J Oral Res
oral and craniofacial sciences

Article

Comparative evaluation of micronuclei in Saudi smokers and non-smokers without any visible oral lesions– A pilot study.

Evaluación comparativa de micronúcleos en fumadores y no fumadores sauditas sin lesiones orales visibles- Un estudio piloto.

Kiran Kumar Ganji.[1]
Srinivas Munisekhar Manay.[2]
Fahad Muqbeel.[3]
Hamad Khalaf.[3]
Naif Tami.[3]
Hassan Ibrahim.[3]
Mohammad Khursheed Alam.[4]

Affiliations: [1]Periodontics section, College of Dentistry, Jouf University, Kingdom of Saudi Arabia. [2]Oral Pathology Section, College of Dentistry, Jouf University, Kingdom of Saudi Arabia. [3]College of Dentistry, Jouf University, Kingdom of Saudi Arabia. [4]Orthodontics section, College of Dentistry, Jouf University, Kingdom of Saudi Arabia.

Corresponding author: Kiran Kumar Ganji. Sakaka 72721, Kingdom of Saudi Arabia. Phone: (9665) 40640338. Email: kiranperio@gmail.com

Receipt: 02/14/2019 **Revised:** 04/23/2019
Acceptance: 05/31/2019 **Online:** 12/30/2019

Cite as:
Ganji KK, Manay SM, Muqbeel F, Khalaf H, Tami N, Ibrahim H & Alam MK. Comparative evaluation of micronuclei in Saudi smokers and non-smokers without any visible oral lesions– A pilot study. J Oral Res 8(6):505-509. Doi:10.17126/joralres.2019.074

Abstract: Objectives: A systematic review was conducted to evaluate effectiveness and safety of beta carotenes for the treatment of oral leukoplakia regarding clinical resolution and prevention of malignant transformation. Material and Methods: The systematic search was conducted in three electronic databases and the study's selection was performed according to pre-set eligibility criteria. Four studies evaluating the efficacy of beta carotenes in oral leukoplakia compared to placebo were included in the review; three of which were assigned for quantitative analysis. Data were extracted, tabulated, quality assessed and statistically analyzed. Results: The meta-analysis revealed that when comparing clinical resolution the beta carotene group favored was favored compared to placebo, with statistically significant difference. However, a meta-analysis comparing beta carotene and placebo groups regarding malignant transformation as a primary outcome failed to show any significant benefit. Furthermore, results showed evidence of beta carotene safety. Conclusion: the overall quality of evidence about efficacy of beta carotene in oral leukoplakia treatment was not high. However, given the obvious safety of this agent, data suggests it could have a promising effect in clinical improvement of oral leukoplakia lesions. However, no evidence supporting its benefits in reducing risk of malignant transformation in these lesions was found. Therefore, further long term, well designed randomized clinical trials are highly recommended.

Keywords: Micronuclei, chromosome-defective; smokers; mouth neoplasms; genomics; biomarkers; control groups.

Resumen: Introducción: el cáncer oral es un problema grave con alta mortalidad y morbilidad, a pesar de la disponibilidad de los mejores tratamientos. Uno de los factores más importantes para una mortalidad tan alta es su diagnóstico tardío. La mejor manera de enfrentar un problema de este tipo es evitar su aparición creando conciencia entre la población y tenendo un diagnóstico más temprano. El cáncer oral es una enfermedad multifactorial, donde el daño genómico tiene un papel. Se ha demostrado que los micronúcleos (MNi) son un biomarcador importante y en este estudio se utilizó como una herramienta para crear conciencia sobre el riesgo de cáncer oral. Objetivo: evaluar y comparar la frecuencia de MNi en fumadores sin ninguna lesión oral visible (Grupo I) y no fumadores sanos (Grupo II). Materiales y métodos: se obtuvieron citoestimuladores de fumadores sauditas (n = 15, Grupo I) sin ninguna lesión oral visible y no fumadores sanos (n = 15, control, Grupo II) y se tiñeron con hemotoxilina y eosina para evaluar la frecuencia de MNi y las observaciones fueron sometidas a análisis estadístico utilizando la prueba t de Student. Resultados: La frecuencia media de MNi en el Grupo I fue significativamente mayor ($p<0.05$) que en el Grupo II. El estudio ayuda a educar, motivar y crear conciencia, alentando así a los pacientes a dejar de fumar, y evitando así el cáncer oral antes de su inicio.

