Journal of Pathology of Nepal (2021) Vol. **11**, 1847 - 1851

Journal of
PATHOLOGY
of Nepal

www.acpnepal.com

Original Article

Frequency of micronuclei in oral mucosal smears of female municipal sweepers with betel quid chewing practice

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Keywords:

Betel quid;
Hard palate;
Micronuclei;
Oral cancer;

ABSTRACT

Background: Oral cancer is common worldwide. Study of micronuclei in exfoliated buccal mucosal cells holds great scope in bio monitoring their carcinogenic potential. Betel quid chewing is one of the most common factors contributing to oral cancers. Ours being a developing country, a study was undertaken among female municipal sweepers, majority of who are betel quid chewers, unaware of the harmful effects of the same. We attempted to cytologically access the presence and compare the proportion of micronuclei in the right, left buccal mucosa and hard palate of female municipal sweepers with and without betel quid chewing practice.

Materials and Methods: This was a cross sectional study with purposive sampling. Buccal smears were analysed from 30 betel quid chewers & non chewer females for micronuclei. Mean and standard deviation were calculated. Independent T test was used for comparison between groups. P value less than 0.05 was considered statistically significant.

Results: Frequency of micronuclei in betel quid chewers and non-chewers was 26.80+/-11.96 and 7.76+/-4.73. The frequency of total micronuclei per 250 cells in cases was 9.3+/-5.37 in right buccal mucosa, 9.43+/-5.66 in left buccal mucosa, 9.43+/- 5.66 in hard palate and 3.33+/- 2.88 in right buccal mucosa, 2.96+/-3.31 in left buccal mucosa, and 2.96+/-3.31 in hard palate in controls.

Conclusion: The mean number of micronuclei in buccal mucosal cells of female municipal sweepers with betel quid chewing practice was significantly higher compared to controls reaffirming their carcinogenic potential and highlighting the need of early screening for oral cancer in susceptible groups.

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Reveived : October 1st 2020 ; **Accepted** : November 2nd 2020

Citation: Shetty A, Verma C, Chowdappa VJ. Frequency of micronuclei in oral mucosal smears of female municipal sweepers with betel quid chewing practice. Pathol Nep 2021;11:1847-51. DOI: 10.3126/jpn.v11i1.31677

INTRODUCTION

Oral cancer is the 6th most common cancer worldwide[1] and constitutes the third most important group of malignancies in India.[2] As per data available from National Cancer Registry Programme, females from our city show the highest age adjustment rate.[3]

It is estimated that betel quid is the fourth most addictive substance in the world after tobacco, alcohol and caffeine.[4] Over 600 million people worldwide constituting about 10% of the world's population, chew betel quid.[5] The International Agency for Research on Cancer (IARC) in 2004 has classified areca nut as group 1 carcinogen 6

with elevated risks noted for oral and pharyngeal cancers.[7] Though the custom of chewing betel quid has existed for thousands of years, modern studies have shown that there is a strong relationship between areca nut/ betel quid chewing and oral cancer.[8] In India, it has been observed that specific occupational groups such as truck drivers, daily wage workers, and municipal sweepers are fond of betel quid chewing habits unaware of its harmful effects.[7] Evaluation of the genotoxic risks in betel quid chewers on the buccal mucosa, observed as DNA damages can be assessed by exfoliated cytology such as micronuclei test.[9] Micronuclei assay is a sensitive, non-invasive and low-cost technique that has been used as a biomarker for the assessment of DNA damage.[10]

Various studies have documented a rate of 16 -37% women with betel quid chewing practice in various regions of India, the higher prevalence being in those of rural, low-income categories and with manual occupations. One such manual occupation is that of municipal sweepers, the majority of who are females.[11] There is paucity in the literature about studies done exclusively in the female population of sweepers with betel quid chewing practice, hence in this, we made an attempt to cytologically assess the presence of micronuclei in exfoliated oral mucosal cells obtained from buccal smear and also to compare the frequency of micronuclei in betel quid chewers and non- chewers in three different sites of the oral cavity.

MATERIALS AND METHODS

This was a cross-sectional study with a purposive sampling method done over a period of two months. The study population was that of female municipal sweepers (also called Paurakarmikas). Study groups: Group I (Cases):30 female betel quid chewers, Group II (Controls): 30 age-matched healthy females with clinically normal oral mucosa with no practice of chewing. Ethical clearance was obtained from the institutional ethical committee. IRC number:(SIMS&RC/IECC/35/2019)

Inclusion criteria: Female municipal sweepers with the practice of chewing betel quid for equal to or more than 10 years.

Exclusion criteria: Females with a history of alcoholism/ smoking, history of previously treated precancerous/ cancerous lesion of the oral mucosa, and consumption of carcinogenic solvents.

