

**CYTOPATHOLOGICAL CHANGES IN ORAL EPITHELIUM
INDUCED BY CRACK COCAINE SMOKING**

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Summary

Crack cocaine, a form of cocaine base, is derived from powder cocaine. Some oral lesions can be developed when crack cocaine is smoked. The aim of this study was to analyze epithelial changes of oral mucosa of crack cocaine users. Oral smears were collected from clinically normal buccal mucosa by liquid-based exfoliative cytology of 52 individuals (32 crack users and 20 non-users). Glass slides were processed, stained by Papanicolaou technique and analyzed by light microscopy. Smears were analyzed according to Papanicolaou classification, grade of inflammation and cellularity. There were a high number of smears classified as class II for crack users, while nonusers were class I (P=0.0004). In crack-user smears classified as class II, the grade of inflammation varied between mild and moderate (P=0.0001). The type of predominant cells on the crack user smears was: 18,8% enucleated superficial cells, 53,1% nucleated superficial cells, 12,5% intermediated cells, and 15,6% nucleated superficial cell = intermediated cell (P=0.0067). These results have demonstrated that crack cocaine was able to induce, principally, inflammatory alterations in the oral epithelium. Thus, periodic oral examinations should be done in those suspect patients of using crack cocaine.

Keywords: Crack cocaine, smoking, cytology, mouth mucosa.

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Introduction

Pure cocaine was first used in the 1880s as a local anesthetic in eye, nose, and throat surgeries. Cocaine was to provide anesthesia as well as to constrict blood vessels and limit bleeding. Many of its therapeutic applications are now obsolete due to the development of safer drugs. Approximately 100 years after cocaine entered into use, a new variation of the substance emerged. This substance, crack, became enormously popular in the mid-1980s due in part to its almost immediate high and the fact that it is inexpensive to produce and buy (6).

Crack cocaine is produced by dissolving powdered cocaine in a mixture of water and ammonia or sodium bicarbonate (baking soda). The mixture is boiled until a solid substance forms. The solid is removed from the liquid, dried, and then broken into the chunks (rocks) that are sold as crack cocaine.

In addition to the usual risks associated with cocaine use (constricted blood vessels; increased temperature, heart rate, and blood pressure; and risk of cardiac arrest and seizure), crack cocaine users may experience acute respiratory problems, including coughing, shortness of breath, and lung trauma and bleeding. Crack cocaine smoking also can cause aggressive and paranoid behavior (5).

Lesions of the mouth, oropharynx and laryngopharynx associated with different forms of cocaine include enamel erosion caused by intraorally applied cocaine hydrochloride, necrotic lesions of the tongue and epiglottis related to smoking free-base cocaine, and laryngeal mucosal burns caused by smoking crack cocaine (4). These lesions are most likely caused by the extreme heat of the smoke. But, it is possible that the chemical content of the smoke contributed to lesion development, but the extent to which these lesions are due to chemical rather than thermal insult is unknown (14).

The aim of this study was to investigate the effect of crack cocaine in epithelial cells of oral mucosa of crack cocaine users.

Methods

The experimental protocol of the present study was approved by Ethics Committee on Human Research at Pontifical Catholic University of Paraná – Curitiba/PR, Brazil.

Subjects

Thirty-two adult crack users (experimental group) and twenty non-users (control group) participated in this study. These crack cocaine users were been treated for detoxification at the Institute of alcoholism prevention and treatment (IPTA, Campo Largo/PR, Brazil). Crack cocaine using was defined as 1 heat-stable rock per day. Name, age, occupation, and relevant medical history were recorded.

Cells collection

Exfoliated cells of the clinically normal buccal mucous membrane were obtained by liquid-based exfoliative cytology. Initially, the mouth was rinsed with water to remove excess of debris and bacteria within the oral cavity. The squamous epithelial cells were collected using cytobrush and kit UCM (Universal Collection Medium of DNA-Citoliq System[®], Digene Brazil).

Cytological preparations

The DNA-Citoliq System[®] allows thin-layer preparations to be provided thanks to a filtration process. An aliquot of 200µL of UCM was filtered through Filtrogene polycarbonate membrane filters[®] (Digene, Brazil), pore size 5µm, diameter 25 mm placed in prepgene press[®] (Digene, Brazil) attached to glass slides. Glass slides were immediately fixed in absolute alcohol for 20 minutes. Smears were then stained with routine Papanicolaou stain.

