INTRODUCTION

Microbial dental plaque causes chronic gingivitis, which is the inflammation of gingiva; without loss of tooth supporting structures or with reduced but with stable periodontium. The treatment of chronic gingivitis depends on the control of dental plaque.[1-4]

Dental plaque formation is prevented by mechanical and chemical plaque control measures. Mechanical plaque control methods include toothbrushing and interdental cleaning aids. Mechanical plaque control measures are sometimes inadequate either due to noncompliance of patient or due to improper technique.[5] Chemical plaque control measures such as mouthrinse are commonly prescribed in clinical practice as an adjunct to mechanical plaque control measures in dental caries, gingivitis, periodontitis and halitosis.[6]

Chlorhexidine (CHX) is the most commonly prescribed mouthrinse in clinical practice and is the gold standard among all the chemical plaque control agents known.[7] CHX was initially used as general disinfectant for washing operation sites in surgery, gynecology, and ophthalmology.[8] Dental plaque inhibition by CHX was first reported by Schroeder[9] in 1969, but CHX was established as an antiplaque and antigingivitis agent in humans by Loe and Schiott in 1970.[10]

The antiplaque action of chlorhexidine is attributed owing to strong attraction between...
positively charged chlorhexidine and negatively charged oral bacteria. CHX causes altered cell permeability and leakage of potassium and phosphorous ions in oral bacteria at low concentration, whereas cytolysis and cell death at high concentration.[11-13]

CHX is available in three formulations; digluconate, acetate, and hydrochloride. The digluconate is water soluble and is most common formulation. The clinical effectiveness of CHX have been proved in several trials,[17,19] however, it has side effects such as discoloration and staining of teeth and oral mucosa, taste alteration, oral erosions, and parotid swelling.[16]

Further, CHX has cytotoxic effect on gingival fibroblasts, epithelial cells, neutrophils, and red blood cells[17-19] and also parachloroaniline, a degradation product of chlorhexidine is mutagenic.[20]

Several biomarkers are used to study the effect of various genotoxic agents on oral epithelial cells in malignant transformation and various biomarkers are used in biomonitoring studies to assist in diagnosis, staging, and evaluation of the risk assessment. The biomarkers give insight in the genetic and molecular transformation which are taking place in the process of oral carcinogenesis[21].One of the most simple and effective biomarker for assessing neoplastic progression in oral mucosal cells is micronuclei.[22] Micronuclei are small cytoplasmic chromatin masses which appear as small nuclei in the cell adjacent to the main nucleus and are formed due to chromosomal damage in basal epithelial cells or due to defect in spindle apparatus during the cell division.[23] There is increase in number of micronuclei in oral epithelial cells when there is malignant transformation from normal mucosa to precancerous and cancerous lesion.[24] The aim of the study was to assess the genotoxic effect of CHX mouthrinse on exfoliated buccal epithelial cells in chronic gingivitis patients using micronucleus (MN) test.

**MATERIALS AND METHODS**

The study was carried out in patients reporting to the outpatient of Department of Periodontology in collaboration of Interdisciplinary Biotechnology Unit of our Institute.

**Inclusion criteria**

Systemically healthy chronic gingivitis patients who had periodontal probing depth of 3 mm or less using William’s Periodontal probe (Silverline, Hufriedy, USA) and gave their written consent were recruited in the study.

**Exclusion criteria**

1. Patients with history of any systemic disease were excluded from the study
2. Smokers, tobacco and pan masala chewers and alcoholics were excluded from the study
3. Patients with dental caries and dental restorations and orthodontic appliances were excluded from the study
4. Patient having any oromucosal lesions, viral diseases, recent vaccinations for the last six were excluded from the study
5. Patient who undergone any radiodiagnostic examinations in the last six months

The patients enrolled in the study were divided into two groups. Group A or controls: Chronic gingivitis patients (n = 101) who were exclusively on mechanical plaque control without any adjunctive chemical plaque control measures.

Group B or cases: Chronic gingivitis patients (n = 255) on mechanical plaque control along with adjunct 0.2% CHX mouthwash (aqueous base).