Palabras Clave: *Micronúcleos con defecto cromosómico; fumadores; neoplasias de la boca; genómica; biomarcadores; grupos control.*

INTRODUCTION.

Oral cancer is a serious health problem the world over. In high risk countries such as Sri Lanka, Pakistan, India and Bangladesh, oral cancer is the most common cancer in men and may represent up to 25% of all new cases of cancer.[1] It is a multifactorial disease that occurs due to activation of oncogenes as a result of mutations in the DNA. Approximately 75% of oral cancers are related to the use of tobacco and alcohol.

In general, many oral cancers pass through clinically evident premalignant mucosal changes which indicate the presence of a risk and present an opportunity for early detection and to intercept the development of a cancerous lesion.

It is very important to prevent cancer at an early stage, as despite the best treatment, the associated morbidity is very high and is dependent on the disease stage at the time of admission. Approximately 50% of the patients are in at an advanced stage at the time of diagnosis. Oral cancer-related mass screening programs do not meet the guidelines for a successful outcome. However, there may be some benefit when focusing on high-risk groups, such as heavy smokers and alcohol-abusers. There still remains a big challenge to reduce the morbidity associated with the treatment of oral cancer without compromising the survival rate.

Studies have shown that there is a marked reduction (50%) in the risk of getting oral cancer if habits such as the use of tobacco are stopped. Hence, it appears that risk-factor reduction may be a very effective tool in decreasing the morbidity and mortality associated with oral cancer. Chronic use of carcinogenic agents brings about irreversible cytogenetic changes such as chromosomal aberrations, exchange of sister chromatids, and micronuclei which can be frequently used as indicators. Micronuclei (MNi) are defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus.

They originate from aberrant mitosis and consist of eccentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated into the daughter nuclei during mitosis. They have proved to be an important biomarker of genomic damage.[1,2]

The MNi test is one of the current rapid, efficient, economical and non-invasive tests, used as an indicator of cytogenetic damage as it provides a quantitative measure of the genotoxic action of carcinogens and mutagens.[1,2]

Extensive research has been carried out and has confirmed that micronuclei frequency is significantly increased in potentially malignant disorders as compared to healthy individuals.

However, not many studies have been carried out to know if there is an increase in the frequency of MNi in smokers without any visible oral lesions. Hence, in this study we intend to analyze and compare the frequency of micronuclei in exfoliated oral epithelial cells of smokers without any visible oral lesion and compare it to that of non-smokers.

MATERIALS AND METHODS.

Source of data

The study was done on male patients attending the dental OPD, College of Dentistry, Jouf University, Sakaka from September 2016 to March 2017.

Inclusion criteria included Non-smokers with apparently healthy mucosa, for the control group; Smokers with apparently healthy mucosa for the study group.

Exclusion criteria included Patients with a history of treatment for any potentially malignant disorder such as leukoplakia, oral verrucous carcinoma, keratoacanthoma, dyskeratosis congenita and oral submucous fibrosis; Smokers and former smokers with oral lesions.

Sampling technique

The samples for the present study were selected by using the systematic random sampling technique until desired sample size was achieved. Fifteen patients with a smoking habit for over 10 years without having any visible oral lesion would constitute Group I, and 15 age-matched normal healthy non-smokers would constitute Group II (control).

This study followed the guidelines of Strengthening the Reporting of Observational studies in Epidemiology (STROBE), and the STROBE checklist was utilized in the preparation of this manuscript.[3]

Study design

After obtaining informed consent from all the patients, they were asked to rinse their mouth with water to remove food debris, and buccal scrapings were obtained using a

ISSN Online 0719-2479 - www.joralres.com © 2019

sterile wooden stick. The cells were then directly spread on a clean microscopic slide to prepare a smear. Then samples were allowed to dry and were fixed with alcohol. The smears were stained with haemotaoxylin and eosin and examined under low magnification (x100) for screening and under higher magnification (x400) for counting the MNi (Figure 1).

A total of 500 cells were assessed for the presence of micronuclei and compared between the groups. The results were then subjected to statistical analysis.

