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ASSESSMENT OF GENOTOXIC EFFECTS OF TOBACCO CHEWING USING MICRONUCLEI ASSAY

Anatomy

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ABSTRACT

Tobacco is the most common misused drug in the world. In India, tobacco is used mainly in smokeless form. Tobacco is a potential carcinogen and its extensive use leads to generation of reactive oxygen species. Micronucleus assay has been used to assess the DNA damage. The aim of the present study was to set up the normal frequency of the presence of micronuclei and other nuclear anomalies in cells of the buccal mucosa and to compare it with tobacco chewers. Most of the tobacco chewers in the present study were mainly from rural areas, which indicates that the subjects living in rural areas are mainly indulged in the tobacco chewing habits. The number of normal cells was less in tobacco chewers and the number of nuclear anomalies was more, which clearly indicates the damage occurring due to tobacco chewing.

KEYWORDS

Tobacco, Micronucleus assay, DNA damage

INTRODUCTION

All over the world a lot of people use one or another drug, such as cocaine, heroin, marijuana, ecstasy, and tobacco, etc. Among misused drugs, tobacco is by far the most commonly used drug in the world. As per Factsheet 2018, India by WHO more than 1 million people died every year because of the use of tobacco, which is 9.5% of all deaths.[1] In India, tobacco is utilized as smoked and smokeless forms, but mainly in the smokeless form.[2] Smokeless tobacco (SLT) is the form of tobacco, which is not ignited before use. Smokeless tobacco includes tobacco or products that contain tobacco and these are used as a chewing or sucking material or by applying to the teeth and gums, or through inhalation. Tobacco is a potential carcinogen and its exposure for a long period leads to the formation of reactive oxygen species (ROS) and thus causing oxidative stress.[3]

Micronucleus assay can be performed on exfoliative cells of the buccal mucosa, so known as "Buccal micronucleus cytome assay (BMCyt)". This assay has been used for measuring biomarkers of DNA damage (micronuclei and nuclear bud), defects in cytokinesis (binucleation) and cell death (karyorrhexis, pyknosis, and karyolysis). Micronuclei are the small structures present in the cells beside the nucleus and they are having same focal plane, texture and staining as the main nucleus. Nuclei of cells with nuclear bud are having one sharp constriction at one end that looks like a bud of nuclear material. Binucleated cells are having two nuclei and these nuclei are normally lies close to each other or sometimes they are even partially attached to each other. The nuclei of karyorrhectic cells lose their integrity, so the chromatin looks aggregated and darkly stained. Pyknotic cells are having a shrunken nucleus, which is smaller in size (nearly 1/3) than the nucleus of the normal differentiating cells. The nucleus of karyolytic cells is known as ghost nucleus, as it only shows few remnants of the main nucleus.

Objectives:

The aim of the present study was to set up the normal frequency of the presence of micronuclei and other nuclear anomalies in cells of the buccal mucosa and to compare it with tobacco chewers. The study also aimed to assess the potential of micronuclei assay as a biomarker for genotoxic damage.

MATERIAL AND METHOD:

The present study was conducted on 50 control subjects that were not having any habits of chewing tobacco and 58 tobacco chewers. The subjects chosen for the present study were the patients visiting to OPD of National Institute of Medical Sciences & Research and Hospital (Jaipur). The study was approved by the Institutional Ethical Committee. The subjects were informed about the study and a written consent was obtained from them. The age of the subjects ranged from 21 years to 75 years. The exfoliated cells of the buccal mucosa were collected by gentle scraping and giemsa stain was used to see the nuclear anomalies. Along with micronuclei other nuclear anomalies were also counted such as binucleated, pyknotic, aryolitic, karyorrhectic cells and cells with nuclear bud.

One way ANOVA analysis was used to determine the significance of the different parameters measured between the controls and tobacco chewers. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS:

In the present study 42 tobacco chewers were from rural areas and 16 were from urban areas, 28 controls were from rural areas and 22 were from urban areas. Most of the tobacco chewers in the present study were mainly from rural areas, which indicates that the subjects living in rural areas are mainly indulged in the tobacco chewing habits. The reason responsible for this is maybe illiteracy and their profession. In the present study majority of the chewers were laborers and as after heavy physical work they will pleasure and relaxed due to chewing tobacco, so they used it. After sometime they get addicted and continue the chewing habit in their whole life.

The number of normal cells in tobacco chewers is very less as compared to controls and the difference is highly significant. The number of nuclear anomalies is even more in tobacco chewers as compared to controls. The number of micronuclei and nuclear budding is more in controls as compared to chewers.