The Group B was further divided into five subgroups based on duration of use of CHX mouthwash as B1 (n = 51): Chronic gingivitis patients on mechanical plaque control along with CHX mouthwash (0.2%) as adjunct for ≤1 week, B2 (n = 51): Chronic gingivitis patients on mechanical plaque control along with CHX mouthwash (0.2%) as adjunct for more than 1 week but ≤2 weeks, B3 (n = 51): Chronic gingivitis patients on mechanical plaque control along with CHX mouthwash (0.2%) as adjunct for more than 2 weeks but ≤4 weeks, B4 (n = 51): Chronic gingivitis patients on mechanical plaque control along with CHX mouthwash (0.2%) as adjunct for more than 4 weeks but ≤12 weeks, B5 (n = 51): Chronic gingivitis patients on mechanical plaque control along with CHX mouthwash (0.2%) as adjunct for more than 12 weeks but ≤24 weeks.

Plaque accumulation and gingival inflammation were measured using plaque index by Silness and Löe,[13] and Gingival index by Loe and Silness,[25] on six index teeth, namely, maxillary right first molar (16), maxillary right lateral incisor (12), maxillary left first premolar (24), mandibular left first molar (36), mandibular left lateral incisor (32), and mandibular right first premolar (44). If any of these index teeth was missing, then whole mouth index was recorded. The four gingival areas of the tooth were examined for scoring, namely, distofacial, midfacial, mesiofacial, and lingual or palatal. The plaque score and gingival score for individual tooth were calculated by adding all the scores from four gingival areas of tooth and then dividing by four. The individual tooth score was added and divided by number of teeth examined to give the plaque index and gingival index, respectively, for a particular person. The mouth mirror, William periodontal probe, light source were used for scoring. The gingival was air-dried with cotton roll or blast of air before proceeding for scoring.

**Micronucleus test**

Buccal mucosal cell collection

Soft toothbrush was used for collection of buccal epithelial cells from each participant by gently scraping inside of the cheek. This toothbrush was then swirled into a test tube containing the buffer solution resulting into a cell suspension.[26]

**Preparation of buffer solution**

0.1M ethylenediaminetetraacetic acid (EDTA), 0.01M Tris HCl, and 0.02M NaCl were dissolved in 1 liter of autoclaved distilled water. The pH of the buffer was adjusted to 7.0 with NaOH.[26]

**Chemicals used**

Tris hydrochloride (SRL, India), EDTA from (SRL, India), Giemsa stain, sodium chloride, methanol, glycerol, and sodium hydroxide pellets (Merck, India).

**Procedure**

Epithelial cells collected from the oral mucosa were in the test tube and were washed twice by centrifugation (Sigma 3-30K
Refrigerated High-Speed Centrifuge) at 8000 rpm for 5 min using the above-mentioned buffer solution.\[27\] This step of washing inactivated the endogenous DNAses, removed bacterial load and cell debris that would otherwise complicate the scoring procedure.\[28\] These cells were then smeared on to clean preheated microscope glass slides and allowed to air dry. The cells were fixed with cold methanol (100%). The slides were kept at 37°C overnight and then stained with 5% Giemsa stain.\[27,28\]

These slides are observed under microscope (Motic B1-220/223 Educational Compound Microscope) to screen 2000 nucleated cells per individual for the presence of MN at the magnification of 100X.\[26\]

The micronucleated cells were identified as DNA-containing structures which were separated from the main nucleus and had the following characteristics:\[26\]
1. Rounded smooth perimeter suggestive of a membrane
2. The diameter was less than a third (1/3\(^{rd}\)) the diameter of the associated nucleus, but large enough to discern shape and color
3. The staining intensity of MN was similar to that of the nucleus
4. The texture of MN was similar to that of nucleus
5. The MN had the same focal plane as the nucleus without overlap or bridge to the associated nucleus.

If a cell contained one MN, it was designated as micronucleated cell with one micronuclei, and if the cell contained two micronuclei, it was designated as micronucleated cell two micronuclei and if the cell contained three micronuclei, it was designated as micronucleated cell containing three micronuclei and so on. The individual scoring was done by adding number of micronucleated cell observed in the slide as total micronucleated cell and total number of micronuclei scored in all micronucleated cells as number of micronuclei.

Thus, total number of micronucleated and total number of micronuclei were taken as genotoxicity measure for a particular subject [Figures 1-4].\[29\]

To remove bias, the slides were coded and were examined under microscope and 2000 cells were screened for micronuclei under microscope at 100X magnification by two examiners.

