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EVALUATION OF GENOTOXIC DAMAGE BY MICRONUCLEI ASSAY IN ORAL MUCOSA OF PATIENTS WITH ORAL SUBMUCOUS FIBROSIS (OSMF)

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ABSTRACT
In oral sub mucous fibrosis (OSMF), lamina propria and deeper connective tissues of the oral mucosa becomes inflamed and fibrotic, which leads to stiffening of the mucosa resulting in difficulty in mouth opening. Chewing of tobacco and its related products are mainly responsible for pre malignant conditions such as OSMF. The genotoxic damage can be assessed by micronuclei assay. The study was done on 30 control and 30 OSMF patients. In OSMF patients frequency of nuclear anomalies were higher as compared to control group. The study conclude that the micronucleus assay can be used in evaluation of genotoxic damage.
KEYWORDS: *Oral mucosa, OSMF, Micronuclei, Nuclear anomalies*

1. INTRODUCTION

Superficial cells of the oral mucosa are constantly being shed, as a result of constant abrasion of the surface tissue. However, it maintains its structural integrity because of cells in the basal layers, which act as stem cells. Any defects in this procedure can lead to developmental anomalies as well as cancerous growth (1).

Oral sub mucous fibrosis was first described by Schwartz (1952) as "atrophia idiopathica (tropica) mucosae Oris" (2) and later Joshi named this condition as oral submucous fibrosis (3). In oral sub mucous fibrosis (OSMF), lamina propria and deeper connective tissues of the oral mucosa becomes inflamed and fibrotic, which leads to stiffening of the mucosa resulting in difficulty in mouth opening. Patients of OSMF have localized burning sensation and develops an intolerance to spicy food. Ulceration and blanching of the mucosa occurs upon further progression of the condition and characteristic fibrotic band forms in the mucosa. According to an epidemiological survey no less than 250,000 persons in India are suffering from this condition (4).

Chewing of tobacco and its related products are mainly responsible for pre malignant conditions such as OSMF. The genotoxic effects of tobacco in oral mucosa can be assessed by various methods, however, the micronuclei assay is gaining attention as it is rapid, simple and sensitive short term assay (5).

Micronuclei (MN) are small extranuclear bodies, which are form by acentric chromosome fragments or whole chromosome that doesn't get included in the daughter nuclei during mitosis. These bodies are formed as an extra nucleus in one of the daughter cells because of failure of attachment of microtubules and successive pulling in an altered direction in comparison to normal chromosome (6,7).

2. OBJECTIVES

The evaluation of micronuclei in patients of oral sub mucous fibrosis is important to identify as OSMF develops within a very short span of time and can lead to oral carcinoma. Chewing of tobacco induces the formation of micronuclei and thus the patients which are at high-risk for developing cancer can be identified by micronuclei assay.

3.METHODOLOGY

A total of 30 patients of OSMF and 30 normal controls was chosen for the present study. The controls did not have the history of tobacco consumption in any form. The OSMF patients chosen for the present study were the patients visiting to OPD of National Institute of Medical Sciences & Research and Hospital (Jaipur). The study was approved by the Institutional Ethical Committee. The subjects were informed about the study and a written consent was obtained from them.

Before sampling each individual was asked to rinse his/her mouth thoroughly with tap water. Oral mucosa cells was collected from each subject using a soft toothbrush gently from the oral mucosa of cheeks.

