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Assessment of Micronuclei in the exfoliated Buccal Mucosal Cells based on the Personnel behavior among the Transgender Population of Tamil Nadu

B.Krishna Prasanth[1], Krishnan Mahalakshmi[2], R.Umadevi[3], S.Kalpana[4], V.M.Anantha Eashwar[5]

1.Research Scholar, Bharath Institute of Higher Education &Research, Epidemiologist &Assistant Professor Department of Community Medicine, Sree Balaji Medical College & Hospital.
2.Professor & Head, Dept of Microbiology, Sree Balaji Dental College & Hospital/ Research lab for oral and systemic health, Bharath Institute of Higher Education &Research.
3.Professor & Head, Department of Community Medicine, Sree Balaji Medical College & Hospital, Bharath Institute of Higher Education &Research.
4.Research Officer, Department of Epidemiology, The Tamilnadu Dr MGR Medical University.
5.Assistant Professor, Department of Community Medicine,Sree Balaji Medical College & Hospital, Bharath Institute of Higher Education &Research.

Corresponding Author:
Dr.B.Krishna Prasanth,
Epidemiologist & Assistant Professor,
Department of Community Medicine,
Sree Balaji Medical College & Hospital,
Bharath Institute of Higher Education & Research, Chennai.
E-mail-**mail2kristain@gmail.com**

ABSTRACT

Introduction

Research on tobacco usage and its complication among transgender populations is limited. Micronuclei have been used for genotoxicity measurement and biomonitoring of different carcinogens. It acts as a chromosomal damage biomarker. Hence, this study was done to measure and compare the number of micronuclei present in the exfoliated buccal cells of the transgender population who are habituated to tobacco/pan users, alcoholic, both alcoholic and tobacco users and those with none of those habits

Methods

An analytical comparative cross-sectional study was conducted among 120 transgender individuals selected by random sampling in Chennai and were divided into four groups with 30 participants in each group. The first group had a habit of chewing tobacco or pan, second only alcohol consumption, third with both tobacco and alcohol and fourth group who were non-habituated with either of those. After obtaining basic information of the individual, buccal mucosa smear was taken and stained with PAP and Giemsa method. All the data were entered in Microsoft excel and analysis was done using SPSS ver-24.

Results

A total of 120 individuals enrolled in this study. The mean age of the population was 29±4.60 years. While comparing the mean micronuclei count, it was significantly less (mean 5.37 with SD 1.12) among those have the habit of only chewing tobacco or pan. But, among alcoholic and alcohol with tobacco cases had more in number (mean 9.27 with SD 4.12 and 7.10 with SD 4.32). There was a statistically significant difference observed between groups.

Conclusion

Early detection of MN in a cell represents an “internal dosimeter” to estimate exposure to genotoxic and carcinogenic agents.

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Transgender with the habit of chewing tobacco with alcohol are having a greater number of micronuclei than the either tobacco or alcohol users, also having higher risk of developing cancer.

Keywords

Transgender, Tobacco, alcohol, micronuclei

INTRODUCTION

The sixth most common malignancy is oral cancer, and it is considered one of the main causes of cancer morbidity. At least 90 percent of all oral malignancies contain oral squamous cell carcinoma. It is by far the most common oral mucosal malignant disease that is life threatening.[(1)] Human beings are exposed to a large number of physical and chemical agents that can induce a variety of health hazards. Genomic damage is probably the most important fundamental cause of developmental and degenerative disease. It is also well established that genomic damage is produced by environmental exposure various factors and mainly, lifestyle factors (e.g. alcohol, smoking, drugs, chewing tobacco. [(2)]

A fundamental factor in the poor prognosis of oral squamous cell carcinoma is that a large proportion of oral cancers have been diagnosed and treated late in advanced stages. It is possible to detect the impact of carcinogens on an exposed population by performing bio-monitoring studies. Early detection of premalignant or cancerous oral lesions can significantly increase survival and reduce treatment-related morbidity to a substantial degree. This genomic damage evaluation would be an excellent biomarker for determining the exposure effects of various life style factors[(3)].

Biomarkers that measure both cell clastogenicity and aneuploidy are micronuclei. They are small extra nuclear bodies, arising from chromosome fragments or whole chromosomes that lag behind during nuclear division at anaphase, thus not included in the daughter nuclei and enveloped by the nuclear membrane, producing small micronuclei.[(4)] In humans, in erythrocytes, lymphocytes, and exfoliated epithelial cells (e.g. oral, urothelial, nasal), micronuclei can be easily measured to obtain a measure of in vivo mediated genome damage. With a procedure that is minimally invasive and painless, oral mucosa gives easy access to sample collection. [(5)]

Transgender population are one of the neglected special vulnerable groups in India who need to be given special attention. Many micronuclei studies report the age and sex of the study subjects, only few studies were able to establish a statistically significant effect by gender or by age.[(6)] A study done to evaluate the oral mucosal lesions among transgender population and found that majority of transgender, were having the habit of chewing smokeless tobacco containing products such as betel nut, betel quid, gutkha, etc. having the habit of alcohol consumption.[(7)]

There is no data available on micronuclei formation in oral epithelial cells of transgender population. Hence, this study tried to assess the micronuclei formation in the transgender population those who have the habit of drug addict, chewing tobacco and alcohol consumption.