Statistical analysis
The statistical analysis was done using Statistical Package for Social Sciences Version 15.0, IBM, USA. statistical analysis software. \(P < 0.05\) was taken as statistically significant.

RESULTS

Demography
The age of individuals ranged from 13 to 73 years. Majority of cases in all the groups were aged 21–40 years followed by those aged <20 years and 41–60 years. The mean age of patients in Group A was 27.89 ± 10.89 years (range 13–73 years) whereas the mean age of patients in different subgroups of Group B ranged from 28.35 ± 10.39 (Group B5) to 35.22 ± 13.68 (Group B3) years. There was no significant difference among groups in terms of age \((P = 0.416)\) [Table 1].

On comparing gender distribution, Group A and Groups B2, B3, and B5, majority of patients were females whereas majority of patients in Group B1 and B4 were males. However, the difference among groups was not significant statistically \(P = 0.965\) [Table 2].

Periodontal health indices
In Group A (control group), mean plaque index was 2.02 ± 0.49. In different subgroups of Group B (study group), mean plaque index ranged from 0.16 ± 0.12 (Group B3) to 0.86 ± 0.51 (Group B1) [Table 3].

In Group A (control group), mean gingival index was 1.98 ± 0.60. In different subgroups of Group B (study group), mean gingival index ranged from 0.06 ± 0.05 (Group B3) to 0.46 ± 0.53 (Group B1) [Table 3].

Genotoxicity measure
Mean number of micronucleated cells was found to be 0.41 ± 0.71 for Group A as compared values ranging from 1.65 ± 2.09 (Group B1) to 11.7 ± 1.87 (Group B5) in different subgroups of Group B, thus showing an incremental trend with increasing duration of use of CHX [Table 4 and Figure 5].

Table 1: Comparison between control and study groups for age

| Age group (years) | Group A \((n=101)\), \(n(\%)\) | Group B1 \((n=51)\), \(n(\%)\) | Group B2 \((n=51)\), \(n(\%)\) | Group B3 \((n=51)\), \(n(\%)\) | Group B4 \((n=51)\), \(n(\%)\) | Group B5 \((n=51)\), \(n(\%)\) |
|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| ≤20              | 30 (29.7)         | 15 (29.4)         | 10 (19.6)         | 9 (17.6)          | 12 (23.5)         | 15 (29.4)         |
| 21-40            | 58 (57.4)         | 28 (54.9)         | 33 (64.7)         | 26 (51.0)         | 28 (54.9)         | 30 (58.8)         |
| 41-60            | 12 (11.9)         | 8 (15.7)          | 7 (13.7)          | 14 (27.5)         | 9 (17.6)          | 6 (11.8)          |
| >60              | 1 (1.0)           | 0                 | 1 (2.0)           | 2 (3.9)           | 2 (3.9)           | 0                 |
| Mean age±SD     | 27.89±10.89 (13-73) | 28.59±10.91 (14-55) | 30.53±10.97 (13-62) | 35.22±13.68 (13-70) | 32.82±13.03 (15-65) | 28.35±10.39 (13-57) |

\(r=15.498\) (df=15); \(P=0.416\). SD – Standard deviation

Table 2: Comparison between control and study groups for gender distribution

| Gender | Group A \((n=101)\), \(n(\%)\) | Group B1 \((n=51)\), \(n(\%)\) | Group B2 \((n=51)\), \(n(\%)\) | Group B3 \((n=51)\), \(n(\%)\) | Group B4 \((n=51)\), \(n(\%)\) | Group B5 \((n=51)\), \(n(\%)\) |
|--------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Female | 46 (45.5)         | 27 (52.9)         | 24 (47.1)         | 24 (47.1)         | 26 (51.0)         | 25 (49.0)         |
| Male   | 55 (54.5)         | 24 (47.1)         | 27 (52.9)         | 27 (52.9)         | 25 (49.0)         | 26 (51.0)         |

\(r=0.967\) (df=6); \(P=0.965\)
Mean number of micronuclei was found to be 0.48 ± 0.80 for Group A as compared values ranging from 2.57 ± 1.64 (Group B1) to 14.5 ± 2.49 (Group B5) in different subgroups of Group B, thus showing an incremental trend with increasing duration of use of CHX [Table 4 and Figure 5]. For both micronucleated cells and micronuclei, analysis of variance revealed statistically significant intergroup differences (P < 0.001) [Table 5].

