

## Letter to the Editor

## Evaluation of apoptotic pathway in oral mucosa by smoking in a Brazilian Outpatient Smoking Cessation Program



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Survivin is a protein that plays an important role in extrinsic apoptotic pathway control, and has activity in the G2/M phase of the cell cycle. It is a component of a temporary chromosomal protein complex (CPC), which is essential for chromosome alignment and cytokinesis during mitosis [1–3]. This protein functions as an apoptosis inhibitor in cell cycle control, conferring a longer survival to the cell [1,2].

To evaluate the apoptotic pathway in oral mucosa by smoking, through the survivin expression, cytological material was collected from 30 patients, who smoked more than 20 cigarettes/day/10 years and with no history of oral malignancy, participating in the Outpatient Smoking Cessation Program of the Heart Institute (Incor), University Hospital, University of São Paulo Medical School (HC-FMUSP), Brazil.

The group included 13 women and 17 men with mean age of 54 years (range: 36–74 years). The patients had been smoking for a median period of 34.5 years (range: 11–65 years), with a mean of 20 cigarettes/day (range: 20–60). Alcohol consumption was reported by 19 participants. Beer was the most frequently consumed beverage.

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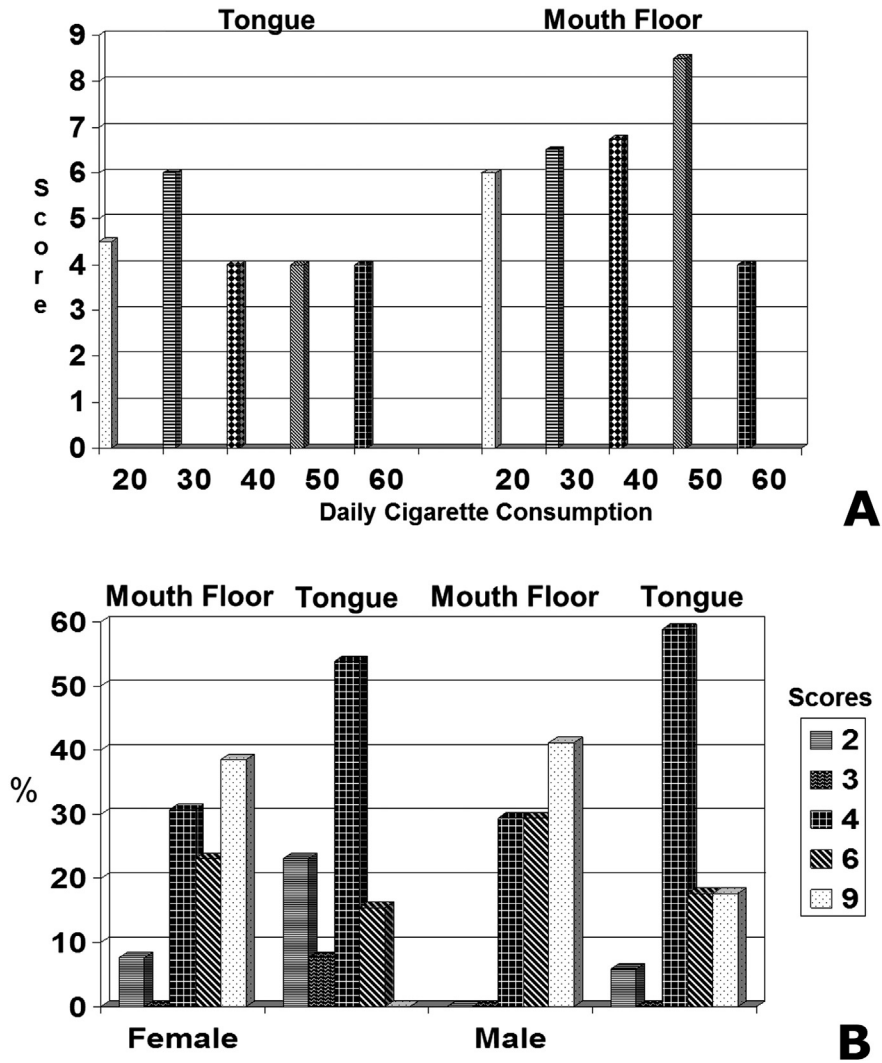
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The study was approved by the Ethics Committee of ICT-UNESP (protocol 026/2008-PH/CEP) and HC-FMUSP (protocol 1362/09) submitted by the Scientific Committee of INCOR.

Oral smears were taken from the left lateral border of the tongue and floor of the mouth of the patients. Immunohistochemistry was performed and anti-survivin antibody (SC-10811, Santa Cruz Biotechnology) diluted 1:100 was used. The cytological material was evaluated by light microscopy at 400× magnification. The smears were analyzed and classified on a scale from 0 to 4 in a previously delimited area measuring 1.5 cm in diameter: 0 = no positive cell; 1 = 25% positive cells; 2 = 50% positive cells; 3 = 75% positive cells, and 4 = 100% positive cells. The intracellular localization of staining was analyzed (nuclear, cytoplasmic, or nuclear/cytoplasmic). Staining intensity in the cells was classified as follows: 0 = no staining; 1 = weak staining; 2 = moderate staining, and 3 = strong staining. The scores obtained by qualitative analysis (0, 1, 2, 3, 4) and by the evaluation of staining intensity (0, 1, 2, 3) were multiplied and provided the final scores (2, 3, 4, 6, and 9) as described by Lo Muzio et al. [1].