The brush will be then swirled into a centrifuge tube containing a buffer solution of pH 7.0 thereby creating a cell suspension. To make the buffer solution (0.1M EDTA, 0.001M TrisHCl and 0.02M NaCl) will be dissolved in sterile 1 liter distilled water. The pH of the buffer will be adjusted to 7.0 with NaOH. Oral mucosa cells will be washed thrice by centrifugation at 1500 rpm for 10 minutes in the buffer solution. Volumes of 15 ml of buffer in a 50 ml conical tube will be used in every washing step. Washing with the buffer leads to the inactivation of endogenous DNAases present in the oral cavity. Gentle pipetting of the cells in the buffer solution reduces clumping and lyses broken cells. Once the cell density (1.5-2 x 10^6/ml) was reached, 50-100 µl of the cell suspension will be laid and spread well on clean, pre-heated (37^{o}C) glass slide and allowed to air dry for 5-10 minutes. The slides will be fixed in methanol, stained with 5% Giemsa and observed under microscope. All slides will be first examined with low power magnification to discard those infected with bacteria and fungi as they interfere with scoring. Only those MN will be scored which will be rounded or oval in shape with a smooth perimeter suggestive of membrane, less than 1/3 the diameter of the main nucleus, of the same, texture and color and refraction as the main nucleus and clearly separated from the main nucleus with no overlap or bridge to the nucleus. Criteria for the cells to be included in the total count will be: intact cytoplasm lying relatively flat, little or no overlap with adjacent cells, little or no debris, nucleus normal and intact, and nuclear perimeter smooth and distinct (8).

Beside micronuclei, other nuclear abnormalities seen in smears of exfoliated buccal cells are: binucleation (presence of two nuclei), pyknosis (small shrunken nucleus), karyorrhexis (nuclear disintegration), karyolysis (nuclear dissolution) and nuclear budding [Shown in figures A to G].

4. STATISTICAL DESIGN

Data was entered into Microsoft Excel spreadsheet. It was analysed using Develve software for statistical analysis. The data between OSMF patients and control groups was analysed by One way Annova Test. A p-value <0.05 was considered as statistically significant.

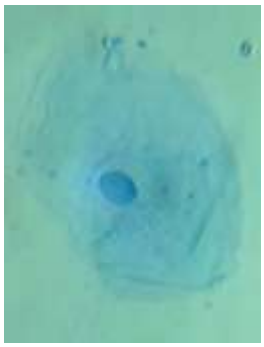
5. RESULTS

The control group has 19 males and 11 females, and in OSMF patient group has 24 males and 6 females. The control had an average age of 49.36 years (age range: 27-74 years), while OSMF group had an average age of 37.76 years (age range: 23-50 years). The normal cells in the control group were 638± 201.14, while it was much lower in OSMF group (170.83± 147.48). The frequency of micronuclei and other nuclear anomalies (except nuclear bud) was comparatively higher in OSMF group as compared to control group and also shows a highly significant difference [P value <0.001 (HS)] (Table 1 and Graph 1).

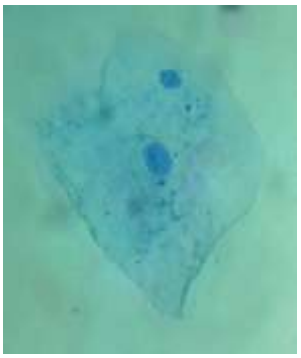
6. CONCLUSION

The increased number of nuclear anomalies and statistical analysis suggest that micronuclei assay can be used as an effective method to predict the genotoxic damage. The micronuclei assay used in the study is simple, rapid and noninvasive. Such a method is cost effective and can be done even in the rural hospitals.

