

Evaluation of Micronuclei in the Exfoliated Buccal Mucosal Cells in Transgender Population

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ABSTRACT

Introduction: Tobacco and alcohol are chemical carcinogen, has genotoxic effects on buccal mucosal cells. Transgender are highly neglected people, who consume more amount of tobacco products and alcohol. The present study aimed to evaluate the micronuclei in the exfoliated buccal mucosal cells in transgender population in & around the Salem district of Tamil Nadu.

Material and methods: This study comprised 120 transgender which were categorized into 4 groups, each consisting of 30; Group A- who had only chewing tobacco, Group B - who had only alcohol consumption, Group C-both the habits, and Group D - control group. Oral buccal mucosal exfoliated cells obtained from healthy mucosa were evaluated formicronucleus were evaluated by Papanicolaou & Giemsa stain. The results are analyzed.

Results: The mean micronuclei frequency noted in study groups was significantly higher. A higher number of micronuclei was observed in those habituated to both habits. Evaluation of micronuclei in PAP-stained smears showed a higher number of micronuclei than Giemsa Giemsa-stained smears.

Conclusion: We observed a greater number of micronuclei in subjects exposed to combined habits. There appears to be no scientific publication on micronuclei assessment in the transgender population. The findings in our study await corroboration by a study of a larger sample in the transgender population.

Keywords: Transgender, micronuclei, Eunuch, PAP, Giemsa

INTRODUCTION

Oral cancer is the sixth most common malignancy, and it is the most common life-threatening oral mucosal malignant disease.¹ Human beings are exposed to various physical and chemical agents that can lead to various health hazards. Genomic damage is considered as the fundamental cause of various developmental and degenerative diseases. Genetic damage is proven to be produced by environmental exposure to genotoxins, lifestyle factors (e.g. alcohol, smoking, drugs, and stress), medical procedures (e.g. radiation and chemicals), micronutrient deficiency (e.g. folate), and genetic factors such as defects in DNA metabolism and or repair.²

The effects of carcinogens on the exposed population can be identified by conducting biomonitoring studies. The genomic damage evaluation would be an excellent biomarker for determining the exposure effects of various lifestyle factors. Almost 90% of human cancers originate from epithelial cells. Oral epithelial cells act as a target site for early genotoxic events caused by carcinogenic agents entering the body via inhalation or ingestion. Analysis of exfoliated buccal cell micronuclei is a proven method of monitoring damage in the human population, which was first proposed by Stich et al and this test still continues to gain popularity as a biomarker of genetic damage due to its non-invasiveness,

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economical & simple technique.³

Micronuclei are biomarkers that measure both the clastogenicity and aneuploidy of cells. It is a small

extranuclear body, that originates from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division, hence not included within daughter nuclei and get enveloped by a nuclear membrane, thus forming small micronuclei.⁴

The transgender population, also known as eunuchs in western countries or hijras in India are present globally. Eunuchs are neglected special vulnerable groups in India who demand special attention. The word “eunuch” springs from a Greek word that means “keeper of the bed”. Very few studies were available on the oral health status of the transgender population. Moreover, no data concerning the transgender population exists in micronuclei detection. Since the habits of smoking & alcohol consumption are reported to be higher among the transgender,⁵ this provoked us to carry out a study on the transgender population. Hence this study aimed to evaluate the frequency of micronuclei by using two different stains namely PAP & Giemsa stains in the exfoliated buccal cells of the transgender population who are habituated to chewing

tobacco, alcohol consumers and who have both habits and to compare the difference among the same.

MATERIALS AND METHODS

This study was conducted in the Department of Oral Pathology of our institution and from dental clinics situated in the vicinity of the transgender colony and also through camps conducted in the transgender population in the Salem district of Tamil Nadu. Institutional Ethical Clearance was obtained (IEC No. 160/KSRIDSR/EC/2016) before the commencement of the study. The study sample comprised 120 transgender subjects who were randomly selected and enrolled, and informed consent was obtained before the study.

