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Original Research Article

Assessment of Micronuclei Frequency in Individuals with a Habit of Tobacco Chewing by Means of Exfoliated Oral Buccal Cells

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Abstract

Background: The majority of malignancies are discovered at an advanced stage, which results in low patient survival rates. Therefore, it appears that early diagnosis of oral malignancies is urgently needed. Micronuclei (MN) of exfoliated buccal cells can be examined to detect genetic damage.

Aim: To carry out assessment of micronuclei frequency in individuals with a habit of tobacco chewing by means of exfoliated oral buccal cells.

Methods and Materials: A total of 100 subjects were examined in the study, which were divided into 2 groups. Each group had 50 subjects. Group1 comprised of the control group which included tobacco non user's individuals i.e. those who are without any habit and Group 2 comprised of smokeless tobacco users. This study was performed to observe and calculate MN index in oral epithelial cells and to compare MN index within above mentioned groups employing feulgen reaction for evaluation of micronuclei.

Results: Maximum mean MN count per 500 cells were in group – 2 (91.40$\pm$29.17) followed by group – 1 (28.96 $\pm$11.20).One way ANOVA test found that mean MN count per 500 cells were not alike in both groups. Post Hoc Turkey HSD Test found in pair wise comparison that there was statistically significant difference ($p<0.05$), between group 1, group 2 with respect to MN count per 500 cells. Table 2 shows that among group 3 subjects, 76% were gutka chewers, 14% were having habit of chewing gutka along with paan, while 6% subjects used gutka in combination with masala, and 4% participants were khaini users. According to table no 3 mean MN count per 500 cells among khaini users was highest (115.00$\pm$24.04) followed by mean MN count per 500 cells (104.33$\pm$19.63) in case of subjects using combination of gutka and masala.

Conclusion: The Micronuclei assay is a useful tool for assessing the severity of premalignant and malignant lesions in tobacco chewers. The micronucleus count is a non-invasive technique used for diagnosis, patient education, widespread population screening, and therapy effectiveness testing.

Keywords: Micronuclei, Tobacco Chewers.

Background

The tenth most common form of cancer in the world is oral cancer. The world's highest rates of tobacco use are found in low- and middle-income nations. China is the world's largest marketer of tobacco, with more than 300 million smokers followed by India having across over 100 million tobacco users. The third most common type of cancer in India is oral cancer. 2 In India, chewing tobacco is more popular than smoking it, particularly in rural regions [1,2]. In 2012, 75,000 to 80,000 new cases of oral cancer were recorded and by 2025, more instances are anticipated. As a result ofthe majority of these malignancies being discovered at an advanced stage, patients have a dismal prognosis and survival rate. As a result, early and prompt identification of oral cancer becomes necessary [3,4].

Micronuclei (MN) from exfoliated oral cells can be examined to detect genetic damage in the general population. This test, which was initially developed by Stitch *et al*., is continuing to gain favour as a biomarker to identify genetic damage due to its inexpensive cost, low level of invasiveness, ease of storage, and convenience of slide fabrication [5]. Micronuclei are defined as cytoplasmic chromatin masses that may be seen under a microscope, have a round to oval form, and are present close to the nucleus. During cellular division, a little extra nucleus is separated from the main one. During cell division, it develops during the metaphase/anaphase transition phase. These structures resemble those found in cellular nuclei during interphase and can be seen in those nuclei [6]. Therefore, the purpose of the current investigation was to assess the genotoxic effects of chewing tobacco using exfoliated buccal mucosa cells and a micronucleus assay.

Methods and Materials

A total of 100 subjects were examined in the study, which were divided into 2 groups.Each group had 50 subjects. Group1 comprised of the control group which included tobacco non user's individuals i.e. those who are without any habit and Group 2 comprised of smokeless tobacco users. This study was performed to observe and calculate MN index in oral epithelial cells and to compare MN index within above mentioned groups employing feulgen reaction for evaluation of micronuclei.

All subjects provided their written informed permission. The concerned institutional ethical committee gave its prior consent. Smoking cigarettes and/or using tobacco for at least the previous six months were a requirement for participation for both smokers and tobacco chewers. Subjects with a history of a recent viral infection, those who had taken antibiotics within two monthsof the sample collection, those who had recently received radiation therapy, chemotherapy, or other potentially genotoxic occupational exposures, and those who had any oral pathological lesions were excluded from the study. Sample, staining, and cytological analysis: Each individual was asked to thoroughly rinse their mouths with tap water prior to sampling. Using a wet wooden spatula, oral smears were made from the exfoliated oral epithelial cells of the buccal mucosa of the control and study groups. For each subject, slide was prepared and stained with feulgen.

