplasia patients by single cell gel electrophoresis assay: A study of DNA damage and repair. Mutat Res 1998;412:195-205.
33. Lou J, He J, Zheng W, Jin L, Chen Z, Chen S, *et al.* Investigating the genetic instability in the peripheral lymphocytes of 36 untreated lung cancer patients with comet assay and micronucleus assay. Mutat Res 2007;617:104-10.
34. Saha DT, Davidson BJ, Wang A, Pollock AJ, Orden RA, Goldman R, *et al.* Quantification of DNA repair capacity in whole blood of patients with head and neck cancer and healthy donors by comet assay. Mutat Res 2008;650:55-62.
35. Szeto YT, Benzie IF, Collins AR, Choi SW, Cheng CY, Yow CM, *et al.* A buccal cell model comet assay: Development and evaluation for human biomonitoring and nutritional studies. Mutat Res 2005;578:371-81.
36. Dhawan A, Mathur N, Seth PK. The effect of smoking and eating habits on DNA damage in Indian population as measured in the comet assay. Mutat Res 2001;474:121-8.
37. Jones NJ, McGregor AD, Waters R. Detection of DNA adducts in human oral tissue: Correlation of adduct levels with tobacco smoking and differential enhancement of adducts using the butanol extraction and nuclease P1 versions of 32P postlabeling. Cancer Res 1993;53:1522-8.
38. Mohankumar MN, Janani S, Prabhu BK, Kumar PR, Jeevanram RK. DNA damage and integrity of UV-induced DNA repair in lymphocytes of smokers analysed by the comet assay. Mutat Res 2002;520:179-87.
39. Hoffmann H, Speit G. Assessment of DNA damage in peripheral blood of heavy smokers with the comet assay and the micronucleus test. Mutat Res 2005;581:105-14.
40. Frenzilli G, Betti C, Davini T, Desideri M, Fornai E, Giannessi L, *et al.* Evaluation of DNA damage in leukocytes of ex-smokers by single cell gel electrophoresis. Mutat Res 1997;375:117-23.