The sample size was estimated by using G power software 3.1.9.4 from a previous article and was estimated as 120 overall which was divided into 30 samples per group. Hence total of four groups were included in the study representing Group A group B group C and group D for the allocation of samples into the group. A random sampling method was implemented in

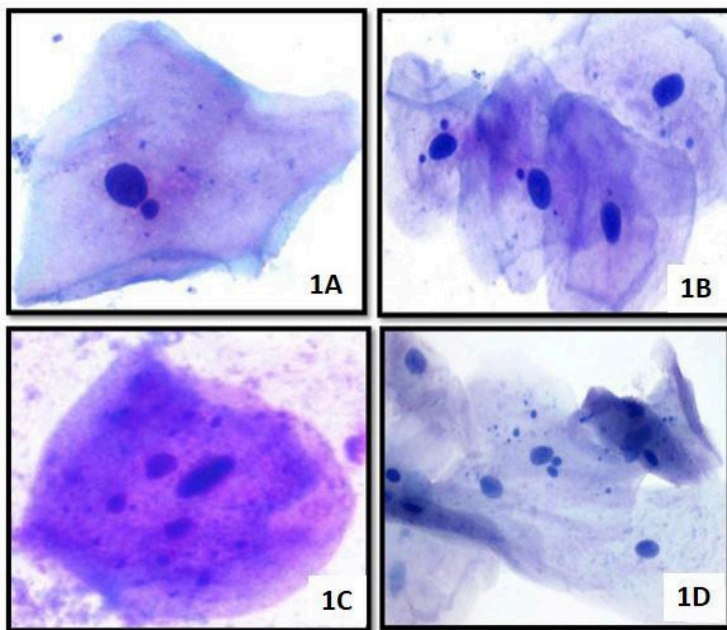


Fig.1: PAP and Giemsa stained micronucleated cells at a magnification of 400X; **1 A** - Giemsa stained smears showing single micronuclei in a cell - (Control group -D); **1 B** - PAP stained smears showing two micronuclei in a cell - (Study group - B); **1 C** -Giemsa stained smears showing three micronuclei in a cell-(Study group - A); **1 D** - PAP stained showing more micronuclei in a cell- (Study group - C)

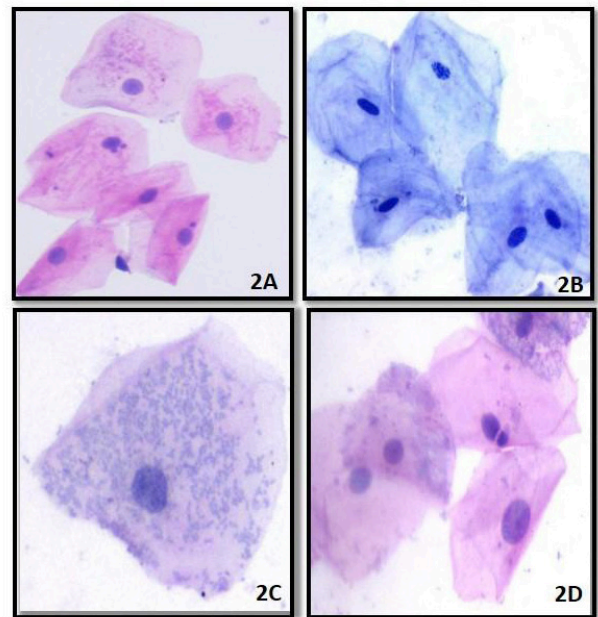


Fig. 2: PAP and Giemsa stained abnormal micronucleated cells at a magnification of 400X; **2A** - PAP stained smear showing nuclear bud; **2B** - Giemsa stained smear showing karyorrhexis. **2C** - Giemsa stained smear showing nuclear debris. **2D**- PAP stained smear showing large diameter micronucleus

TABLE 1: Comparison of mean micronuclei values between the four groups by two different staining (Pap and Giemsa stain)

| Smear | Group A n(30) Mean ± SD | Group B n(30) Mean ± SD | Group C n(30) Mean ± SD | Group D n(30) Mean ± SD | p value |
|-----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---------|
| Pap staining | 4.7±1.92 | 2.8±0.48 | 10.5±5.33 | 1.67±4.43 | 0.001** |
| Giemsa staining | 3.7±1.48 | 2.0±0.69 | 7.07±4.93 | 0.07±0.25 | 0.001** |

the study. The study population was divided into four groups, namely group A – transgender subjects who are habituated to only chewing tobacco (n=30), group B – transgender subjects, who are habituated to alcohol (n=30), group C- transgender subjects who are habituated to both alcohol & chewing tobacco (n=30) and group D - (Control group) - Transgender subjects without any habits (n=30).