Statistical Analysis

The results between the Group I and Group II were compared with a Student's t-test to evaluate the statistical significance between the two groups.

RESULTS.

The number of MNi in smokers ranged between 89 to 159 (MNi cells) per 500 assessed cells. An average of 100 cells was found to be the highest frequency. (Figure 2) In case of non-smokers the frequency was found to be 30 (MNi cells) per 500 counted cells. (Figure 3) A statistically significant difference was found between group I and group II with respect to the number of micronuclei present. (Table 1)

Figure 1. Hemotaoxylin and eosin stained cytosmear showing the presence of micronuclei. (X400 magnification).

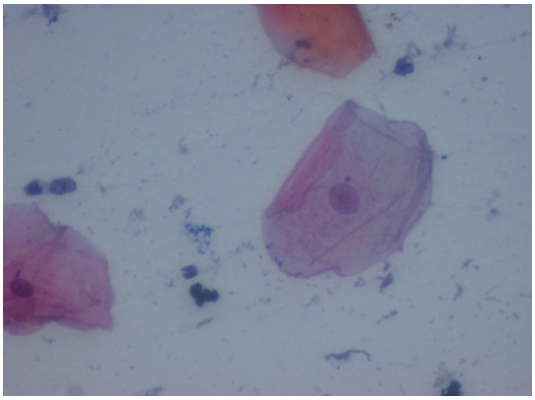

Figure 2. Frequency distribution of number of MNi in smokers (n=15)

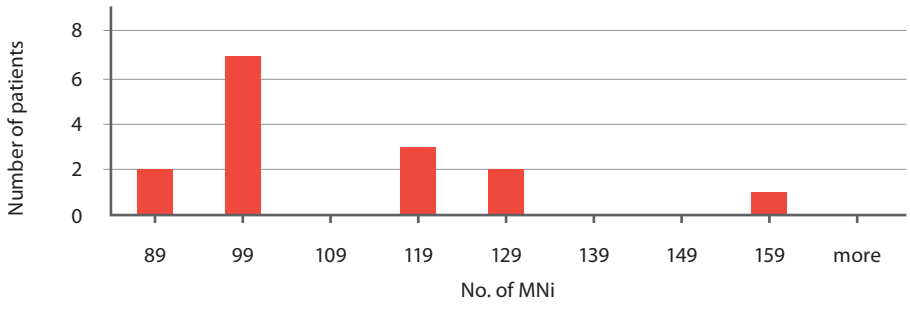

Figure 3. Frequency distribution of number of MNi in Non-smokers (n=15)

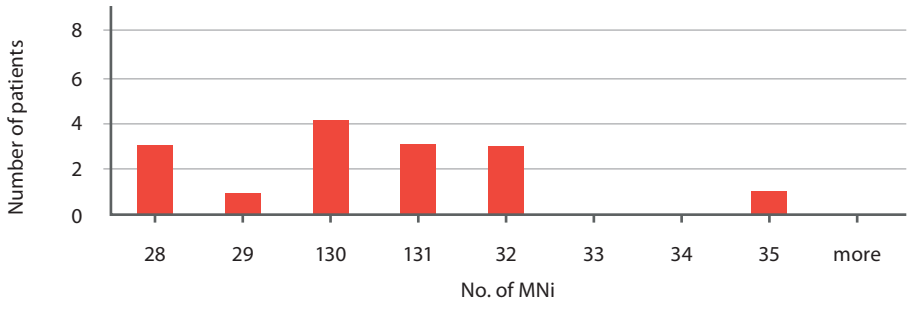

Table 1. Comparison of mean values of micronuclei in smokers and non-smokers

Group	N	Mean(SD)	Student t Test (p<0.05)
Group I (Smokers)	15	104.26(±17.51)	.000*
Group II (Non-smokers)	15	30.13(±2.65)	

DISCUSSION.

Micronuclei (MNi) denote small, additional nuclei formed by the exclusion of chromosome fragments or whole chromosomes lagging at mitosis. MNi rates, therefore, indirectly reflect chromosome breakage or impairment of the mitotic apparatus. Throughout the last few years, MNi in oral exfoliated epithelial cells have been widely used as biomarkers of chromosomal damage, genome instability and cancer risk in humans.