Materials and methods

A comparative cross-sectional study was conducted among 120 transgender population based on their habit of chewing pan/tobacco or alcohol selected randomly by simple random sampling from in and around Chennai city.

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Transgender with suspected cancer, smokers, herpes infection and under treatment for chronic infection were excluded from the study. Cases were enrolled after obtaining informed consent. The participants were divided into four groups with each containing 30 participants. Group one who had only chewing pan or tobacco, group two had the habits of only alcohol intake and group three had both the habits of chewing tobacco and alcohol and fourth group who were not habituated to any form of tobacco usage and alcohol consumption. Clinical examination of the subjects was done and demographic data, history and the relevant details were recorded with the help of a pre-tested structured questionnaire. After obtaining due informed consent, oral mucosal cells were scrapped from clinically normal appearing buccal mucosa in relation to premolar-molar area, above and below the occlusal plane using slightly moistened wooden spatula. The cells were immediately smeared in microscopic slides and were fixed under 95% ethanol. Observation was completed within a week of sample collection. Cytosmear analysis was done by Giemsa stain. PAP staining and processing steps were carried out in room temperature. The procedure developed by Tolbert et al., [8] were followed for micronuclei assessment. According to Tolbert et al procedure, the following extra nuclear cytoplasmic DNA fragments are counted as micronuclei with the following criteria. Micronuclei must be clearly separated from the main nucleus, Micronuclei must have a smooth, oval or round shape, Texture similar to nucleus. The criteria for excluding cells for micronuclei assessment by Tolbert et al., were also followed. The cells with the following features were not taken for micronuclei assessment as Cells with two nuclei, Dead or degenerating cells (karyolysis, karyorrhexis, nuclear fragmentation),Nuclear blebbings (micronucleus- like structure connected with the main nucleus with a bridge), and anucleated cells.

Statistical analysis

All the data were entered in Microsoft excel and analysis were using SPSS-version 24. The mean micronuclei comparison between groups were done by Kruskal - Wallis test, inter group comparison done by chi-square test.

Results

Around 120 transgenders were selected for this study. All the transgenders were transitioned from male to female. The mean age of the selected individuals was 29.5 with SD±4.60 and the age ranged between 20-40 years. Around 75% (90) of them were done their high school level education and 30% (30) were illiterate. The mean age of all the four groups was almost similar. (Table 1)

Table-1: Age distribution among cases and control

Personnel habit of transgender	N	Age	
		Mean age	SD
Pan or Tobacco Chewers (Gr-1)	30	29.65	5.41
Both tobacco Chewer & Alcoholics (Gr-2)	30	29.57	4.06

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Alcoholics (Gr-3)	30	29.05	4.01
Without any habit (Gr-4)- control group	30	30.15	5.45

Table 2: Comparison of micronuclei count among various groups by Pap smear using one way Anova

Groups	N	Pap Smear				F Value	P-value
		Min	Max	Mean	SD		
Pan or Tobacco Chewers (Gr-1)	30	3	9	5.37	1.12	88.52	0.001*
Both tobacco Chewer & Alcoholics (Gr-2)	30	5	25	7.10	4.32		
Alcoholics (Gr-3)	30	4	26	9.27	4.12		
Without any habit (Gr-4)- control group	30	1	2	1.01	0.56		

P Value< 0.05, Statistically significant at 95% Confidence Interval

While comparing the mean micronuclei count identified by PAP Smear, it was significantly less (mean 5.37 with SD 1.12) among those have the habit of only chewing tobacco or pan. But, among alcoholic and alcohol with tobacco cases had more in number (mean 9.27 with SD 4.12 and 7.10 with SD 4.32). While comparing the groups, there was a significant difference observed as p-value 0.001. (Table 2)

The below figure shows the detection of micronuclei by PAP and Giemsa staining. (Fig-1)

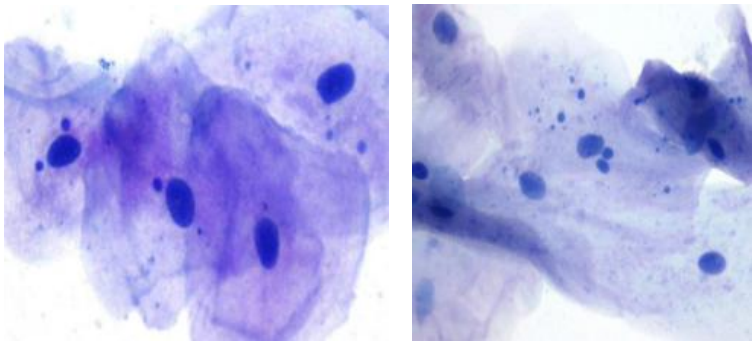

Figure-1: Micronuclei present in buccal mucosa- detected by PAP and Giemsa staining