**DISCUSSION**

The genotoxicity of CHX was measured in terms of number of micronucleated cells and number of micronuclei observed per 2000 buccal epithelial cells of the individual.

On comparing number of micronucleated cells in Group A (0.41 ± 0.71) with subgroups of Group B; Groups B1, B2, B3, B4 and B5. There was an incremental trend in number of micronucleated cells from B1 (1.65 ± 2.09), B2 (4.88 ± 2.09), B3 (5.20 ± 2.62), B4 (10.0 ± 2.09) to B5 (11.7 ± 1.87) in the sequence A < B1 < B2 < B3 < B4 < B5 (P < 0.001) [Tables 4-6 and Figure 5].

Likewise, on comparing number of MN in Group A (0.48 ± 0.80) with subgroups of Group B; Groups B1, B2, B3, B4 and B5. There was an incremental trend in number of micronuclei cells from B1 (2.57 ± 1.64), B2 (6.00 ± 1.92), B3 (6.69 ± 2.21), B4 (13.8 ± 2.72) to B5 (14.5 ± 2.49) in the sequence of A < B1 < B2 < B3 < B4 < B5 (P < 0.001) [Tables 4-6 and Figure 5].

In a similar study, Erdemir et al.[30] studied the cytotoxicity of three commercial mouthrinses klorhex (0.2% CHX...
In a more recent study, Carlin et al.\(^{[31]}\) compared the DNA damage and cellular death in buccal mucosa cells of patients which were exposed to various commercially available five mouthrinse; Listerine\(^{c}\) Cepacol\(^{d}\), Plax alcohol-free\(^e\), Periogard\(^f\), and Plax Whitening\(^g\). Seventy-five individuals were recruited in the study and were divided into five groups containing 15 people each and were exposed to respective commercial mouthrinse. Exfoliated buccal mucosa cells were taken before exposure and after 2 weeks of mouthrinse exposure. Furthermore, blood was also taken from three healthy donors for \textit{in vitro} study. The result showed that Periogard\(^f\) (0.12% CHX) caused increase in Micronucle frequency by 1.8% after 2 weeks of exposure with statistically significant difference (\(P < 0.05\)) as compared to before. The single-cell gel (comet) assay demonstrated DNA damage both in blood lymphocytes and buccal mucosa of individuals who were exposed to 0.12% CHX digluconate for 2 weeks.

Likewise, Eren et al.\(^{[32]}\) in their study evaluated the genotoxicity of CHX using comet assay (single cell gel electrophoresis, or SCGE). Thirteen individuals were asked to rinse their mouth with 0.12% CHX solution for 18 days. Buccal epithelial cells and peripheral blood lymphocytes were obtained from all participants at baseline and at the end of experiment. A statistically significant amount of DNA damage in both buccal epithelial cells and blood lymphocytes was observed. The same findings were seen by Ribeiro et al.\(^{[33]}\) in peripheral blood and oromucosal cells using MN test and comet assay (single cell gel electrophoresis, or SCGE) in Wistar rats.

These above findings demonstrate genotoxic effects of CHX on buccal epithelial cells. Our results are in agreement with previous various previous studies done by Erdemir et al., 2007, Carlin et al., 2012, and Eren et al., 2002,\(^{[30-32]}\)

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**Table 3: Comparison between control and study groups for periodontal health indices**

| Parameter        | Group A (n=101) | Group B1 (n=51) | Group B2 (n=51) | Group B3 (n=51) | Group B4 (n=51) | Group B5 (n=51) |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Plaque index     | 2.02±0.49       | 0.86±0.51       | 0.71±0.53       | 0.16±0.12       | 0.52±0.32       | 0.74±0.16       |
| Gingival index   | 1.98±0.60       | 0.46±0.53       | 0.45±0.41       | 0.06±0.05       | 0.31±0.29       | 0.18±0.28       |

**Table 4: Comparison among control and different study subgroups for number of micronucleated cells and total number of micronuclei**

| Parameter          | Group A (n=101) | Group B1 (n=51) | Group B2 (n=51) | Group B3 (n=51) | Group B4 (n=51) | Group B5 (n=51) |
|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Micronucleated cells | 0.41±0.71       | 1.65±2.09       | 4.86±2.09       | 5.20±2.62       | 10.0±2.09       | 11.7±1.87       |
| Micronuclei        | 0.48±0.80       | 2.57±1.64       | 6.00±1.92       | 6.69±2.21       | 13.8±2.72       | 14.5±4.29       |