Healthy transgender subjects with either chewing tobacco or alcohol consumers or with both habits with clinically normal-appearing oral mucosa were included in this study. Transgender subjects with potentially malignant lesions or conditions suggestive of cancer, subjects under radiation therapy, history of any viral infections, and smoking individuals were excluded from this study. A clinical examination of the subjects was done, and demographic data and the relevant details were recorded. The subjects were asked to rinse their mouth with water. Oral

mucosal cells were scrapped from clinically normal-appearing buccal mucosa in relation to the premolar-molar area, above and below the occlusal plane using a slightly moistened wooden spatula. The cells were immediately smeared onto, pre-cleaned, number-coded microscopic slides, and were fixed in 95% alcohol and stained with PAP and Giemsa stain. Slides were blinded with respect to patient details and were evaluated for the number of micronuclei under 100X magnification in the binocular microscope. (Olympus BX43, Olympus Corporation, Tokyo, Japan) The micronuclei were scored following the criteria outlined by Tolbert et al., 1992. The details were recorded, tabulated, and subjected to statistical analysis. [Figure 1 and 2]

STATISTICAL ANALYSIS

All the parameters were tabulated and assessed for statistical significance using statistical packages of social science (SPSS) software. The mean micronuclei comparison between all four groups was done by the Kruskal - Wallis test and the intergroup comparison was done by the Bonferroni test. In the entire statistical tool, p value less than 0.05 was statistically significant.

RESULTS

The mean micronuclei by using Pap-stained smear in group A, group B, group C, and group D were 4.7, 2.8, 10.5 & 1.67 respectively. Similarly, the mean micronuclei by using Giemsa-stained smear in group A, group B, group C, and group D were 3.7, 2.0, 7.07, and 0.07, respectively. In both the staining, the mean value of micronuclei was found to be higher in subjects who are habituated to both alcohol & chewing tobacco, followed by subjects habituated to only chewing tobacco habituated to consuming alcohol alone. Comparison of mean micronuclei between the four groups by two different staining (Pap smear and Giemsa smear) was done using the Kruskal-Wallis Test. The mean micronuclei value among the four groups in both the stains (Pap and Giemsa) showed a statistically significant value (p < 0.001). [Table 1]

Multiple comparisons between the intergroup with Pap stain and Giemsa stain were done by using the Bonferroni correction test for the intergroup comparison. In Pap staining, all four groups were compared with the inter groups in which group A showed a significant difference with P values 0.54, 0.00, 0.00 with groups B, C, and D which infers that in inter-group comparison there was a good statistically significant difference among them. Likewise in group B when compared with intergroups, there was a non-significant difference between group B and D with a P value of 0.772, which represents group B and D showed no variation in the smear and when group C is compared with intergroups, it shows all the three intercomparisons showed a very high statistical significant difference with P value 0.00 which info there was a perfect difference among the groups.

Likewise, when the Giemsa stain was compared among the four groups, in which group A showed a significant difference with P values of 0.00, 0.00 with groups C and D but when compared with group D it showed a P value of 0.58 with no statistical significant difference representing no difference

TABLE 2: Multiple comparisons between intergroup (Pap & Giemsa stain)

| Bonferroni test for multiple groups | | | | | |
|-------------------------------------|--------|--------|------|-------------------------|-------------|
| STAIN | GROUPS | GROUPS | Sig. | 95% Confidence Interval | |
| | | | | Lower Bound | Upper Bound |
| PAP | A | B | .054 | -.0212 | 3.9546 |
| | | C | .000 | -7.6879 | -3.7121 |
| | | D | .000 | 1.1121 | 5.0879 |
| | B | A | .054 | -3.9546 | .0212 |
| | | C | .000 | -9.6546 | -5.6788 |
| | | D | .772 | -.8546 | 3.1212 |
| | C | A | .000 | 3.7121 | 7.6879 |
| | | B | .000 | 5.6788 | 9.6546 |
| | | D | .000 | 6.8121 | 10.7879 |
| | D | A | .000 | -5.0879 | -1.1121 |
| | | B | .772 | -3.1212 | .8546 |
| | | C | .000 | -10.7879 | -6.8121 |
| Giemsa | A | B | .058 | -.0341 | 3.5674 |
| | | C | .000 | -5.1008 | -1.4992 |
| | | D | .000 | 1.8992 | 5.5008 |
| | B | A | .058 | -3.5674 | .0341 |
| | | C | .000 | -6.8674 | -3.2659 |
| | | D | .028 | .1326 | 3.7341 |
| | C | A | .000 | 1.4992 | 5.1008 |
| | | B | .000 | 3.2659 | 6.8674 |
| | | D | .054 | 5.1992 | 8.8008 |
| | D | A | .000 | -5.5008 | -1.8992 |
| | | B | .028 | -3.7341 | -.1326 |
| | | C | .000 | -8.8008 | -5.1992 |

