Micronuclei as prognostic marker: A clinicopathologic study

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Abstract

Background: Tobacco products attribute to oral cancer. Chronic smoking causes permanent damage to the oral mucosal cells and results in micronuclei (MN) formation.

Aims and Objectives: The present study design was to assess the MN number in different locations of the oral cavity and to assess the damaging effects in patients who smoke and those who had left the habit recently.

Materials and Methods: Three groups were included in the study. The first group included smokers; the second group included those who recently quit the smoking habit; and the third group included those without habit. Cytosmears were taken and stained with rapid Papanicolaou. MN were observed using a polarized light microscope.

Results: Mean values of MN were observed more in the buccal mucosa, followed by lower labial mucosa and floor of the mouth. A significant increase in MN was observed in smokers group, a significant decrease in recently quit habit group and no MN observed in the control group.

Conclusion: The genotoxic effects of tobacco smoke cause MN, and the counts can be used as a non-invasive early diagnostic tool. This finding was important in using MN detection in various areas of the mouth and a prognostic indicator to determine smoking cessation.

Introduction

Cancer is one of the most common causes of high number of deaths all over the world. The incidence of cancer is increasing day by day as there is an increase in the number of people getting addicted to smoking and other tobacco related products. Tobacco is undoubtedly the leading cause of cancer and people consuming the same are at higher risk in the progression of this dreadful disease. The most common form of tobacco consumption in India is gutkha which is a mixture of betel nut and dry powdered tobacco. The use of these tobacco products is very popular among younger generations of our society, and as it is very addictive, it is used extensively in India. It may be in the form of gutkha, quid, etc. Long-term consumption of these products causes potentially malignant disorders such as leukoplakia and erythroplakia if not stopped would cause oral cancer. These byproducts of tobacco cause irreversible damage to the genes at the molecular level. Thus, the cytopathic effects of tobacco cause extensive damage in the nucleus and cytoplasm, and this form of cytogenetic damage can be used as a potent biomarker to measure the extent chromosomal damage caused by it.

According to the WHO, oral cancer is one of the most common cancers in the world, which is increasing every year and is also the leading cause of death. Oral cancer is one of the major health problems. According to the WHO, there are two types of oral lesions viz., precancerous lesions and precancerous conditions. The WHO defined oral premalignant lesion in 1978 as “a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart.” The oral premalignant condition is defined as “a generalized state associated with a significantly increased risk of cancer.”

Micronuclei (MN) are used as a biomarker for assessing damage to the cell for determination of population at risk. MN is studied from exfoliated buccal mucosa of smokers. MN are abnormal fragments of chromosomes that are formed during the mitosis and finally present as a separate smaller nuclei.
Thus, MN estimation detects the amount of cytogenic damage caused by toxic agents such as smoking and use of other tobacco products.\(^5\)

The formation of MN in living cell is a signal of genetic damage which has occurred, and it reflects any cytopathic and carcinogenic exposure of chemicals. Since MN are associated with alterations and deletions in genes of the chromosomes, it has been used as an indicator of genotoxic exposure since eight decades.\(^4\)

**Aims and objectives**

The present study design was to assess the MN number in different locations of the oral cavity (buccal mucosa, lower labial mucosa, and floor of the mouth) and to assess the damaging effects in patients who smoke and those who had left the habit recently (<1 month).

**Materials and Methods**

This study was conducted during a time period of 3-month, from July 2014 to September 2014. The sample consisted 45 healthy male subjects, which included 15 non-smokers, 15 smokers, and 15 those recently quit smoking habit in the age range of 20-30 years, who attended the Department of Oral Medicine and Radiology, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bengaluru. The smokers had an average of 10-15 cigarettes for more than 6-7 years duration. Clinically examined patients with no history or sign of potentially malignant disorders were included in the study. The polarized microscope was used, to eliminate false positive results. Individual’s oral cavity was thoroughly rinsed with clean tap water. The exfoliated cells were taken from buccal mucosa, lower labial mucosa, and floor of the mouth with moistened wooden spatula. The sample was spread on the middle third of a clean dried glass slide and fixed immediately using 100% ethyl alcohol for staining with Papanicolaou (PAP) stain. Rapid PAP stain kit was used for staining the slide with PAP stain. MN were observed using the polarized microscope under ×40 magnification.

Criteria for assessing MN as given by Heddle and Countryman\(^6\) are:
1. Diameter <1/3rd of the main nucleus
2. Non-refractility
3. Same color as or slightly lighter than the nucleus
4. Located within three or four nuclear diameters of a nucleus and not in contact with the nucleus
5. Not more than two MN associated with one nucleus.