Table 1: Comparison of Mean ± SE of different nuclear anomalies (NA) among tobacco chewers (TC) and controls

Nuclear Anomalies (NA)	Control	Tobacco Chewers	P value
Number of Normal cells	593.2 ± 222.29	430.86 ± 209.54	<0.001 (HS)
Number of cells with micronuclei	0.58 ± 0.20	0.46 ± 0.09	0.59 (NS)
Bi-nucleated cells	0.74 ± 0.24	3.41 ± 0.18	<0.001 (HS)
Pyknotic cells	0.08 ± 0.06	5.15 ± 0.29	<0.001 (HS)
Cells with nuclear bud	0.1 ± 0.05	0.03 ± 0.02	0.28 (NS)
Karyorrhectic cells	0.04 ± 0.02	1.12 ± 0.16	<0.001 (HS)
Karyolytic cells	0.04 ± 0.02	2.15 ± 0.14	<0.001 (HS)

DISCUSSION:

Use of smokeless tobacco is a major etiological factor which is causing significant morbidity and mortality. Chewing of tobacco as a part of custom in rural India is followed for many centuries. However, use of tobacco has increased significantly because of large scale production and social marketing, which has even attracted the youth. The literature is replete with data which is thoroughly showing light on the genotoxic and carcinogenic potential of tobacco, but still major attention is diverted towards smoking as compared to chewing tobacco.

According to **Thomas et al.**[4] (2007) for prediction of degenerative changes micronuclei scoring along with other nuclear abnormalities

must be scored. In the present study, the view point of Thomas et al. was also considered and on that basis along with micronuclei other nuclear abnormalities were also scored.

According to **Anand and Kanchan**[5] (2017) over 80% tobacco users belong to rural areas. Similar to our study, **Gupta et al.**[6] (1990) on a survey in 7 rural areas of India reported that use of tobacco was 44% in Bhavnagar (Gujarat) and it was 74% in Srikakulam (Andhra Pradesh). **Bala DV et al.**[7] (2006) also found in their study that the majority of the tobacco users were laborers.

Almost similar to the present study, mean micronucleated cells count was reported by **Ozkul et al.**[8], (1997) and **Sellapa et al.**[9], (2009). Contrary to the present study, the scoring of micronuclei reported by **Palaskar and Jindal**[10] (2010), and **Agarwal et al.**[11], (2016) in tobacco chewers as well as in controls was very high.

The study clearly indicates that the micronuclei assay is a relevant biomarker for assessment of genotoxic damage. However, there is still need for large group study to clearly define the normal frequency of micronuclei and other nuclear anomalies.

REFERENCES

1. World Health Organization. India tobacco fact sheet. 2018. Available from: www.who.int/iris/bitstream/10665/272672/1/wntd_2018_india_fs.pdf.
2. Deliana K, Dave D. Smokeless tobacco use in India: role of prices and advertising. Soc Sci Med. 2015; 138: 82-90.
3. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res. 2008; 87: 14-32.
4. Thomas P, Holland N, Bolognesi C, et al. Buccal micronucleus cytome assay. Nat Protoc. 2009; 4(6): 825-837.
5. Anand A, Kanchan I. The risk of hypertension and other chronic diseases: comparing smokeless tobacco with smoking. Front. Public Health. 2017; 5: 1-6.
6. Gupta PC, Mehta FS, Pindborg JJ, et al. Primary prevention study of oral cancer among Indian villagers, eight year follow-up results. IARC scientific publication. International agency for research on cancer. 1990.
7. Bala DV, Bodiwala IN, Patel DD, Shah PM. Epidemiological determinants of tobacco use in Gujarat state, India. IJCM. 2006; 31(3): 173-176.
8. Ozkul Y, Donmez H, Erenmemisoglu A, et al. Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis 1997; 12: 285-287.
9. Sellappa S, Balakrishnan M, Vellingiri B. Mixture of betel leaf, areca nut and tobacco chewing is a risk factor for cytogenetic damage in construction workers from south India. Braz J Oral Sci. 2009; 8(3): 145-148.
10. Palaskar S, Jindal C. Evaluation of micronuclei using Papanicolaou and May Grunwald Giemsa stain in individuals with different tobacco habits- a comparative study. JCDR. 2010; 4: 3607-3613.
11. Agrawal V, Dubey I, Mishra KB. Evaluation of micronucleus frequency in oral exfoliated buccal mucosa cells of smokers and tobacco chewers: a comparative study. Int J Res Med Sci. 2016; 4: 3130-3133.