Summary & Conclusion
Chlorhexidine is the most widely prescribed and the gold standard of all mouthrinses used in clinical practice. It is used as an adjunct to mechanical plaque control measures. It was first introduced as skin disinfectant in surgical specialties for washing operation sites. Its dental plaque inhibition was first investigated by Schroeder in 1969, but antiplaque and antigingivitis action in humans were established by Loe & Schiott in 1970.

Chlorhexidine is a symmetrical molecule made up of two biguanide group and two 4-chlorophenyl rings joined by central hexamethylene bridge and is positively charged at physiological pH. Owing to its positive charge it attracts negatively charged oral bacteria and causes alteration in cell permeability leading to leakage of potassium and phosphate ions at low concentration and cytolysis and cell death at high concentration.

Chlorhexidine has broad spectrum of activity against gram positive and gram negative bacteria, fungi and viruses. Its clinical efficacy has been proved in several clinical trials but it has certain side effects as well such as tooth staining, oral discoloration, Oral ulceration & hypersensitivity reactions, taste alterations, parotid swelling and viral infections.

Also, various invitro, animal and human studies have shown cytotoxic and genotoxic effect of chlorhexidine on epithelial cells, fibroblasts, odontoblasts, erythrocytes, lymphocytes and various cell lines.

With the wealth of evidence supporting cytotoxic and genotoxic effects of chlorhexidine, The present study was done to assess the genotoxic effects of chlorhexidine mouthwash on buccal epithelial cells of its users and Micronuclei test was used as measure of genotoxicity, as it is very simple and a valid test for assessing genotoxicity in-vivo. Also, Micronuclei test is economical and can be done in large number of patients in epidemiological studies.

We employed a case control and crossectional study design in which chronic gingivitis patients who were exclusively on mechanical plaque control measures were recruited as Controls (Group A) and chronic gingivitis patients who were on mechanical plaque control measures along with adjunct 0.2% chlorhexidine gluconate.
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twice daily mouthrinse for 60 seconds were recruited as Study group (Group B). Group B was split into 5 subgroups; B1, B2, B3, B4, B5 on increase in duration of usage of mouthrinse from ≤1 week to ≤24 weeks. Buccal cells from these patient were taken with soft toothbrush and number of micronucleated cells and number of micronuclei were visualized using giemsa stain and calculated under microscope. The genotoxic measure was measured as number of micronucleated cell and total number of micronuclei observed per 2000 buccal epithelial cell in each participant.

The mean number of micronucleated cells control (Group A) and study (Group B) were 0.41±0.71 and 6.68±4.20 respectively and mean number of micronuclei in control (Group A) and study (Group B) were 0.48±0.80 and 8.70±5.15 respectively. The above results show significant difference (p<0.001) in mean number of micronucleated cells and total number of micronuclei in control (Group A) and Study (Group B) groups.

The null hypothesis which was stated at the beginning of the study that Chlorhexidine is not genotoxic to exfoliated buccal epithelial cells in chronic gingivitis patients has been rejected (p<0.001), thereby implying genotoxic effects of chlorhexidine on buccal epithelial cells of its users which is evidenced by significantly increased micronucleated cells and number of micronuclei in the buccal epithelial cells.

Also, on comparing mean number of micronucleated cells and total number of micronuclei among Control (Group A) and Study subgroups (B1, B2, B3, B4, B5) there was incremental trend in micronucleated cells and number of micronuclei with increase in duration of chlorhexidine use (p<0.001).

The genotoxic effect of chlorhexidine mouthwash on buccal epithelial cells, limits it use as antiplaque agent and suggests its more prudent use after weighing potential risks and benefits and therefore its unscrupulous and unwarranted use should be avoided. The results of this study have substantiated the need for formulating guidelines regarding the use of chlorhexidine as an antiplaque and antigingivitis agent in clinical practice, considering its cytotoxic and genotoxic effects.

Our study also puts in impetus for development of newer formulations which are non-cytotoxic and non-genotoxic and have equivalent antiplaque and antigingivitis effects as chlorhexidine.
The limitations of our study also justifies more studies with more robust study design taking due considerations of all potential cofounders so as to study the genotoxic effects of chlorhexidine mouthwash on oral epithelial cells, inorder to ascertain its clinical use and formulate guidelines of chlorhexidine usage in clinical settings.