Original Research Paper

Volume-8 | Issue-5 | May-2018 | PRINT ISSN No 2249-555X

Dental Science

ASSESSMENT OF CYTOTOXIC AND GENOTOXIC POTENTIALS IN TOBACCO CHEWERS

Dr. G. Kiranmai MDS — Assistant Professor of Dental Surgery, Dept. of Dental Surgery. ACSR Medical College, Nellore, AP.

Dr. P. Vani Priya MDS* — Assistant Professor of Dental Surgery, Dept. of Dental Surgery. SVIMS, Tirupathi, AP. *Corresponding Author

ABSTRACT Smokeless tobacco is the cheapest, least taxed and most commonly used tobacco products in India. They are highly addictive and high in carcinogens. Tobacco has addictive properties due to the presence of nicotine which causes signs of dependence of tolerance in the consuming adult. The extent and increasingly speed of the tobacco addictions are believed to be directly proportional to the biological strength of nicotine addiction. The oral cavity suffers from potentially harmful effects due to the habit of tobacco and tobacco mixtures chewing. The main effects on the hard tissue of the oral cavity are on the teeth. It caused severe wearing of the enamel covering which may result in increased dentinal sensitivity. Present study was carried out using 150 individuals (118 men, 32 women; age range 25-56 years) who are having a habit of chewing tobacco from 5-25 years were tested along with 150 controls with same age group. Samples were cultured and prepared for chromosomes and DNA was extracted and gel electrophoresis was done to find out the DNA damage levels. In this study we have scored the numerical and structural chromosomal abnormalities and DNA damage of the presented samples have been calculated. Although significant numerical aberration observed, structural abnormalities were notably seen in a few cases. When compared with controls tobacco-chewing habitués does increase induced significant mitodepression. The demonstration of chromosomal damage in lymphocytes from tobacco chewers strongly indicates that tobacco should be categorized as a human carcinogen and major risk factor for oral, throat and pharynx cancers.

KEYWORDS : DNA, genotoxic, cytotoxic, tobacco, chromosomes

1.Introduction

Traditionally the people of south eastern India and the eastern mediterranean have used a number of different plant products for chewing (1). Tobacco when it was introduced into this part of the world in the 16th century was, therefore readily accepted as an additional ingredient in the various chewing mixtures. The forms in which tobacco is taken orally vary widely in their manner of preparation, ingredients and the manner in which they are used. Much of the tobacco in the world is used without combustion. Rather it is placed in contact with mucous membrane, through which nicotine is absorbed to provide the pharmacological effect (2). The tobacco and lime mixture is probably the most common variant of chewing tobacco without betel nut .The mixture is known as 'khaini' in the northern part of India, and it is popular in other parts as well (3). To prepare the quid, the user places a small amount of tobacco in the palm; a dash of lime is flicked by a thumb or forefinger, and it is mixed and rubbed vigorously with the tobacco in the hand. The mixture is then ready for use and is placed in the mouth. The exact placement of the tobacco and lime mixture in the mouth varies among the people of different by regions. The most common sites of oral cancers and precancers also vary correspondingly in those regions (4).

The oral cavity suffers from potentially harmful effects due to the habit of tobacco and tobacco mixtures chewing (5). The main effects on the hard tissue of the oral cavity are on the teeth. It caused severe wearing of the enamel covering which may result in increased dentinal sensitivity (6). Root fractures have also been reported in long term chewers probably as a result of increased masticatory load and repetitive masticatory stress during chewing betel quid with tobacco (7,8). Discolouration of teeth with brownish staining occurs in chronic users with poor oral hygiene, Report on the effect of betel quid chewing on dental caries is contradictory (9). Some studies have reported that prevalence of dental caries is higher in non chewers than in chewers, while others have shown that there is no difference in the occurance of dental caries in betel quid chewers and non chewers (10).

Incidence of gingivitis has been found to be higher among betel quid chewers with tobacco. Loss of periodontal attachment and calculus formation has been found to be higher in betel quid with tobacco chewers (11). Areca nut chewing activates sympathoadrenal response and increases plasma concentration of adrenaline and nor adrenaline. It also increases central sympathetic activity in humans, leading to increased heart rate and increased blood flow through the common and external carotid arteries (12). Increase in serum homocysteine levels which is a risk factor for heart disease and betel quid chewing has been reported to be associated with homocysteine level in chewers. Exposure to environmental carcinogens can induce DNA lesions; elicit infidelity of DNA repair, and cause the instability phenomenon, and subsequent consequences as e.g., chromosomal breakage syndromes and neoplastic diseases (13). Chromosomal breakage syndromes are since the environmental chemical exposure level continuously increases with the increasing number of existing and commercially available chemicals (14). Tobacco has addictive properties due to the presence of nicotine which causes signs of dependence of tolerance in the consuming adult (15). The extent and increasingly speed of the tobacco addictions are believed to be directly proportional to the biological strength of nicotine addiction. Follow-up investigation of the frequency of structural and numerical Chromosome Aberrations (CA) and DNA damage in peripheral blood samples from Guntur General Hospital.