Morphologic analysis

Each slide was assessed using the light microscopy by binocular Olympus BX50 microscopy[®] (Olympus, Japan). All cellular features were coded according to Papanicolaou classification. Normal, inflammatory, reactive, degenerative and neoplastic conditions of oral epithelial cells were considered. Inflammatory cytological results were categorized as mild (1-5 cells/field), moderate (6-20 cells/field) or severe (>20cells/field). The type of predominant cell (cellularity) in each smear was analyzed too.

Statistical analysis

All data were tabulated and statistical tests were performed with SPSS for Windows 13.0 (SPSS Inc., Chicago, Illinois, USA). Significant statistical differences between groups were examined using Mann-Whitney U test. Differences were considered statistically significant when $P < 0.05$.

Results

The results of diagnosis obtained by Papanicolaou classification are showed in Table 1. Twenty (62.5%) smears obtained of crack cocaine users exhibited inflammatory cells and were classified as class II (Figure 1A and 1B). In 10 crack users, the smears were normal. Two smears showed insufficient quantity of epithelial cells to be assessed; then, they were classified as class 0.

TABLE 1 – Papanicolaou classification in crack cocaine users and nonusers smears.

Class	Experimental		Control		<i>P value</i>
0	2	6,25%	0	-	0,0004*
I	10	31,25%	19	95%	
II	20	62,5%	1	5%	
III	0	-	0	-	
IV	0	-	0	-	
V	0	-	0	-	
Total	32	100%	20	100%	

*Mann-Whitney U test: $P < 0.05$

All smears classified as class II were analyzed according to the grade of inflammation (Table 2). In crack cocaine-user smears, the grade of inflammation varied between mild and moderate (Figure 2A and 2B). Only, one smear showed mild inflammation to the control group ($P=0,0001$).

TABLE 2 – Grade of inflammation in crack cocaine users and nonusers smears.

Inflammation	Experimental		Control		<i>P value</i>
Mild	14	70%	1	100%	0,0001*
Moderate	6	30%	-	-	
Severe	-	-	-	-	
Total	20	100%	1	100%	

* Mann-Whitney U test: $P < 0.05$

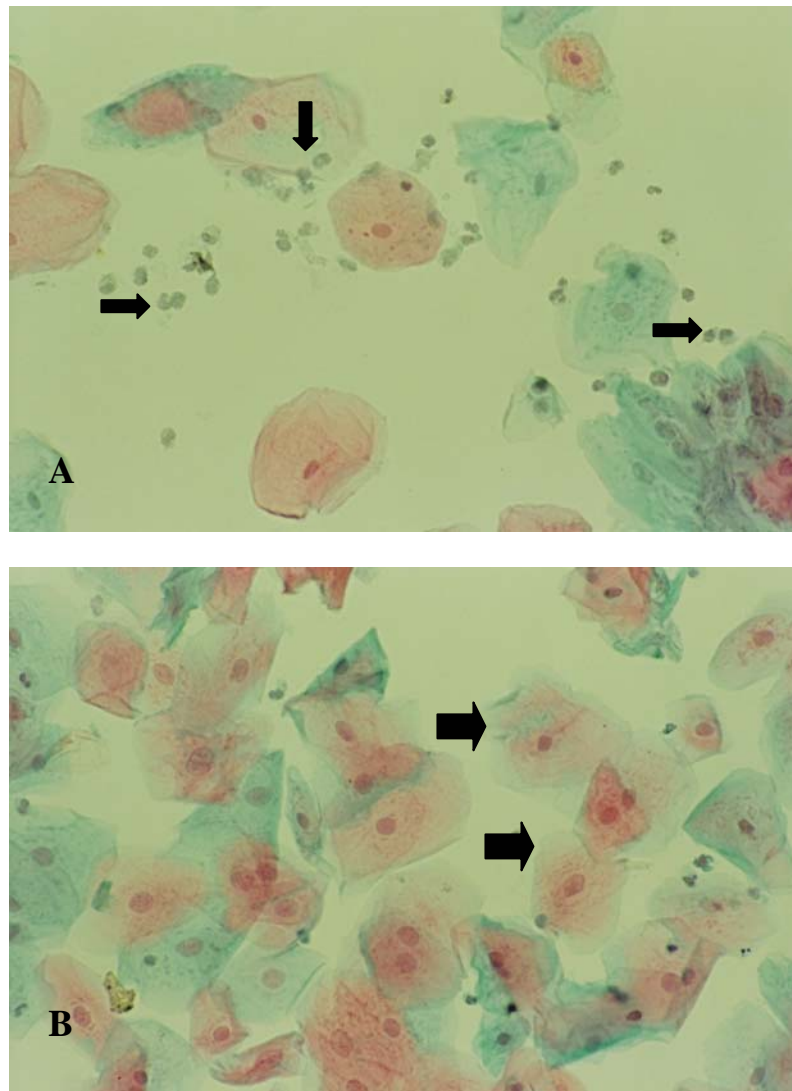


Figure 1 – Crack cocaine cytological smears of normal buccal mucous membrane. A – Class II of Papanicolaou classification. Inflammatory cells (arrows). B – Class I of Papanicolaou classification. Normal squamous cells (arrows). (Papanicolaou stain, original magnification x 400).

