Cytological grading: An alternative to histological grading in oral squamous cell carcinoma

ABSTRACT

Background: Micronuclei (MN) in oral exfoliative cells have been shown to indicate a disparaging change in genetic information of the cell. Recent studies showed correlation between the frequency of MN and severity of this damage. Grading of lesions can be used to determine the austerity of this damage. Aims: The aim of this study is to compare the MN frequency in oral exfoliated cells of normal and oral squamous cell carcinoma (OSCC) individuals and to cytologically grade the frequency of MN in cytological smears and to correlate it with histological grading. The objective is to ascertain whether MN frequency in oral exfoliated cells can be a parameter for grading of OSCC.

Settings and Design: The study group comprises of 40 subjects (20 controls and 20 OSCC patients) in the age group of 45-85 years.

Materials and Methods: The cytosmear was obtained from each group and stained with Papanicolaou (PAP) stain. Twenty cells from each slide were counted for MN and cytological grade of OSCC was assigned based on the average frequency of MN. Cytological grade was correlated with histological grading and the data were recorded. Student’s t-test and Spearman’s correlation were used for the analysis of the data.

Results: Average frequency of MN was 2.5 times higher in OSCC patients when compared to that in controls and the difference was found to be highly significant. Sixty percent correlation was found between cytological grade and histological grade of OSCC and the difference between them was not significant.

Conclusions: Cytological grading can be used in grading OSCC, and MN insinuates genotoxic damage occurring in the epithelial cells.

Key words: Cytological grade; histological grade; micronuclei (MN); oral squamous cell carcinoma (OSCC)

Introduction

Squamous cell carcinomas (SCC) constitutes about more than 90% of head and neck cancers. They form the sixth most common cancer worldwide and sixth common cause of death in males and seventh common cause of death in females in India. In spite of enormous progress in detection and treatments over recent decades, the overall 5-year survival rate is reduced to 35%, since most of the patients present with metastatic disease at the time of diagnosis. It was estimated that head and neck SCC has an overall 5-year survival rate 75% if detected early.

Chromosomal deletions, translocations, and structural abnormalities are the various complex karyotypes that are characteristic of oral cancer. Damage to the spindle apparatus
of the cell leads to exclusion of whole chromosome during anaphase resulting in bigger MN (aneugenic effect), whereas structural aberrations result in chromosomal fragments forming smaller MN (clastogenic effect). Thus chromosome fragments or whole chromosomes that are not incorporated in the main daughter nuclei during nuclear division gives rise to MN that are induced in oral exfoliated cells by an array of genotoxic agents and carcinogenic agents in tobacco, betel nut, and alcohol. Based on the studies conducted by Bernneke and Mather, MN have been used as an indicator of genotoxic exposure due to its association with chromosomal aberrations. MN in exfoliated oral epithelial cells exemplifies a preferred target site for early genotoxic events induced by carcinogenic agents.\[1-9\]

Applying this thought, the micronucleus test was applied to oral exfoliated cells taken from the lesional site.

**Materials and Methods**

The study group consisted of totally 40 subjects who were divided into 2 groups:

a. Group I: 20 healthy subjects without any tobacco consumption habit and any other systemic diseases,

b. Group II: 20 histopathologically diagnosed OSCC patients.

The importance and need for the study was explained to each individual and after clinical examination, the relevant data were noted and an informed consent was obtained from the study samples.

A cytobrush was used to obtain the smear of exfoliated cells from oral cavity (buccal mucosa in the control group and margins of the lesion in the OSCC group). It was slightly moistened before applying on the mucosa. To remove food debris and necrotic slough, if any, the patients were also asked to rinse the oral cavity before taking the samples to ensure good-quality cytosmear. A light gentle pressure was applied while scrapping. Smears were prepared and were fixed in 95% alcohol and stained with Papanicolaou (PAP) stain.

**Measurement of micronuclei**

Twenty cells from each PAP-stained slide was checked for the presence of MN under OLYMPUS (Noida, India) binocular research microscope model CX21i and counting for MN was done under 400× magnification according to method given by Countryman and Heddle\[7\] with some modifications. According to the criteria put forward by Countryman and Heddle,\[7\] the size of MN should be at one-third the size of the nucleus. The criterion regarding the assessment of size of MN has been modified in the present study. Different sizes of MN, except for the extranuclear structures that appear pinpoint under 400×, have been included.

The average frequency of MN was further tabulated based on the following formula:

\[
\text{Average frequency of MN} = \frac{\text{total number of MN}}{20 \text{ cells with MN}}
\]

Based on the average frequency of MN, a cytological grade of OSCC was determined [Table 1].

**Histopathological grading of OSCC**

Histopathological grading of OSCC was done according to the system proposed by Anneroth et al.\[10\] Degree of keratinization, nuclear pleomorphism, number of mitotic figures, pattern of invasion, stage of invasion, and plasma lymphocytic infiltration were the six parameters used to determine the score. Each parameter is given a score of 1-4. Then, the malignancy point score was calculated as follows:

\[
\text{Malignancy point score} = \frac{\text{total score}}{\text{total number of parameters used}}
\]

Based on the malignancy point score, a histopathological grade was assigned to each case of OSCC [Table 2].

The average frequency of MN was compared between groups I and II and also the obtained cytological grade based on average frequency of MN in group II was correlated with histopathological grade of group II based on malignancy point score.

Student’s t-test and Spearman’s correlation were used to statistically analyze the data.