Sample collection was done after obtaining consent from the subjects. Basic data on betel quid chewing practice and demographic details were noted. Procedure for taking smear: The subjects were asked to rinse their mouth with water. Buccal mucosal cells were scraped from the right and left buccal mucosa and hard palate (3 slides per case) with a wooden spatula and spread on the slides to collect the cells. The slides were fixed in 95% ethyl alcohol. Cytological staining: PAP stain was used as it gives good nuclear chromatin details and cytoplasmic transparency. Counting the cells: Initially, the slides were focused at 40X magnification under a microscope to observe for adequacy. A thinly spread area with minimal overlapping of cells was chosen for counting. A minimum of 500 cells was counted in an orderly zig-zag manner. Then under 100X Magnification, micronuclei were assessed and the number of micronuclei per 250 cells was calculated.

Scoring criteria: Tolbert et el[12] developed the criteria for identifying micronuclei which consist following parameters:

1. Rounded smooth perimeter suggestive of a membrane
2. Less than a third the diameter of the associated nucleus, but large enough to discern shape and color.
3. Staining intensity to the nucleus
4. Texture similar to the nucleus
5. Same focal plane as the nucleus
6. Absence of overlap with a bridge to the nucleus

Statistical analysis was done using SPSS version 20. The findings were presented as mean and standard deviation. An Independent T-test was used for comparison between groups. A P-value less than 0.05 was considered statistically significant.

RESULTS

The study was a definitive expression of micronuclei in cells cultivated from each smear. Micronuclei were single or multiple in many cells (fig. 1A and B). The micronuclei frequencies are presented in Tables1 and 2. As per the age-adjusted group majority of the females were from the third decade of life. Also, when calculated the total average of micronuclei (right, left buccal mucosa & hard palate together in a single study participant) in chewers was found to be 26.80+/- 11.96 and that in non-chewers was found to be 7.76+/- 4.73. As per the above table, a highly significant difference was noted between the frequency of micronuclei among chewers and non-chewers ($p < 0.001$)

DISCUSSION

Betel quid has an immense role in changing the oral pathology and in the biogenesis of cancer.[13,14] Bloching et al. suggested micronuclei to be a cellular alteration formed due to genotoxic effects of the potential carcinogens which can be used as a prognostic biomarker of cancer.[15] In a

Table 1: Frequency of micronucleated cells in controls

Right buccal mucosa			left buccal mucosa			Hard palate		
Frequency Of MN	No. Of controls with MN	% controls	Frequency of MN	No of controls	% controls	Frequency of MN	No. Of controls	% controls
0	3	10	0	4	13.33	0	9	30
1	6	23.33	1	12	40	1	5	16.66
2	8	26.66	2	3	10	2	5	16.33
3	3	10	3	4	13.33	3	4	13.33
4	1	3.33	4	2	6.66	4	1	3.33
5	4	13.33	5	1	3.33	5	2	6.66
6	2	6.66	6	1	3.33	6	1	3.33
7	1	3.33	7	1	3.33	7	1	3.33
8	0	0.00	8	1	3.33	8	1	3.33
10	2	6.66	10	0	0.00	10	1	3.33
11	0	0.00	11	1	3.33	11	0	0

MN= Micronuclei

Table 2: Frequency of micronucleated cells in cases

Right buccal mucosa			left buccal mucosa			Hard palate		
Frequency Of MN	**No. Of controls with MN**	**% Cases**	**Frequency of MN**	**No of cases**	**% cases**	**Frequency of MN**	**No. Of cases with MN**	**% cases**
3	2	6.66	3	1	3.33	3	4	13.33
5	5	16.66	5	4	13.33	4	4	13.33
6	1	3.33	6	3	10	5	2	6.66
7	3	10	7	4	13.33	6	4	13.33
8	4	13.33	8	4	13.33	8	5	16.66
9	1	3.33	9	3	10	9	3	10
10	3	10	10	3	10	10	4	13.33
12	2	6.66	12	1	3.33	12	1	3.33
13	0	0.00	13	1	3.33	13	0	0.00
14	2	6.66	14	1	3.33	14	0	0.00
15	3	10	15	1	3.33	15	1	3.33
22	1	3.33	22	2	6.66	22	2	6.66
25	1	3.33	25	1	3.33	25	0	0.00

MN= Micronuclei

study conducted by the international collaborative project on micronuclei, frequency in different populations and cell types, an increase in the micronuclei frequency in the target tissues and peripheral lymphocytes in cancer patients was found which further supports micronuclei to be a reliable cytogenetic biomarker.[16] Therefore, in the present study, analysis of micronuclei in the epithelial cells has been used as it is a sensitive method for monitoring genetic damage in the human population. The micronuclei are the chromatin-containing bodies that represent fragments or even whole chromosomes that were not incorporated into the daughter cell nuclei at mitosis.[17]

In the early 1970s, the term 'micronucleus' was first time suggested by Boller and Schmidt and it was Heddle who showed that this is a simple point of identification to detect the genotoxic potential of mutagens after in vivo exposure of animals using the bone marrow erythrocytes.[18] Theodor Boveri originally observed the fact that abnormal nuclear morphologies commonly occur in cancer. Micronuclei are also referred to as Howell-jolly bodies; discovered by haematologist William Henry Howell and Justin Marie jolly in erythrocytes.[19] Oral mucosal smears are one of the time tested quick, inexpensive, and a reliable mode of obtaining a sample to study the micronuclei.