*. The mean difference is significant at the 0.05 level.



among the group A and group B. In group B again there was no statistical difference between group A and group B the remaining other groups C and D showed a very high significant difference. There was a statistical significant difference among all the intergroup comparisons which infers that in group C and group D when compared with the other remaining groups. [Table 2]

DISCUSSION

Cancer is an epidemic among non-communicable disease and is a major cause of morbidity and mortality worldwide. The development of cancer is a multistage process involving the accumulation of genetic, epigenetic, and phenotypic alterations due to exposure of carcinogens. Oral mucosa is the first line of contact with various hazardous agents. It also provides the first barrier against potential carcinogens and is therefore susceptible to DNA damage by these agents before reflecting any systemic condition.² Micronuclei are individualized structures within the cytoplasm of interphasic cells measuring between one-fifth to one-third of the size of the main nucleus, observed in the same plane as the nucleus and presenting similar staining and chromatin distribution.⁶ Among various staining methods, DNA-specific stains and non-specific stains are preferred for staining nuclei, micronuclei, and other nuclear anomalies in buccal exfoliated cells. PAP and Giemsa are most commonly used DNA non-specific stains.⁷

Eunuchs are also named "hijra," which represents the third gender or "male-to-female" transgender people. Many of the micronucleus studies were conducted in chewing tobacco, smoking tobacco, and alcohol in takers of different genders (male and female), to explore their health risks. However, no information was available on the micronuclei induction in the transgender or Eunuchs, who were exposed to different lifestyle factors. This signifies the importance of conducting the genotoxicological bio-monitoring among the transgender population. Saravanan et al., in 2006 studied about oral health status of the eunuch population.⁸ Similar studies on the oral health status of transgender were also conducted by Torwane et al., in 2015, M. Ovia, et al in 2019 & Manpreet K et al in 2021.^{9,10,11} Their studies showed, that the Transgender had the habit of smoking tobacco, chewing smokeless tobacco-containing products such as betel nut, betel quid, gutkha, etc alcohol consumption, and low oral hygiene status. To the best of our knowledge, except for the above-mentioned studies of the oral health status of the transgender, micronuclei evaluation in clinically normal buccal mucosa of transgender, who are habituated to chewing tobacco and alcohol has not been reported in the literature. Hence the present study was designed with micronuclei induction due to chewing tobacco, alcohol consumption, and in combination among transgender population in and around Salem district of Tamilnadu.

In this study, the mean micronuclei by using Pap-stained smear in group A, group B, group C, and group D were 4.7, 2.8, 10.5 and 1.67 respectively. Similarly, the mean micronuclei by using Giemsa-stained smear in group A, group B, group C and group D were 3.7, 2.0, 7.07 and 0.07 respectively. Comparison of mean micronuclei between the four groups by two different staining (Pap and Giemsa stain) showed a statistically

significant value ($p < 0.001$). A literature search using the Google Scholar and PubMed data bases using the search words 'Micronuclei Transgender', and 'Eunuch Micronuclei' did not reveal any relevant study. Hence all our findings of micronuclei evaluation have been indirectly cor-related with those studies observed on normal males or females as no such evaluation exists in the transgender population.

In this study, mean number of micronuclei in the control group (group D) by using PAP stain was 1.67. This value was similar to the mean micronuclei value of the control group in the study done by Baxi and Gohil in 2017 (mean -1.5) but not similar to study done by Bansal et al., 2012 (mean - 4.17).