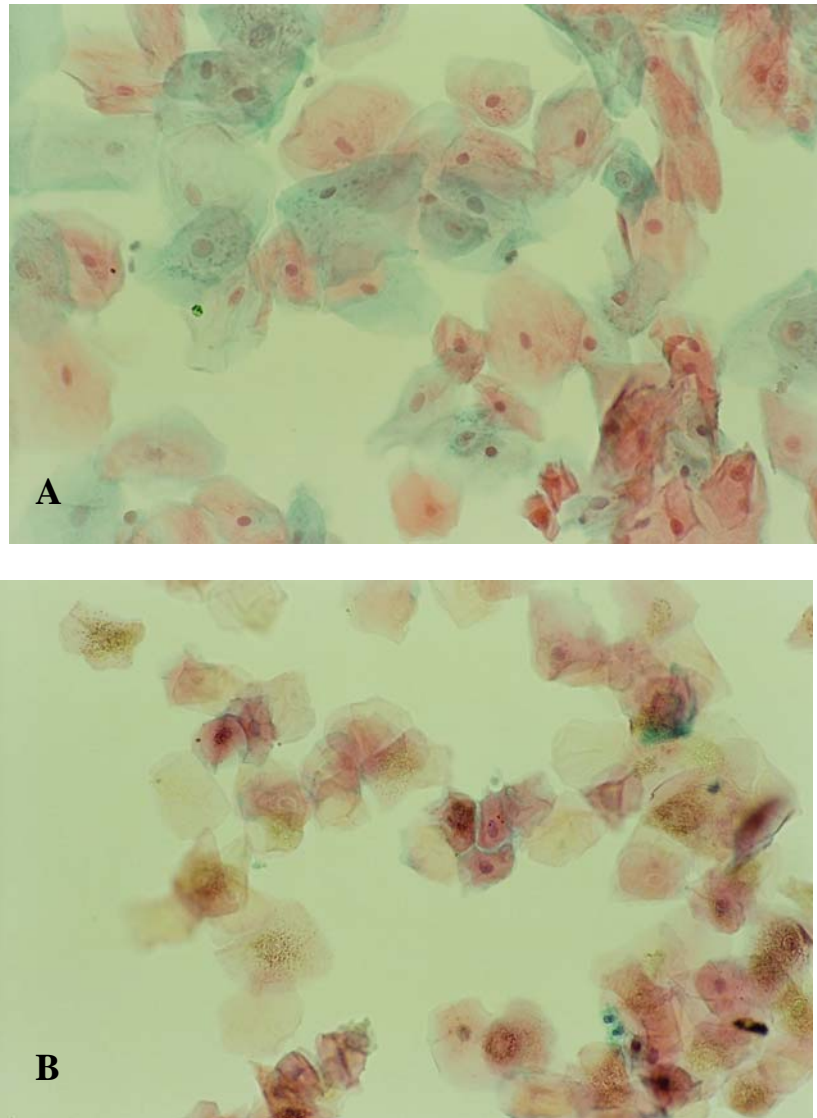


Figure 2 – Crack cocaine cytological smear of normal squamous cells. A - Abundant nucleated superficial cells. B – Numerous enucleated superficial cells (Papanicolaou stain, original magnification x 400).

In crack cocaine users, the type of predominant cells on the smears was: 18,8% enucleated superficial cells, 53,1% nucleated superficial cells, 12,5% intermediated cells, and 15,6% nucleated superficial cell = intermediated cell. In non-crack cocaine users, 30% nucleated superficial cells, 10% intermediated

cells, and 60% nucleated superficial cell = intermediated cell. When comparing crack cocaine users and nonusers, a greater percentage of nucleated superficial cells was observed in experimental group ($P=0.0067$) (Table 3).

TABLE 3 – Cell distribution in crack cocaine users and nonusers smears.