Various studies from 1985 till date have shown a significant increase in the frequency of micronuclei in betel quid chewers as compared to healthy individuals.[19,20] Studies have shown a dose-response relationship, suggesting an increased relative risk of oral cancers with increasing frequency of areca nut chewing.[21] A study by Gene Chen et al

Table 3: p-value of micronuclei per 250 cells

Group		Number	Mean of Micronuclel	Standard deviation	P-Value
Right buccal mucosa	Cases	30	9.30	5.37	<.00100
	Controls	30	3.33	2.88	
Left buccal mucosa	Cases	30	9.43	5.661	<.00100
	Controls	30	2.96	3.316	
Hard Palate	Cases	30	9.43	5.661	<.00100
	Controls	30	2.96	3.316	
Total micronuclei	Cases	30	26.80	11.96	<0.001
	Controls	30	7.76	4.73	

Table 4: Comparison of buccal mucosal micronuclei frequency in various studies

	Fareed et al [25]	Agrawal et al [2]	Jyoti et al [20]	Sudha et al[17]	Present study
Chewers	3.56+/-0.719	44.3+/-12.1	21.3+/-1.788	1.90+/-1.03	26.8+/-11.96
Non-chewers	0.75+/-0.171	41.0+/-14.4	4.56+/-0.331	0.81+/-0.66	7.76+/-4.73

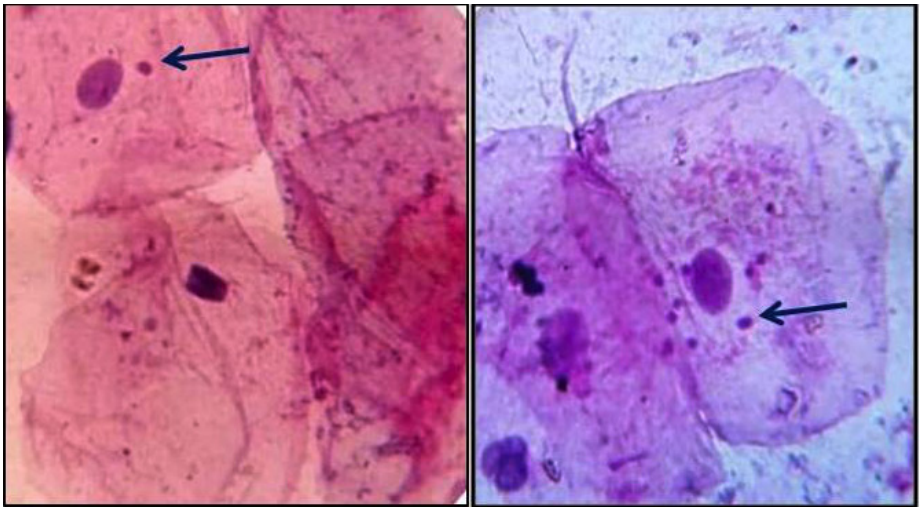

Figure 1: *Buccal mucosal cells showing micronuclei (arrow) (Pap stain, X 400)*

stated a hypothesis that there is a relationship between betel quid chewing and its effect on pregnancy.[22] To prove this hypothesis various studies have been conducted. A research study done in Taiwan pointed out the relationship between betel quid chewing and an adverse birth outcome like low birth weight, spontaneous abortion, and preterm birth among women who chewed betel quid during gestation.[23,24]

All the studies shown above demonstrate a significantly higher number of micronucleated cells in betel quid chewers as compared to healthy individuals. The present study shows a mean micronucleus of 26.8+/- 11.96 which is comparable to the findings of Khan et al[20] who reported a mean value of 21.3+/- 1.788.

Sufficient and compelling evidence shows that the constituents of betel leaf, areca nut, and tobacco have cytogenic, genotoxic, and mutagenic effects on mammals. Similar work was conducted by Kayal et al 1993 in north Indian subjects and it was reported that the chewing of areca nut alone or in combination with betel leaf and lime, caused damage to the oral mucosa.[17] Higher prevalence of betel quid chewing is seen in people of rural, low-income categories and with manual occupations. One such manual occupation is that of municipal sweepers, the majority of who are females, hence this study. The assessment of this category of workers provides an opportunity to assess the relationship between occupation and oral awareness, attitude, and oral health behaviour. At the same time, it underlines the importance of implementing early screening for oral cancers in the above-mentioned susceptible groups.[18,25]

CONCLUSIONS

From the present study, we conclude that the frequency

of micronuclei was found to be significantly increased in betel quid chewers as compared to non-chewers, affirming the fact that habitual female chewers like the municipal sweepers in our society might be at increased risk of developing oral cancers. The findings further highlight the need for increased awareness about the ill effects of habitual betel quid chewing practice among the susceptible population.

Conflict of Interest: None

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