The mean MNi frequency noted in the present study was significantly higher (p<0.05) in smokers than in non-smokers. These results are in accordance with the findings of Grover *et al.,*[1] and Anila *et al.*[4] The results of our study are in contrast to the findings in a similar study carried out by Nerseseyan *et al.,*[5] in which a statistically significant difference in MNi counts between smokers and nonsmokers was detected, only with DNA nonspecific stains but not with DNA-specific stains.

Casartelli *et al.,*[6] determined that the gradual rise in MNi frequency from normal mucosa to precan-cerous lesion to carcinoma represents a link of this biomarker with neoplastic progression.

Oliveira *et al.,*[7] demonstrated that the action of genotoxic agents (tobacco and alcohol) causes alterations in the frequency of micronuclei and metanucleated anomalies. According to Samanta *et al.,*[8] the various possible mechanisms for MNi formation in preneoplastic conditions include chromosomal aberrations, chromosome loss/breakage, mitotic apparatus dysfunctions, aneuploidy, and genetic instability. First, MNi formation is generally considered as a manifestation of genetic damage or chromosomal breakage.

However, many factors such as radiation, drugs, pollutants, even the normal aging process may be responsible for MNi formation.[9,10] Selection of buccal mucosa site for smear collection is justified as this site is more vulnerable to external insult from possible carcinogens. Limitations of the present study include small sample size and that the presented results are confined to a male population only. The study helped us in identifying changes at a cellular level, before lesions become apparent, creating awareness, and motivating and educating the patients about the ill effects of smoking, and to some extent to advocate for the cessation of the habits associated with these cellular changes, to prevent oral cancer before its onset.

CONCLUSION.

A statistically significant difference was found between smokers without visible oral lesions and non-smokers regarding the number of micronuclei present. There is evident damage to the cell nucleus in smokers although there is no clinical evidence of a white lesion.

Conflict of interests: The authors declare no conflict of interest.

Ethics approval: Ethical approval granted by the Office of Scientific Committee for Research Ethics, College of Dentistry, Jouf University.

Funding: The research is self-funded.

Author's contribution: All authors contributed to the manuscript.

Acknowledgements: Special thanks to Dr.Bhilal Tantry for his histological readings and support.

ISSN Online 0719-2479 - www.joralres.com © 2019

REFERENCES.

1. Grover S, Ahmed MB, Telagi N, Shivappa AB, Nithin K. Evaluation of diagnostic reliability of micronuclei in potentially malignant disorders of oral cavity. CHRISMED Journal of Health and Research. 2014;1(1):15.
2. Grover S, Mujib A, Jahagirdar A, Telagi N, Kulkarni P. A comparative study for selectivity of micronuclei in oral exfoliated epithelial cells. J Cytol. 2012;29(4):230.
3. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for reporting observational studies. International Journal of Surgery. 2014;12(12):1495-9.
4. Anila K, Kaveri H, Naikmasur VG. Comparative study of oral micronucleated cell frequency in oral submucous fibrosis patients and healthy individuals. 2011.
5. Nersesyan A, Kundi M, Atefie K, Schulte-Hermann R, Knasmüller S. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiology and Prevention Biomarkers. 2006;15(10):1835-40.
6. Casartelli G1, Bonatti S, De Ferrari M, Scala M, Mereu P, Margarino G, Abbondandolo A. Micronucleus frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. Anal Quant Cytol Histol. 2000;22(6):486-92.
7. Oliveira LU, Lima CF, Salgado MA, Balducci I, Almeida JD. Comparative study of oral mucosa micronuclei in smokers and alcoholic smokers. Anal Quant Cytol Histol. 2012;34(1):9-14.
8. Samanta S, Dey P. Micronucleus and its applications. Diagn Cytopathol. 2012;40(1):84-90.
9. Dindgire SL, Gosavi S, Kumawat R, Ganvir S, Hazarey V. Comparative study of exfoliated oral mucosal cell micronucleus frequency in potentially malignant and malignant lesions. International Journal of Oral and Maxillofacial Pathology. 2012;3(2):15-20.
10. Jyoti S, Khan S, Afzal M, Siddique YH. Micronucleus investigation in human buccal epithelial cells of gutkha users. Advanced biomedical research. 2012;1(1):35.