The patients were divided into five subgroups according to the number of cigarettes smoked per day: 20–29, 30–39, 40–49 cigarettes, 50–59, and more than 60 cigarettes. The expression of survivin in the lateral border of the tongue and floor of the mouth according to daily cigarette consumption is illustrated in Fig. 1A. Fisher's exact test showed no association between daily cigarette consumption and survivin expression scores obtained for the tongue ( $p = 0.68$ ) or floor of the mouth ( $p = 0.69$ ). There was also no association between the quantity of expression of survivin and the duration of smoking ( $p = 0.21 > 0.05$  and  $p = 0.69 > 0.05$ , Fisher's exact test). For this association, the group was divided according to the median duration of 34.5 years. Expression of survivin was higher in the floor of the mouth when compared to the lateral border of the tongue ( $p = 0.001$ , Mann–Whitney test).

The evaluation of consumption of alcoholic beverages showed no difference in the expression of survivin between genders was observed for the floor of the mouth ( $p = 0.65$ , Mann–Whitney test), but the difference was almost significant ( $p = 0.059$ ) for the tongue. The Mann–Whitney test revealed no difference in the expression of survivin between ages for the floor of the mouth ( $p = 0.87$ ) or tongue ( $p = 0.38$ ). These results are shown in Fig. 1B.



**Fig. 1.** A: Graph showing the expression of survivin according to daily cigarette consumption. B: Graph showing the expression of survivin in cytological smears obtained from male and female patients according to the total number of cases studied (n = 30), score, and intraoral localization.

Survivin expression is considered to be a predictor in cases of carcinomas and potentially malignant disorders [1–3].

In the present study, cytological material obtained from clinically healthy mucosa of smokers, who are probably in the initiation stage of carcinogenesis, was analyzed. Cigarette smoking is known to trigger the process of oral carcinogenesis, but there are no studies in the literature investigating the association between smoking and survivin expression in oral mucosa that presents no clinically detectable alterations. The two intraoral sites studied (tongue and floor of the mouth) are associated with an increased risk of oral squamous cell carcinoma (OSCC) [4,5]. Comparison between these sites revealed a significantly higher expression of survivin in the floor of the mouth. This finding might be related to the combined action of other carcinogens, such as alcoholic beverages, in the floor of the mouth whose anatomic structure favors the retaining of these agents, increasing the time of contact with the mucosa.

The number of cigarettes smoked per day was not associated with the expression of survivin in the tongue or floor of the mouth. The same was observed for smoking duration in agreement with previous studies [6,7].

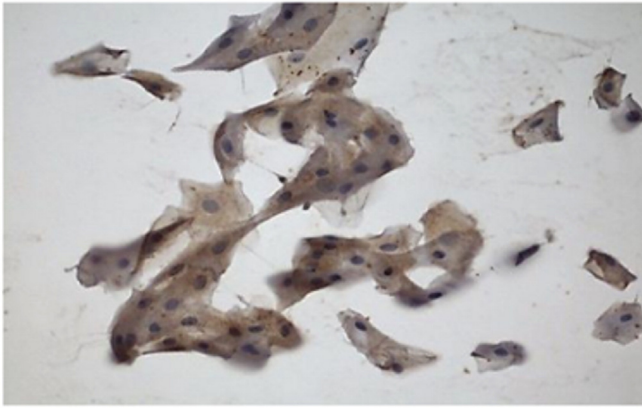
Alcohol consumption did not exert a significant effect on the expression of survivin, although its expression was higher in the floor of the mouth than in the tongue. Alcohol increases the permeability of the cell membrane, facilitating the penetration of the carcinogen into the cell [8], especially in areas where saliva is retained such as the borders

and ventral surface of the tongue, floor of the mouth and retromolar trigone.

No significant difference in the expression of survivin in the floor of the mouth was observed between genders, whereas this difference was borderline significant in the tongue border, with higher scores being observed in males. Survivin expression was more uniform in subjects younger than 54 years when compared to the group of 54 years or older. This finding might be explained by the higher epithelial turnover in younger individuals, which facilitates the elimination of altered cells [9].

Analysis of the intracellular localization of survivin expression showed 100% cytoplasmic staining (Fig. 2). Cytoplasmic survivin regulates the apoptosis and nuclear survivin cell division, and in this case the cytoplasmic expression represents apoptosis induction caused by smoking in the oral mucosa [10]. In the present study, it was not possible to associate cytoplasmic expression of survivin with the prognosis. However, monitoring smokers is important considering that cytoplasmic expression of survivin was observed in all cases of chronic smokers studied here.

The evaluation of the expression of survivin in the oral mucosa of chronic smokers oral mucosa indicates that there is an apoptotic induction in early stages of oral carcinogenesis, especially caused by smoking. These results also highlight the importance of smoking cessation.



**Fig. 2.** Positive expression of survivin in exfoliative cytology specimens of smokers (magnification: 200×).

#### Conflict of interest

The authors declare that they have no conflict of interest.

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