Each individual had at least 1,000 cells seen, and MN frequency was graded using Tolbert *et al* criterias. To be classified as a micronucleus, the suspected nucleus must satisfy the following requirements: (a) a rounded, smooth perimeter suggestive of membrane; (b) a diameter less than one-third that of the main nucleus, but large enough to distinguish shape and colour; (c) staining

intensity similar to that of the nucleus; and (d) the same focal plane as the nucleus.

Analytical statistics

With SPSS version 20, statistical analysiswas carried out. The results are shown as a mean and a standard deviation. One way ANOVA was used to analyse the mean difference in micronuclei between the three groups, and a p value of less than 0.05 was regarded as statistically significant.

Results

Table 1 shows that maximum mean MN count per 500 cells were in group – 2 (91.40±29.17) followed by group – 1 (28.96 ±11.20). One way ANOVA test found that mean MN count per 500 cells were not alike in both groups. Post Hoc Turkey HSD Test found in pair wise comparison that there was statistically significant difference ($p<0.05$), between group 1, group 2 with respect to MN count per 500 cells. Table 2 shows that among group 3 subjects, 76% were gutka chewers, 14% were having habit of chewing gutka along with paan, while 6% subjects used gutka in combination with masala, and 4% participants were khaini users. According to table no 3 mean MN count per 500 cells among khaini users was highest (115.00±24.04) followed by mean MN count per 500 cells (104.33±19.63) in case of subjects using combination of gutka and masala. Individuals having a habit of using gutka alone showed mean MN count/500 cells (92.32±31.07) which was lower than above two categories but study participants using combination of gutka along with paan showed lowest mean MN count/500 cells(74.14±12.28) as compared to all other categories in group – 2.

ANOVA test was applied which showed that there existed a significant difference ($p<0.05$) in mean MN count/500 cells between individuals using various categories of chewing tobacco in group 2. Table 4 indicates that average duration of habit of tobacco chewing was 12.50±7.40. Similarly, average quantity (number of packets) per day consumed by tobacco chewers in group 2 was 8.18±6.09 which relates with total amount of tobacco consumed per day (2.69±2.55) on an average. When this was expolated on yearly based consumption of tobacco, it came out to be 983.31±929.79. When correlation between various tobacco chewing or tobacco use indicators and MN count per 500 cells was calculated, significant positive correlation ($p<0.05$) with all parameters was observed.

Average total quantity per day and average duration (years) of tobacco chewing habit were most correlating indicators with values of 0.6172 and 0.6425 respectively. (Table 5)

Table 1: Comparison of study groups w.r.t. MN count per 500 cells.

Group	N	Mean	SD	Median	Min.	Max.	95% CI	'p' Value*
Non-tobacco	50	28.96	11.20	26	15	55	25.777-	<0.001
Users (1)							32.143	
Tobacco	50	91.40	29.17	85	45	180	83.11-	
chewers (2)							99.69	

* ANOVA - Analysis of Variance **Post hoc Tukey HSD Test

Table 2: Distribution of tobacco chewers (group 2) according to type of chewing tobacco used.

Tobacco type	No.	%
Gutka	38	76.00
Gutka+Masala	3	6.00
Gutka+Paan	7	14.00
Khaini	2	4.00
Total	50	100.00

Table 3: Comparison of type of chewing tobacco used with MN count/500 cells within group 3.

Tobacco type	N	Mean	SD	Median	Min.	Max.	95% CI	'p' Value*
Gutka	38	92.32	31.07	85	45	180	82.105 - 102.527	0.225
Gutka+Masala	3	104.33	19.63	93	93	127	55.570 - 153.097	
Gutka+Paan	7	74.14	12.28	79	56	89	62.785 - 85.500	
Khaini	2	115.00	24.04	115	98	132	-101.005 - 331.005	

* ANOVA - Analysis of Variance

Table 4: Habit indicators of smokeless tobacco chewers (group 2)

	Mean	SD
Average quantity (no.)/day	8.18	6.09
Average Tobacco Qt/day	2.69	2.55
duration of habit(yrs)	12.50	7.40
Average Cumulative Consumption of tobacco	15270.87	23512.26
Average yearly Consumption of tobacco	983.31	929.79
Duration in mouth	16.30	5.79

Table 5: Correlation of MN per 500 cells with various habit indicators of tobacco chewers.