2.Methodology

EDTA and Sodium heparinised peripheral blood samples were collected from 150 individuals (118 men (78.7%) and 32 women (21.3%), 25-55 age group who are having a habit of chewing tobacco from 5-25 years were tested along with 150 controls with same age and gender matched with no history of exposure to clastogenic and/or aneugenic agents and of socio-economic level also similar to that of the experimental subjects. The study was done in the period of December 2016 to August 2017 and samples were collected from who consulted the Dept. of Dental Surgery, GGH, Guntur for dental check up. Informed consent was taken from the patients. These individuals were interviewed about their tobacco use and examined for the presence of oral leukoplakia and other precancerous lesions. The study procedures used in the present study were approved by the local ethical committee. Sodium heparin blood samples were cultured and prepared chromosome using standard protocol. DNA was extracted using Quiagen DNA extraction kit from EDTA blood and extracted DNA samples were processed for gel electrophoresis (2, 4).

2.1 Microscopic Evaluation

Chromatid and chromosomal breaks and fragments were the most frequent chromosomal aberrations found. Results were expressed as percentage of aberrant cells. Scoring of the all parameters including MI is done using Upright Light Microscope.

3. Results

The present study involves 150 samples of tobacco chewing

population, have been tested for genotoxic and cytotoxic effects with 150 sample with same age groups from Government General Hospital, Guntur. In this study we have studied the numerical and structural abnormalities of chromosomes have been scored (Figure 1). And also Mitotic Index (MI) and DNA damage of the participating samples have been calculated (Figure 1- 3) (Table 1). No significant numerical aberrations have been observed. But structural abnormalities are very significantly seen in few cases. Genotoxicology monitoring including cytogenetic investigations has been performed in several control and tobacco chewers as it is summarized on Table including the control data.

Table shows Mitotic Index, chromosomal aberrations of cultures from the samples of tobacco chewing population. The mean value of MI of control is 5.2303 whereas samples Mitotic Index are 2.6019. When compared to control values tobacco chewing habitues samples does increase induced significant mitodepression. Significant DNA damage or DNA fragmentation was observed in subjects when compared to the control population.

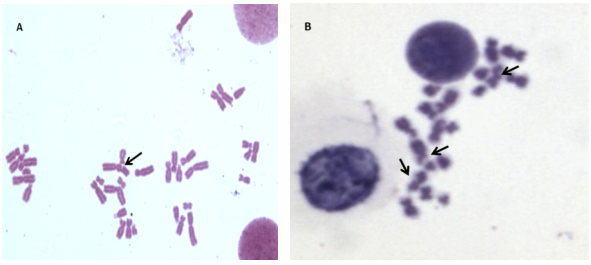

Fig-1(A) shows the chromosomal break in chromosome No.1.
Fig-2(B) shows the chromosomal break and gaps in chromosomes

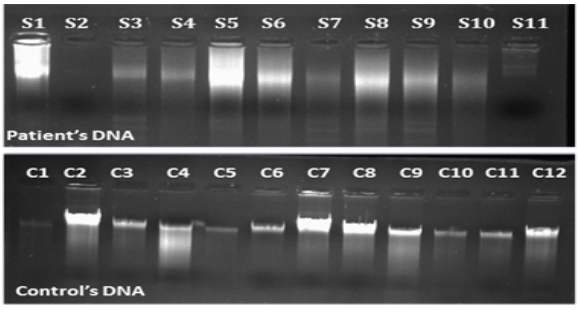

Fig-3 shows the DNA damage in patient's in samples when compared to the control group.

Table 1. Mitotic index, chromosomal aberrations of cultures

Category	No. Of samples	No. of metaphases scored per sample	Mitotic Index	Chromosomal breaks and gaps Mean ± S.E
Controls	150	100	5.2303 +/- 0.63567	1.5074 +0.65919
Tobacco chewers	150	100	2.6019 +/- 0.5163	22.4103 +2.3125*

The mean chromosomal anomalies frequency in control is 1.5074, whereas tobacco chewing population 22.4103 chromosomal breaks and gaps per metaphase on an average.