**Table 1: Cytological grade of OSCC based on average frequency of micronuclei**

| Cytological grading of OSCC | Average frequency of micronuclei |
|-----------------------------|----------------------------------|
| Grade I                     | 3-5                              |
| Grade II                    | 5.1-7                            |
| Grade III                   | 7.1-9                            |

**Table 2: Histological grade of OSCC based on malignant point score**

| Histological grading of OSCC | Malignant point score |
|-----------------------------|-----------------------|
| Grade I                     | 1-2                   |
| Grade II                    | 2.1-3                 |
| Grade III                   | 3.1-4                 |
Results

The average frequency of MN in group I was found to be 42.35 ± 6.73 and in group II was found to be 107.25 ± 28.58 with the P value highly significant (<0.001) [Table 3]. The frequency of MN in group II is 2.5 times that of in group I. Group I showed an average of MN ranging from 1.6 to 2.9 and group II showed an average of MN ranging from 3.35 to 8.55. Based on these average frequencies of MN, cytological grade was given for group II.

Out of 20 individuals, 9 (45%) showed cytological grade I, 9 (45%) showed cytological grade II, and 2 (10%) showed cytological grade III.

When histological grade was determined, it was noted that 13 out of 20 (65%) individual showed histological grade I, 7 (35%) showed histological grade II, and 0 (0%) showed histological grade III. When cytological grade was compared with histological grade, it was observed that out of 20 individuals, cytological grade of 12 (60%) was correlating with respective histopathological grade. The P value between them was found to be insignificant (P = 0.583) [Table 4].

Discussion

MN are extranuclear cytoplasmic bodies formed by chromosome fragments or whole chromosomes, which lag behind at anaphase during nuclear division and are induced by various genotoxic agents. Hence, both structural and numerical chromosomal aberrations can be measured using MN.

In the present study, oral exfoliated cells from the buccal mucosa have been collected. Exfoliative epithelial cells have been used as OSCC is an epithelial malignancy, and they represent the target site for genomic damage induced by the carcinogens. These exfoliated cells can be collected using wooden spatula, metal spatula, or cytobrush. But for collecting large numbers of cells, cytobrushes appear to be the most effective tool. Hence, in our study, cytobrushes were used for collecting exfoliated epithelial cells.

DNA specific stains, such as 4′,6-diamidino-2-phenylindole, feulgen, acridine orange, are superior in staining MN when compared to nonspecific DNA stains. Though PAP stain is a nonspecific DNA stain, we have used it in our study as it is the most commonly used stain for exfoliative cytology, very simple to use, less time-consuming, and is easily available.

Countryman and Heddle criteria has been used by slightly modifying it by including all the structures smaller in size than nucleus as MN and did not include the one which are of pinpoint size when seen under 400× magnification [Figure 1]. The structures similar to MN can be excluded by fine-tuning the microscope whereby these structures are lost. This indicates that these structures are not in one plane, and hence not to be considered as MN. The neutrophils and some inflammatory cells appear hyperchromatic and create confusion with MN should also be excluded [Figure 2]. Countryman and Heddle criteria for scoring MN was also used by Jadhav et al but they included the structures ranging from one-third to two-third the size of the nucleus as MN.

In our study, the MN was scored in the cells that contain MN and the average was calculated similar to Jadhav et al based on the following formula: Average frequency of MN = total number of MN/total number of cells with MN. This was in contrast to the other studies where irrespective of whether the epithelial cell contains MN or not, 1,000-10,000 cells were counted for MN and the average frequency was calculated. In the present study, the number of cells counted with MN was standardized to 20 cells that were missing in the study done by Jadhav et al. and hence the following formula is used: Average frequency of MN = total number of MN/20 cells with MN.

The average frequency of MN was ranging from 3.35 to 8.55 that differed from the average frequency of MN in OSCC in Jadhav et al where it ranged from 1.4 to 3.1. This difference may be due to the inclusion of the structures smaller than one-third the size of parent nucleus as MN.

In the present study, the criteria laid down by Anneroth et al. was used to grade the histological sections that
were lucratively used in the studies done by Pavle et al.\textsuperscript{[14]} and Jadhav et al.\textsuperscript{[5]}

The frequency of MN in OSCC was found to be increased (2.5 times) when compared to controls and the difference was found to be highly significant ($P < 0.001$) that was in consistent with the findings of other studies.\textsuperscript{[5,14]} This increase is due to the genotoxic effects of tobacco, alcohol, and various carcinogens that cause increase in MN production in this group.

The present study compared frequency of MN in the form of cytological grading with the histological grading of OSCC. 60\% correlation was found between cytological grading and histological grading which was similar to the result found in the study done by Jadhav et al. with 75\% correlation indicating cytological grading can be used alternatively to histological grading.\textsuperscript{[5]} Statistical analysis between cytological and histological grade of OSCC showed no significance ($P = 0.583$) signifying there is no difference between the two methods. This indicates that cytological grading can also be used to grade OSCC.

Controls also contain MN but the average frequency of MN in OSCC showed a highly significant augmentation of MN of about 2.5 times when compared to that of the controls indicating the cytogenetic damage in this group caused by tobacco, alcohol, and other carcinogens. Sixty percent correlation was found when the cytological grade and histological grade were compared. Cytological grading and histological grading showed no significant difference indicating that cytological grading can also be used in grading OSCC, and MN connotes genotoxic damage occurring in the cells.

\textbf{Conclusion}

Cytological grading is at par with histological grading and cytological grading can be used as an alternative to it.

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\textbf{Conflicts of interest}

There are no conflicts of interest.

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