Predominant cell	Experimental	Control	<i>P value</i>
Enucleated superficial cell	6 18,8%	0 -	
Nucleated superficial cell	17 53,1%	6 30%	
Intermediated cell	4 12,5%	2 10%	
Nucleated superficial cell = Intermediated cell	5 15,6%	12 60%	0,0067*
Basal cell	0 -	0 -	
Total	32 100%	20 100%	

* Mann-Whitney U test: $P<0.05$

Discussion

Since liquid-based cytology was developed in the 1990s various comparative studies have shown that it can offer significant information about epithelial cells of the oral mucosa (18). This technique results in slides with high a cellularity dispersed in a homogeneous thin layer. Blood, inflammatory cells and mucus are reduced and distributed randomly throughout the slide. The clear background obtained enhances sensitivity and quality of the results (13). Thus, the aim of this study was to analyses epithelial cells of the oral mucosa of crack cocaine users using this technique.

Cocaine was first introduced to medicine in 1884 by Köller as a local anesthetic, but the earliest known use of cocaine was by sub-Andean Indians. The leaves of the coca plant were chewed and their stimulating properties help them tolerate working at higher altitudes. Cocaine is an alkaloid prepared from the leaves of the *Erythroxylon coca* plant (6). When smoked, it produces a near-instantaneous euphoric effect due to its rapid absorption via the pulmonary circulation (2). This drug is able to block the reuptake of norepinephrine and dopamine of the sympathetic nervous system. It has a marked psychomotor stimulating effect that provokes euphoria, verbiage, motor activity, and an amplification of the sensation of well-being similar to the effect of amphetamines (12).

Bronchial biopsy specimens and brushing of crack cocaine users revealed more frequent cell alterations than nonsmokers. The most common alterations observed were: basal cell hyperplasia, cell disorganization, nuclear variation, basement membrane thickening, and subepithelial inflammation (3). Simultaneously, crack cocaine is able to induce pulmonary microvascular injury (2).

Our results have clearly demonstrated that oral mucosa of crack cocaine users suffers cell alterations too. Twenty (62,5%) of the oral smears were classified by Papanicolaou as class II. In this smears, it could be observed many inflammatory cells, principally, leukocytes (polymorphonuclear). Besides, in these smears, the grade of inflammation varied of mild-to-moderate ($P < 0.05$). These results have demonstrated that an inflammatory reaction is elicited by crack cocaine smoking in the oral mucosa, equally, as occurs in bronchial mucosa. Nevertheless, it is unclear how crack cocaine induces these alterations. Clinically, there are some case reports of dental and oral inflammatory lesions induced by crack cocaine smoking (14).

In general, crack cocaine users state that feel a sensation of burning mouth during the crack cocaine smoking. Besides, crack cocaine abusers frequently use other types of drugs, such as: marijuana, alcohol and tobacco. When smoked associated to tobacco, crack cocaine appears to augment the bronchial damage induced by tobacco smoke, but no additive effect was noted between cocaine and marijuana (8).

Actually, it is known that cocaine is a potent immune modulator. In a habitual exposure of the lung to cocaine, it is able to impair the function and/or cytokine production of macrophages. The ability of macrophages to kill both bacteria and tumor cells are severely limited. Besides, macrophages from cocaine users were not able to use nitric oxide as an antibacterial effector molecule. These effects induced by cocaine may be an enhanced susceptibility to infectious disease, cancer, and AIDS in their users (1).

In this study, the oral smears were collected from clinically normal buccal mucous membrane for both crack cocaine users and nonusers. The smears showed cells of all the epithelial strata, since non-keratinized cells (blue cells) to completely keratinized ones (enucleated superficial cell). Three types of oral mucosa has been described whose the individual structure and composition are related to their functional requirements: lining, masticatory and specialized. Lining epithelium is normally non-keratinized (16). Thus, it could be expected that cellularity had changed in oral mucosa of crack cocaine users. The effects of

heat and chemical components of crack smoke on the oral epithelium probably have induced an adaptive response in the cells. A higher number of enucleated and nucleated superficial cells in the smears of crack cocaine users can be explained partly by acceleration in the process of differentiation. This fact would justify an increase in the relative number of superficial cells and, clinically, an oral mucosa would be exhibiting hyperkeratosis. According to these results, it would be necessary histopathologic studies of oral mucosa from crack cocaine users.

In general, crack cocaine abusers smoke this drug associated to alcohol and tobacco (7,10). The effects of smoking crack cocaine in combination with tobacco also show a trend toward additivity, suggesting that smoking more than one substance may be especially injurious to oral mucosa (3).

This present study has shown that crack cocaine is able to induce inflammatory changes in oral epithelium. Thus, a methodical and thorough examination of the oral mucosa should be carry out in crack cocaine users as part of a routine dental inspection. Dental practitioners should be able to identify oral crack cocaine-induced lesions. Early recognition of mucosal changes in the mouth, which result from crack cocaine smoking, is also essential for the early diagnosis and prompt treatment.

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