**Table 5: Analysis of variance for micronucleated cell and micronuclei count among control and different study subgroups**

| Parameter          | Source of variance | Sum of squares | df  | Mean square | F       | Significant |
|--------------------|--------------------|----------------|-----|-------------|---------|-------------|
| Micronucleated cells | Between groups     | 6255.9         | 5   | 1251.182    | 390.419 | <0.001      |
|                    | Within groups      | 1121.7         | 350 | 3.205       |         |             |
|                    | Total              | 7377.6         | 355 |             |         |             |
| Micronuclei        | Between groups     | 10,394.0       | 5   | 2078.793    | 557.719 | <0.001      |
|                    | Within groups      | 1304.6         | 350 | 3.727       |         |             |
|                    | Total              | 11,698.5       | 355 |             |         |             |

For both micronucleated cells and micronuclei, analysis of variance revealed statistically significant intergroup differences (\(P < 0.001\)).

**Table 6: Between control (Group A) and study groups (Group B) differences in micronucleated cell and micronuclei count (Tukey honest significant difference test)**

| Comparison          | Micronucleated cells | Micronuclei |
|---------------------|----------------------|-------------|
|                     | Mean difference | SE  | \(P\) | Mean difference | SE  | \(P\) |
| A versus B1         | −1.241              | 0.308  | 0.001 | −2.093           | 0.332 | <0.001 |
| A versus B2         | −4.476              | 0.308  | <0.001 | −5.525           | 0.332 | <0.001 |
| A versus B3         | −4.790              | 0.308  | <0.001 | −6.211           | 0.332 | <0.001 |
| A versus B4         | −9.614              | 0.308  | <0.001 | −13.995          | 0.332 | <0.001 |
| A versus B5         | −11.261             | 0.308  | <0.001 | −18.333          | 0.332 | <0.001 |
| B1 versus B2        | −3.235              | 0.355  | <0.001 | −3.431           | 0.382 | <0.001 |
| B1 versus B3        | −3.549              | 0.355  | <0.001 | −4.118           | 0.382 | <0.001 |
| B1 versus B4        | −8.873              | 0.355  | <0.001 | −11.196          | 0.382 | <0.001 |
| B1 versus B5        | −10.020             | 0.355  | <0.001 | −11.902          | 0.382 | <0.001 |
| B2 versus B3        | −0.314              | 0.355  | <0.001 | −0.686           | 0.382 | 0.470     |
| B2 versus B4        | −5.137              | 0.355  | <0.001 | −7.765           | 0.382 | <0.001 |
| B2 versus B5        | −6.784              | 0.355  | <0.001 | −8.471           | 0.382 | <0.001 |
| B3 versus B4        | −4.824              | 0.355  | <0.001 | −7.078           | 0.382 | <0.001 |
| B3 versus B5        | −6.471              | 0.355  | <0.001 | −7.784           | 0.382 | <0.001 |
| B4 versus B5        | −1.647              | 0.355  | <0.001 | −0.706           | 0.382 | 0.437     |

SE – Standard error
Our findings show that on increasing the duration of CHX use increases the total number of micronucleated cells and micronuclei in buccal epithelial cells of its users. This suggests that CHX causes cumulative genotoxicity and is dependent on time of exposure [Tables 4-6 and Figure 5].

Our observations have been substantiated by Lessa et al.,[34] who in their in vitro study exposed cultured odontoblast-like cells (MDPC-23) to different concentrations of CHX (0.06%, 0.12%, 0.2%, 1% and 2%) for 60 s, 2 h or 60 s with 24 h recovery period using MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium) assay. They showed that CHX exhibited dose-dependent toxic effect on odontoblast-like cells. The cytotoxic effect of CHX was more pronounced on increasing the contact time of CHX with cells.

Similarly, Giannelli et al.[35] in their in vitro study exposed osteoblastic, endothelial, and fibroblastic cell lines to various concentrations of CHX (0.0025%, 0.005%, 0.0075%, 0.01% and 0.12%) for different times and tested for cell viability and cell death. They found that CHX causes cell damage in concentration and time-dependent manner.

Furthermore, Bonacorsi et al.[36] in their study evaluated CHX-induced cytotoxicity using MTT assay on murine peritoneal macrophages and showed that cytotoxicity of CHX is dependent on concentration and time of exposure.