Table 3: Comparison of micronuclei among various groups using Giemsa Staining using one-way ANOVA

Groups	N	Giemsa Stain				*F value*	P-value
		Min	Max	Mean	SD		
Pan or Tobacco Chewers (Gr-1)	30	3	7	3.17	1.12		

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Both tobacco Chewer & Alcoholics (Gr-2)	30	1	21	4.10	2.69	87.75	0.001*
Alcoholics (Gr-3)	30	0	23	6.07	3.93		
Without any habit (Gr-4)- control group	30	0	1	0.07	0.25		

P Value< 0.05, Statistically significant at 95% Confidence Interval

While comparing the mean micronuclei count by Giemsa staining, there was a statistically significant difference observed between cases and control groups (p value-0.001). (Table 3)

Discussion

According to the World Health Organization (WHO) evaluation, WHO reported that tobacco caused 5.4 million deaths a year worldwide during the 20th century, 100 million tobacco deaths occurred and if the existing trends persist, it is expected to rise to 1 billion during the 21st century.(9) Micronuclei are distinctively individualized structures within the cytoplasm of interphasic cells measuring between one fifth and one third of the size of the main nucleus, observed in the same plane as the nucleus and presenting similar staining and chromatin distribution. Oral mucosa is the first line of contact with different hazardous agents; it provides the first barrier against potential carcinogens; although makes prone to DNA damage, a finding that is relevant since it is estimated that 90% of all cancers are derived from epithelial cells.[(2)]

In this study, the mean age of the selected individuals were 29.5 with SD±4.60 and the age ranged between 20-40 years. Majority of them were done their high school level education. Smoking, alcohol intake and diet are the lifestyle factors that are associated with genetic damage. Several studies have been performed on the basis of various types of tobacco and alcohol. However, there was no evidence available on the induction of micronuclei in transgender people who were currently exposed to various life-style factors such as smokeless/smoking tobacco, intake of alcohol, and unhealthy diet. But, this study findings showed, the mean micronuclei count, was significantly less (mean 5.37 with SD 1.12) among those have the habit of only chewing tobacco or pan. But, among alcoholic and alcohol with tobacco cases had more in number (mean 9.27 with SD 4.12 and 7.10 with SD 4.32). There was a significant difference observed between micronuclei compared between the four groups (p-value 0.001). The synergistic effect of tobacco smoking and alcohol consumption as evidenced by increased micronucleus count was also observed by in 1983 by Stitch and Rosin.[(16)] Most of the previous studies[(10-13)] have also shown that, relative to non-users, subjects who smoked or chewed tobacco of some form had a much higher frequency of oral lesions. In chewing tobacco, smoking tobacco, alcohol consumption in different genders, investigate micronucleus to identify the health risk. This means that genotoxicological biomonitoring is important for the transgender community to be carried out. This study was conducted to evaluate the development of micronuclei due to pan/tobacco chewing and alcohol habits among the transgender population in and around Chennai City. This study shows that the development of micronuclei was high among alcoholics and chewing tobacco alcoholics relative to tobacco chewers alone. It is said that oral activities such as tobacco and alcohol intake are important etiological factors for cytological changes

leading to carcinogenesis. Tobacco and alcohol intake are linked to about two-thirds of squamous cell carcinoma and 75 percent of head and neck cancers.[10] Micronuclei derive from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have not been integrated during mitosis into the daughter nuclei. Chromosomal breakage and Dysfunction of the mitotic apparatus are major mechanisms leading to the micronuclei formation.[14] Two fates for micro nucleated cells are premature chromosome condensation/chromothripsis and the elimination of micro nucleated cells by apoptosis.[15] In those accustomed to alcohol and chewing tobacco use the higher micronuclei count than either of the behaviors suggests the synergistic effect of chewing tobacco and alcohol to cause further nuclear damage, contributing to malignancy. This observation is confirmed by our findings, which were highly statistically significant (P value < 0.001). As observed in this study, the additive effect of tobacco and alcohol in causing DNA damage leading to further micronuclei formation was recorded earlier. Various staining methods are available to stain micronuclei, of which PAP and Giemsa are most commonly used DNA- nonspecific stains. In the analysis of micronuclei, they are usually considered more useful than more costly DNA specific fluorescent acridine orange stain. Further studies are required to assess the micronuclei formation by different tobacco products in different forms and alcohol consumed by the transgender society.

Conclusion

This study was conducted to assess the presence of micronuclei in the buccal mucosa among tobacco users and alcoholic transgender. It can be concluded that due to the synergistic effect of alcohol and tobacco, more micronuclei are present in those consuming both tobacco and alcoholic than those who consume either tobacco or alcohol alone. Detection of micronuclei is a reliable, cost effective and minimally invasive biomonitoring tool for the early detection of oral cancer especially among marginalized population like transgenders.

Conflicts of Interest: None

Ethics committee approval: This study was duly approved by the ethics committee of Sree Balaji Medical College & Hospital, Chennai.

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