**Statistical analysis**

Data were entered in Microsoft Excel and analyzed using SPSS. Results were analyzed using t-test.

In the above test, \(P < 0.05\) was accepted and indicated statistical significance.

**Results**

The frequency of distribution of MN is shown in Table 1 with mean and standard deviation of 0.00 ± 0.00 in non-tobacco habit group, 4.16 ± 2.549 in smoker group, and 1.71 ± 1.058 in recently quit smoking tobacco group. MN in three different sites from three different study groups are shown in Table 2 with mean and standard deviation in non-tobacco habit group in buccal mucosa, lower labial mucosa, and floor of the mouth (0 ± 0.0), (0 ± 0.0), and (0 ± 0.0), respectively; (2.53 ± 0.915), (1.67 ± 0.976), and (0.93 ± 0.594), respectively, in recently quit smoking group; and (7.13 ± 1.767), (3.53 ± 0.743), and (1.80 ± 0.941), respectively, in smoking group. Graph 1 represents the mean value of MN in various study groups. The mean difference between the number of MN in smokers and non-tobacco habit group was 7.133 was statistically significant (\(P = 0.00\)). Figure 1 shows the MN as seen in smoker’s group, recently quit habit group, and non-tobacco habit groups of patient.

**Discussion**

Oral pharyngeal precancer and cancer are the most common conditions seen in India and also other countries. Cigarette and bidi smoking is regarded as the main etiologic agents in the pathogenesis of oral cancer. Thus, any detection tool to assess precancer and cancer will be of utmost importance to diagnose patients at higher risk and take necessary counseling for the patients to stop the habit and advice treatment which will be beneficial for the patient. The toxic effect to the genes can be assessed by MN assays in the smokers, and these patients are susceptible to damage to DNA and subsequently cause mutation and cause cancer. These will assess the amount and level of smoking which causes cancer.\(^3\)

A variety of ingredients contained in cigarette smoke is genotoxic.\(^3\) Various contents of the smoke of tobacco cause chromosomal alterations, so these cytotoxic effects can be used as a biomarker. Hence, the mutations in the chromosomes caused by smoking is regarded as the initiation point of cancer.\(^7,8\)

**Graph 1:** Mean value of micronuclei in different study group.
Environment, biology, and genes play an important role in finding the impact in human life and assessing risk factors for cancer. There are different forms of tobacco in the market and it one of the causative agents for oral cancer since years, and any way to find out the population at risk is considered of utmost importance. MN are a potent tool to study any subgroup of the population at risk. MN are an important marker in the cells of the oral cavity which is obtained from the scrapings of the oral mucosa to know about cellular and nuclear damage. Genetic and chromosomal alterations can be studied in epithelial cells of the oral mucosa.

Smoking cigarette and bidi is regarded as one of the potential etiopathologic agents in the formation of MN and helps in assessing the population at risk their occupational and environmental risk. There is an association between MN and smoking according to some studies, but some studies did not show any link between smoking habit and MN.

Table 1: Micronuclei in different study groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Mean difference</th>
<th>t-test</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>Non-tobacco habit group</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>20-30</td>
<td>Smoker group</td>
<td>4.16</td>
<td>2.549</td>
<td>4.156</td>
<td>10.936</td>
<td>0.00</td>
</tr>
<tr>
<td>20-30</td>
<td>Recently quit smoking tobacco</td>
<td>1.71</td>
<td>1.058</td>
<td>1.711</td>
<td>10.850</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* t-test cannot be computed because the standard deviation is 0. ** P<0.05 statistically significant, SD: Standard deviation
The present study evaluated the mean average of MN in non-smokers, smokers, and patient with recently quit the habit in buccal mucosa, lower labial mucosa, and floor of the mouth. It showed the mean value in case of smokers were higher (7.13 ± 1.767) in buccal mucosa compared with recently quit and non-tobacco habit group. As the groups were for their mean difference, it was statistically significant (P < 0.05). This finding was with accordance with the study done by Palaskar and Jindal,[12] Patel et al.[13] and Bansal et al.[1]

### Conclusion

The MN assay is a simple, practical, inexpensive, and a non-invasive diagnostic tool for a population with oncogenic risks after exposure to different forms of tobacco products. Thus, it acts as a useful indicator to assess the level of smoking cessation.

### Clinical significance

MN assay is an inexpensive and non-invasive diagnostic tool for assessment of carcinogenic risk after exposure to tobacco.

### References

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