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# Assessment of frequency of micronucleated exfoliated buccal cells in relation to oxidative stress in oral lichen planus in coastal Karnataka, India

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## ABSTRACT

**Objective:** To assess the frequency of micronucleus in exfoliated buccal mucosa cells of patients with oral lichen planus (OLP) in relation to free radical toxicity since OLP is considered to be a precancerous lesion. **Methods:** The micronucleus frequency in exfoliated buccal mucosa cells of patients with OLP was assessed and compared to those in healthy subjects. **Results:** A significant increase ( $P < 0.004$ ) in frequency of micronucleated exfoliated cells (MEC) was observed in patients with OLP when compared to normal subjects. **Conclusions:** It can be concluded that DNA damage in MEC of OLP patients may be a consequence of increased free radical toxicity.

## 1. Introduction

Invasive techniques to study the impact of environment and life style factors on genomic stability in cells obtained from human population are very critical. In this milieu, a safer, more economical and non-invasive method of obtaining cells especially exfoliated buccal cells in diseased conditions like oral lichen planus (OLP) to study micronucleus is a more viable proposition<sup>[1]</sup>. OLP is an immunologically mediated mucocutaneous disease that affects the oral mucosa with a variety of clinical presentations. The most prevalent type was the reticular type. It was located most frequently on the buccal mucosa followed by the tongue and the alveolar ridge.

OLP is generally considered a prelude to oral squamous cell carcinoma<sup>[2]</sup>. Further, oral practices such as smoking, chewing tobacco and betel nut make the population more susceptible to the dangers associated with these habits in the present study. It has been demonstrated earlier that there is a decreased antioxidant defence and increased oxidative damage to lipids, DNA and proteins in lichen planus<sup>[3]</sup>. Therefore, the purpose of this study was to assess the DNA damage due to oxidative stress in OLP by

measuring the quantity of micronucleated exfoliated cells (MEC) of the buccal cavity in these patients.

## 2. Materials and methods

### 2.1. Study design: case-control study

#### 2.1.1. Subjects (patients and controls)

Eighteen patients (males 11, females 7 with a mean age of 45 years) with biopsy proven, untreated OLP lesions with onset of symptoms of 6 weeks duration, participated in this study. The exclusion criteria included subjects with diabetes or any other illness, alcoholics, smokers, post-operative cases, subjects under medication and subjects with unhealthy oral cavity. The study protocol was approved by the Institutional Ethical Committee and informed consent was obtained from all the subjects. The study was carried out during the period of October, 2007 to February, 2009 and samples (convenient sampling) were collected from patients attending the Department of Oral Medicine and Radiology, Manipal College of Dental Sciences and associated hospitals (tertiary care), in coastal Karnataka, South India. The control group comprised healthy subjects who visited the hospital for a routine health checkup ( $n=20$ , males 10, females 10, with a mean age of 41 years).

#### 2.1.2. Methods

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The subjects were asked to rinse their mouth thoroughly with plain water. Buccal scrapings were obtained from the subjects prior to breakfast. The patients were asked to scrape the lesions with the help of a wooden spatula. After 5–6 gentle strokes the spatula was dipped in sterile phosphate buffered saline (PBS, pH 7.4). Normal (control) scrapings were also obtained in a similar manner.

## 2.2. Micronucleus assay

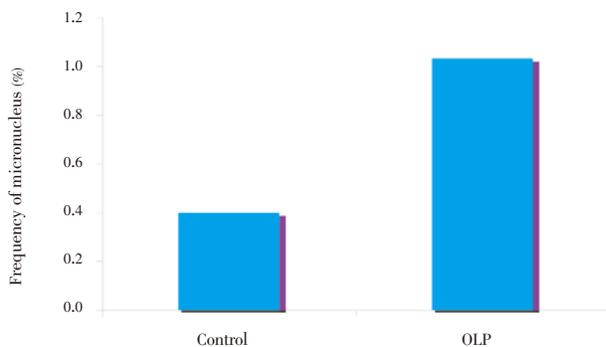
The micronucleus assay was carried out according to the method of Tolbert *et al*[4]. Briefly, the cell suspension was washed by centrifugation at 2000 rpm for 5 minutes twice. The cells were fixed with freshly prepared Carnoy's fixative (methanol: glacial acetic acid in a ratio of 5:1) and kept at 4 °C for at least 30 minutes for proper fixation of the cells. The cell suspension was later centrifuged at 2000 rpm for 5 minutes and the pellet was re-suspended with fresh fixative. The cells were later spread on clean pre-chilled slides. The air dried slides were stained with acridine orange (0.002% in Sorenson's buffer, pH 6.8) and scored under fluorescent microscope at 400× magnification using the criteria described by Fenech and Morley[5]. At least 400 cells were scored from each slide and expressed as percent micronucleated cells.

## 2.3. Statistical analysis

Statistical analysis was performed by the unpaired Student's *t* test using the SPSS package version-11.  $P < 0.05$  was considered statistically significant.

## 3. Results

There was a significant increase ( $P < 0.004$ ) noted in the frequency of MEC in OLP patients ( $1.03 \pm 0.17$ ,  $n = 18$ ) when compared to controls ( $0.40 \pm 0.09$ ,  $n = 19$ ) (Figure 1).



**Figure 1.** Frequency of micronucleus (%) in exfoliated buccal cells in controls and OLP patients.

## 4. Discussion

Micronuclei have been reported as markers for high cancer risk as they arise in response to carcinogens[6]. They can be detected in exfoliated cells and used as an indicator of recent DNA injury within oral mucosa[7–9]. The frequency of MEC is markedly elevated in tissues from which carcinoma arises[6].

The relationship between oxidative stress and etiopathogenesis of cancer is well documented[10]. Since OLP is a pre-cancerous lesion, free radical toxicity will make such patients susceptible to damage (DNA damage) by oxidative stress[11]. Hence, the frequency of MEC in OLP could increase in such cases, as observed in the present study. Further, we have also reported a significant increase in malondialdehyde (lipid peroxidation marker) and a marked decrease in total antioxidant and protein thiol levels in serum of patients suffering from OLP[12]. Therefore, a marked increase in the frequency of MEC in OLP could probably be due to increased oxidative stress in this study.

## Conflict of interest statement

We declare that we have no conflict of interest.

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