The mean value of micronuclei in the control group (group D) by using Giemsa stain in this study was 0.07. This value was similar to the mean micronuclei value (2.80 \pm 1.00) of the control group in the study of Metagud and Neelesh in 2018 and not similar to study reported by Arul et al in 2017 (mean value -6.64).¹²⁻¹⁵

The number of micronuclei in the control group of the previous studies (in male & female) varied from 1.5 to 9.12 in PAP smear and from 0.5 to 14.84 in Giemsa smear. Our findings in the control group values were 1.67 for PAP and 0.07 for Giemsa and thus lie within this wide range.

Since the usage of tobacco in various forms and alcohol consumption is popular among the transgender, in the present study habituation to chewing tobacco and alcohol intake are included as parameters. Oral habits such as tobacco and alcohol consumption are considered to be an important etiologic factor for carcinogenic cytological change. Around two-third of squamous cell carcinoma and 75% of head and neck cancers have been attributed to tobacco and alcohol.¹⁶ The toxic effects of alcohol are believed to be mediated by damage to the DNA indirectly via several mechanisms, such as via induction of oxidative damage, DNA adducts, DNA cross-links and DNA strand breaks.¹⁷

In the PAP stain, mean micronuclei value (4.77), among tobacco chewers (group A) seen in this study was lower than reported by Bansal et al., 2012 (mean - 24.13).¹³ But this observation was approximately similar to those reported by Baxi and Gohil, 2017 (mean - 2.1). In Giemsa stain, mean micronuclei value (3.77), among tobacco chewers (group A) seen in this study was lower than reported by Jyoti et al., 2012 (mean - 17.4). But this observation was similar to those reported by Pradeep et al., 2014 (mean - 1.67).^{12, 13, 18, 19}

In PAP stain, the mean micronuclei value (2.80), in those habituated to alcohol only (group B) seen in this study was lower than reported by Arul et al., 2018 (mean - 13.98). In Giemsa stain, the mean micronuclei value (2.00) in those habituated to alcohol only (group B) seen in this study was lower than reported by Arul et al., 2018 (mean - 8.69).¹⁵

In this study, in PAP and Giemsa stain, the mean micronuclei value of in those habituated to both chewing tobacco and alcohol (group C) was 10.47 and 7.07 respectively. A thorough review of the literature did not reveal any study of micronuclei assessment in clinically normal appearing mucosa among male and female genders with these habits namely, chewing tobacco and alcohol consumption for comparison with our study.



In this study, we observed that the mean micronuclei count was higher in the study group when compared to the control group. Among the study group, the mean micronuclei count was higher in group C followed by group A and B. The higher micronuclei count in those habituated to alcohol and tobacco chewers than those with either of the habits represents the synergistic effect of chewing tobacco and alcohol to cause more nuclear damage, leading to malignancy. The synergistic effect of tobacco smoking and alcohol consumption as evidenced by increased micronucleus count was also observed by in 1983 by Stich and Rosin.²⁰

Although PAP and Giemsa stains are known to be non-specific DNA stains, they are generally considered as more useful and cost-effective when compared to DNA-specific stains.²¹ Evaluation of micronuclei in PAP-stained smears showed higher number of micronuclei than Giemsa-stained smears in the control group and all study groups (A, B and C). This shows that PAP stain is better than the Giemsa stain in the evaluation of micronuclei. Similar findings were reported by Palaskar and Jindal in tobacco smokers with control group.²²

While designing and conducting the study, utmost care was taken to ensure the accuracy and reliability of the results obtained. Still, while using a non-DNA specific stain like PAP stain, Giemsa stain, there are inherent chances of counting particles other than micronuclei such as keratohyaline granules and bacteria, which take up the stain, as micronuclei. Limitations of our study includes, using a DNA specific stain, can eliminate the inherent chances of counting particles other than micronuclei such as keratohyaline granules and bacteria, which take up the stain, as micronuclei. Further studies with increased sample size are required to assess the micronuclei induction of different tobacco products in different forms and alcohol consumed by the transgender society.

CONCLUSION

The study design was framed in such a way that the evaluation of micronuclei was done in apparently healthy subjects using a reliable cost-effective and minimally invasive bio-monitoring tool. From the results, we observed a greater number of micronuclei in subjects exposed to combined habits (chewing tobacco and alcohol) when compared to either of the two habits individually. There appears to be no scientific publication on micronuclei assessment in the transgender population. The findings in our study await corroboration by a study of a larger sample with different types of tobacco and alcohol habituation in transgender population which might help create awareness among the transgender group.

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