Likewise, Hidalgo and Dominguez[37] studied the mechanisms underlying CHX-induced toxicity on human fibroblasts using ATP-dependent Luciferin-Luciferase reaction using commercial ATP Bioluminescence Assay Kit (Boehringer Germany). Human fibroblast was exposed to range CHX concentrations of 0.00005%–0.025% for 3, 6, 8, or 24 h in the absence or presence of different concentrations of fetal calf serum (2, 5, and 10%). They observed that CHX causes time-dependent cytotoxicity.

Chang et al.[38] also observed the cytotoxic effects of CHX on human periodontal ligament cells to be concentration and exposure time dependent.

The cytotoxicity of CHX was first shown by Helgeland et al.[39] who showed cytotoxic effects of CHX on human epithelial cells at very low concentration of 0.05 mM and also inhibition of Na K ATPase on the membrane of erythrocytes. CHX at concentration of 0.01% or greater when exposed to cell cultures of human gingival fibroblasts and HeLa cells causes death of these cells.[37]

Seymour et al. and Watts et al. both investigated the effect of CHX on neutrophil chemotaxis and found a dose-related decrease in the chemotaxis on increasing CHX concentrations from 0.002% to 0.2% in aqueous solutions, and complete cell lysis was seen at 0.2% concentration.[40,41]

Mariotti and Rumpf[42] showed that CHX caused dose-dependent reduction in cellular proliferation of gingival fibroblasts.

Thus, above evidence portrays CHX as a potential genotoxic and cytotoxic agent and also conforms our observations.

The genotoxicity of CHX can be studied by various biomarkers. The biomarkers have been classified on three purposes; the first purpose is to give account about deleterious chemical exposure. Second, to show the biological effects on target tissue and third, to give information about individual susceptibility.[43] There are various assays that have been developed as potential biomarkers in biomonitoring studies which include assessing metaphase chromosomal aberrations, sister chromatid exchanges, and host-cell reactivation. These methods are quite laborious and time-consuming and require highly trained technicians to read and interpret the slides. Therefore, there is great enthusiasm about micronuclei as genotoxic biomonitoring tool.[44]

MN arise from acentric fragments or whole chromosomes which are not included in the main nuclei of the daughter cells. The micronuclei are formed when the cells are exposed to the substances which cause chromosomal breakage (clastogens) and also affect the spindle apparatus (aneugens). MN test in exfoliated buccal mucosa cells have been systematically used in genetic biomonitoring of populations exposed to various genotoxic agents such as tobacco products, pesticides, and alcohol consumption.[45,46]

The advantage of micronuclei is its simplicity, relative ease of scoring, economical in terms of cost, and required person-time resources. Moreover, precision is obtained from scoring large number of cells. MN cell index reflects genomic instability and increased frequency of micronuclei indicates risk of malignancy.[46,47] Micronucleus is multiple endpoint test which indicates chromosomal aberration.[45,46]

We took buccal epithelial cells for assessing genotoxic effect of CHX in our study because it is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to the individuals. It is frequently used in molecular epidemiological studies for investigating the impact of nutrition, lifestyle factors, genotoxin exposure and genotype on DNA damage and cell death.

Furthermore, the buccal epithelial cells are of ectodermal origin and 90% of all cancers are of ectodermal origin in humans. Therefore, buccal mucosa can be used to monitor early genotoxic events which enter the body either through ingestion or inhalation.[50]

The limitations of our study is that it is a cross-sectional study design and it does not give highest level of evidence. Thus, our study calls for undertaking robust study designs such as longitudinal studies and randomized control trials using specialized test such as micronuclei test using DNA specific stains and comet assay.

Our findings question the long-term use of CHX as adjunct chemical plaque control agent and its usage can only be justified after taking into account the risk to benefit ratio. The patient should be educated and motivated for maintaining adequate plaque control which can be achieved by demonstration of proper toothbrushing technique and correct use of interdental plaque control aids and also periodic reevaluations at regular interval recall visits.
CONCLUSION

The study gives us an indication of potential genotoxic effects of CHX mouthwash on buccal epithelial cells, and therefore, it validates the claim for search of an antiplaque agent which has same efficacy as CHX but without genotoxic effects.

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Conflicts of interest

There are no conflicts of interest.

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