	N	Correlation coefficient	'p' Value	95% Confidence interval for r
Average Cumulative Consumption of tobacco	50	0.5708	<0.0001	0.3477 to 0.7327
Average quantity (no.)/day	50	0.6172	<0.0001	0.4091 to 0.7642
Average Tobacco Qt/day	50	0.5207	0.0001	0.2834 to 0.6979
Average yearly Consumption of tobacco	50	0.5207	0.0001	0.2834 to 0.6979
duration of habit(yrs)	50	0.6425	<0.0001	0.4434 to 0.7811
Duration in mouth	50	0.3112	0.0278	0.03600 to0.5426

Discussion

In order to identify chromosomal loss or mitotic spindle failure brought on by aneugenic causes, the Micronuclei assay may be an excellent biomarker. The usefulness of this test for this reason has been emphasised in numerous research.With the aid of a micronucleus assay onbuccal mucosa cells that had been exfoliated, the current study sought to assess the genotoxic consequences of chewingtobacco [7].

In this study, group 2 had the highest mean MN count per 500 cells (91.40+29.17),

followed by group 1 (28.96 +11.20).

The mean MN count per 500 cells was not the same in both groups, according to a one- way ANOVA test. In a pair wise comparisonusing the Post Hoc Turkey HSD Test, it was discovered that there was a statistically significant difference (p 0.05) between group 1 and group 2 in terms of the MN count per 500 cells. In group 3, 76% of the participants chewed gutka; 14% did the same with paan; 6% did so with masala; and 4% used khaini. The highest mean MN count per 500 cells was found among khaini users (115.00+24.04), followed by subjects who combined gutka and masala (104.33+19.63). Those who regularly use gutka alone had mean MN counts per 500 cells that were lower than those in the previous two groups (92.32+31.07), whilethose who combined gutka and paan had the lowest mean MN counts per 500 cells (74.14+12.28) of any of the other categories in group 2.

ANOVA test was applied which showed that there existed a significant difference ($p<0.05$) in mean MN count/500 cells between individuals using various categoriesof chewing tobacco in group-2. Average duration of habit of tobacco chewing was 12.50$\pm$7.40. Similarly, average quantity (number of packets) per day consumed by tobacco chewers in group 2 was 8.18$\pm$6.09 which relates with total amount of tobacco consumed per day (2.69$\pm$2.55) on an average. When this was expolated on yearly based consumption of tobacco, it came out to be 983.31$\pm$929.79. When correlation between various tobacco chewing or tobacco use indicators and MN count per 500 cells was calculated, significant positive correlation ($p<0.05$) with all parameters wasobserved.

The two indicators with the highestcorrelation coefficients, average daily consumption and average chewing habit duration (years), had values of 0.6172 and 0.6425, respectively. The buccal mucosa is negatively affected by tobacco use, including chewing and smoking. The three most harmful substances found in tobacco are nicotine, tar, and polycyclic hydrocarbons [8]. Based on a higher incidence of micronuclei in tobacco chewersand tobacco chewers who also smoke, this study discovered that tobacco has significant genotoxic effects on the buccal epithelial cells in both smoking and smokeless types. These results are entirely consistent with prior studies. Kassie F et al. investigated the interaction between chewing tobacco use and tobacco smoking on the production of micronuclei in oral epithelial cells [9].Tobacco soluble saliva compounds mayenter the basal layer of the epithelium and interfere with the reproductive system of the underlying actively dividing cell population,leading to genotoxicity and nuclear aberration formation, according to a statement made by Livingston GK et al [10].

Nagler R et al. added that the associations between the less reactive free radicals produced during chewing and smoking tobacco and the active reactive metals in saliva increase the likelihood of genotoxicity. In their study, Palaskar et al. discovered that those who use smokeless tobacco have a higher incidence of micronuclei than people who use traditional tobacco [11].

The oral buccal cell micronucleus is considered as an indicator of chromosomal damage that is typically brought on by genotoxic chemicals originating from nicotine, tobacco, and alcohol-related compounds [12,13]. The development of micro nucleated cells as a result of these carcinogens' harmful effects is a representation of such chromosomal damage. Because oral mucosal cells are a readily accessible tissue that can be harvested without putting patients under stress, adverse effects are typically observed in these cells. The greater frequency of nuclear alterations in oral mucosal cells from smokers and drinkers raises the possibility of a high-risk oral cancer population. Thus, it appears that analysing the micronucleus within the oral epithelial cells is an appropriate method for the research's objectives [14,15].

Conclusion

The Micronuclei assay is a useful tool for assessing the severity of premalignant and malignant lesions in tobacco chewers. The micronucleus count is a non-invasivetechnique

used for diagnosis, patient education, widespread population screening,and therapy effectiveness testing.

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