Discussion

In our study we have received surprising results. We found structural abnormalities, but no detectable levels of numerical abnormality. Table reveals the genetic damage in tobacco chewing habitués. Chemical constituents in the tobacco leaf such as NNK and PAHs require metabolic activation to exert their carcinogenic effects (16-18); there are competing detoxification pathways, and the balance between metabolic activation and detoxification differs among individuals and will affect cancer risk (17). Statistical analysis indicates there is marginal increase or significant increase in chromosomal abnormality rates in observations (19). Chewing tobacco is highly addictive. In the duration of a half hour chew, the average smokeless tobacco user ingests an amount of nicotine which is equivalent to the amount in 4 cigarettes Chamberlain (20). It would take nearly 60 cigarettes to equal the amount of nicotine in a single can of chewing tobacco. In the present study Significant DNA damage or DNA fragmentation was observed in subjects when compared to the control population. Our findings may indicate an emerging public health problem, since our subjects were young and adult have lesions that could be signs for increased risk of developing oral malignancies (21).

5. Conclusion

Various studies showed that the tobacco chewing and tobacco smoking effects on bio molecule damage. In the present context we have under taken the study to assess the DNA damage using 150 peripheral blood samples from tobacco chewing habitues for in vitro cultures. The present study provides the reproducible evidence regarding genotoxicity and cytotoxicity of tobacco consumption. The demonstration of chromosomal damage and DNA damage in lymphocytes from tobacco chewing habitues strongly indicates that tobacco should be categorized as a human carcinogen and major risk factor for oral cancer and throat and pharynx cancers. In view of these findings, the present study indicates that tobacco users should be considered a high risk group and need to be monitored for health hazards including cancer (20-22).

6. References

1. Agrawal S, Karan A, Selvaraj S, Bhan N, Subramanian SV, Millett C. Socio-economic patterning of tobacco use in Indian states. Int J Tuberc Lung Dis 2013;17:1110-7.
2. Braakhuis BJ, Nieuwint AW, Oostra AB, Joenje H, Flach GB, et al. (2016) Sensitivity to chromosomal breakage as risk factor in young adults with oral squamous cell carcinoma. *J Oral Pathol Med* 45: 189-192.
3. Burrell RA, McGranahan N, Bartek J, Swanton C (2013) The causes and consequences of genetic heterogeneity in cancer evolution. *Nature* 501: 338-345.
4. Chandirasekar R, Kumar BL, Sasikala K, Jayakumar R, Suresh K, et al. (2014) Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. *Mutat Res Genet Toxicol Environ Mutagen* 767: 21-27.
5. DeMarini DM. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. Mutat Res 2004;567:447-74.
6. Gupta P C, Pindborg JJ and Mehta F S 1982 Comparision of carcinogenicity of betel quid with and without tobacco: an epidemiological review; Ecol. Dis.1 213-219.
7. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144:
8. 646-674.
9. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancers. Nat Rev Cancer 2003;3:733-44.
10. Johnson N. Tobacco use and oral cancer: a global perspective. J Dent Educ 2001;65:328-39.
11. Kashyap B, Reddy PS. Micronuclei assay of exfoliated oral buccal cells: means to assess the nuclear abnormalities in different diseases. J Cancer Res Ther 2012;8:184-91.
12. Li CC, Shen Z, Bavarian R, Yang F, Bhattacharya A (2018) Oral Cancer: Genetics and
13. the Role of Precision Medicine. *Dent Clin North Am* 62: 29-46.
14. Niaz K, Maqbool F (2017) Smokeless tobacco (paan and gutkha) consumption, prevalence, and contribution to oral cancer. *Epidemiol Health* 39: e2017009.
15. Proia NK, Paszkiewicz GM, Nasca MA, Franke GE, Pauly JL. Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer--a review. Cancer Epidemiol Biomarkers Prev 2006;15:1061-77.
16. Sapkota A, Gajalakshmi V, Jetly DH, Roychowdhury S, Dikshit RP, Brennan P, et al. Smokeless tobacco and increased risk of hypopharyngeal and laryngeal cancers: a multicentric case-control study from India. Int J Cancer 2007;121:1793-8.
17. Silverman S. Oral Cancer. 5th ed. Atlanta, USA: American Cancer Society; 2003.
18. Strickland SS. Anthropological perspectives on use of the areca nut. Addict Biol 2002;7:85-97.
19. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat Res 1992;271:69-77.
20. World Health Organization (WHO). WHO Report on the Global Tobacco Epidemic, 2008: The MPOWER package. Geneva: WHO; 2008.
21. World Health Organization (WHO). The global burden of disease: 2004 update. Geneva: WHO Press; 2008.
22. World Health Organization, Tobacco Free Initiative (TFI). Tobacco facts [Internet]. 2005 [cited 2014 Aug 10]. Available from: http://www.who.int/tobacco/mpower/tobacco_facts/en/