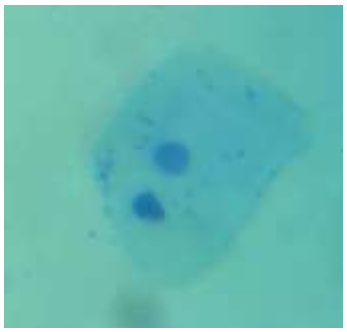
7. FIGURES



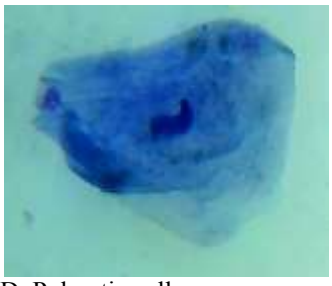
A. Normal cell



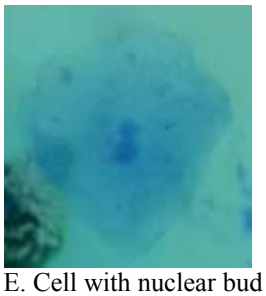
B. Cell with 1MN



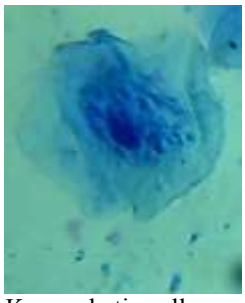
C. Binucleated cell



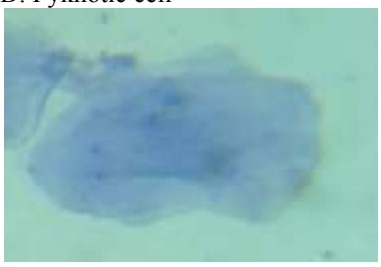
D. Pyknotic cell



E. Cell with nuclear bud



F. Karyorrhetic cell



G. Karyolytic cell

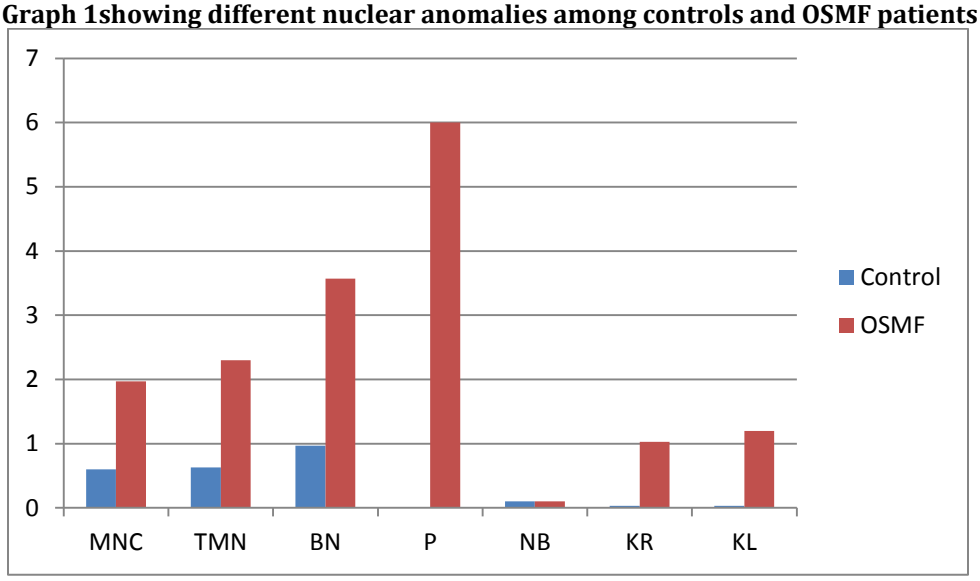
8. GRAPHS AND TABLES

Table 1: Mean ± SD of different nuclear anomalies among controls and OSMF patients

Nuclear Anomalies	Control (C)	OSMF
Number of cells with micronuclei (MNC)	0.60 ± 1.50	1.97 ± 1.33*
Total number of micronuclei (TMN)	0.63 ± 1.52	2.30 ± 1.39*
Bi-nucleated cells (BN)	0.97 ± 2.01	3.57 ± 2.99*
Pyknotic cells (P)	0.00	6.00 ± 4.09*
Cells with nuclear bud (NB)	0.10 ± 0.40	0.10 ± 0.31**
Karyorrhetic cells (KR)	0.03 ± 0.18	1.03 ± 1.03*
Karyolytic cells (KL)	0.03 ± 0.18	1.20 ± 1.06*

*P value is <0.001 (HS); **P value is Non-significant

Graph 1showing different nuclear anomalies among controls and OSMF patients



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