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CYTOMORPHOMETRIC PARAMETERS OF ORAL MUCOSA OF TOBACCO, ALCOHOL and MATE CONSUMMERS

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INTRODUCTION: Drinking mate is a widespread habit in several countries in South America, also extended to other countries in the rest of the world like Germany, Syria, Lebanon, Israel. Hot mate is considered a risk factor for esophageal, oral and oropharyngeal cancer. Many studies have attempted to detect early cytological changes indicatives of cell damage in exfoliated cells of the oral mucosa of smokers and drinkers. Some authors reported a significant reduction in the mean cytoplasmic area of cells taken from normal oral mucosa of alcohol-dependent patients (Ogden GR et al 1999, Reis et al 2006) and in tobacco users (Seifi et al 2014 , Khot 2015), some of them with increase of the nuclear area and an increase of the N/C ratio (Hashemipour et al 2013). In the last decade many authors studied micronuclei and nuclear changes associated with malignancy. Nevertheless there are no reports evaluating the effects of mate (M), or its combination with tobacco (T) and alcohol (A) in cells from the oral mucosa.

OBJECTIVE: To study cytomorphometric changes in cells of the normal oral mucosa in healthy volunteers who consumed T, A, M and their combinations (TA, TM, AM, TAM) .

MATERIALS AND METHODS:

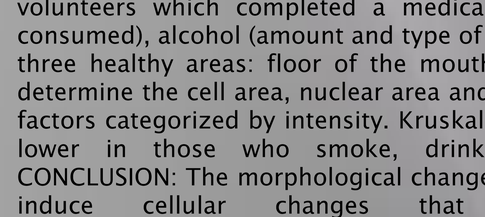
This work was approved by the Institutional Ethical Committee N°1619. (2010). Exclusion criteria: pregnant women, <18 years, diabetics, addicted to drugs consumption, immunocompromised patients, those with more than one year of leaving the cigarette smoke, patients with cancer or who have received antineoplastic therapy. After signing an informed consent, a complete clinical record was performed by calibrated professionals with a detailed questionary related to tobacco, alcohol and mate was applied. The intensity of consumption in his life was obtained, and then categorized:

- **History of tobacco** smoking: age at started, age quit, smoking duration, number of cigarettes/day in different periods of his life, and type of Cigarettes: blond, black, with o without filter according to international rating tables. The total amount of cigarettes consumed was calculated. (Biondi et al)
- **History of alcohol**: age at started, age quit, type of beverage, an average of cc in each period of his life. The total of alcohol consumed in grams was obtained.
- **Mate consumption**: It was assessed taking into account the amount of daily consumption (in cc), and the temperature: hot or warm. (Sewaram et al 2003).

Cell samples from 3 areas from the right side, were obtained with citobrush: **A**- Floor of the Mouth FM, **B**- Buccal Mucosa BM, **C**- Soft Palate SP. Fixed with alcohol 96°. Papanicolaou stained An average of 55 cells by location were studied Image J program was used for the morphometric analysis: cell área (Ca), Nuclear area (Na) were measured and then Nucleus-Cell relation (N/C) was obtained. Then the association with the habits categorized, was analyzed; Kruskal Wallis was applied using InfoStat/Professional program, versión 1.1 Faculty of Agronomic Sciences National University of Córdoba.

80 VOLUNTEERS	
Male	39 (48%)
Female	41 (52%)
Mean age	48 years (18-87)
Cells counted	4.485 cells. 181 cell /volunteer, 3 locations each: 60 cell/location
	Image J program

Type of beverages consumed n: 44

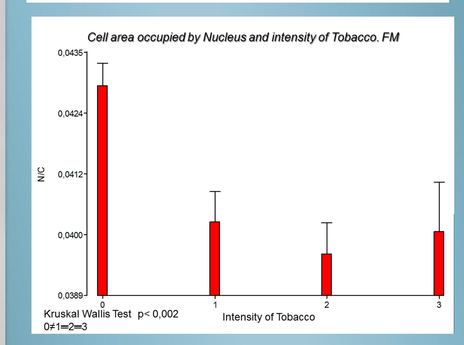


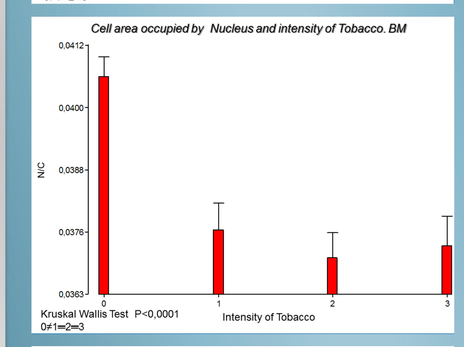
Tobacco consumption

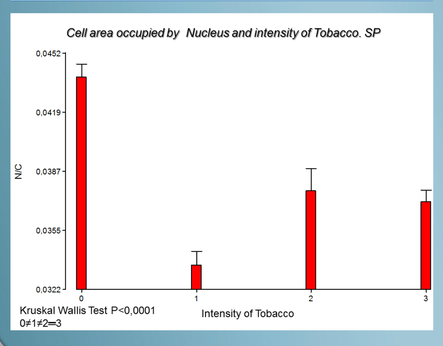
Blond filter cigarettes users: 45 (56%)
ẋ years : 30
ẋ cig.: 230,000 (2190- 830,000)
ẋ cig. / day: 21

Categorization of Tobacco consumption

Category	Amount of cigarettes
1	< 100.000
2	100.000-200.000
3	> 200.000







Alcohol consumption

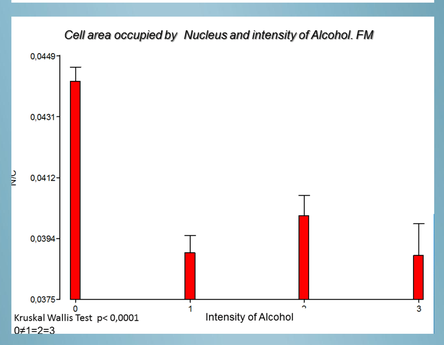
drinkers: 44 (55%)
x alccohol: 380.000g
x years: 24 years
x/day: 43 g (2 drinks wine/day)

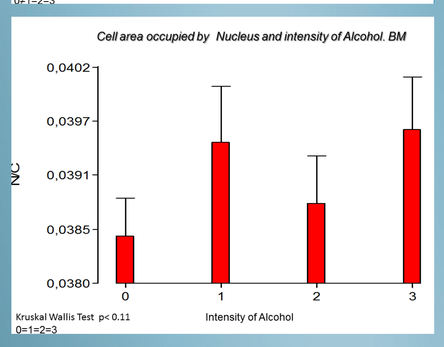
Categorization of Alcohol consumption

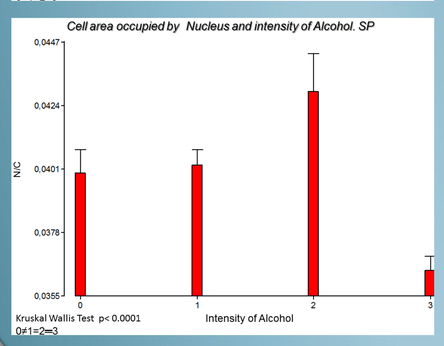
Category	Alcohol in grams
1	< 200.000
2	200.000-400.000
3	> 400.000

Beverage	Alcohol % (range)	Grams/L Mediane g/L	Equivalence in drinks
Red wine	12-14,5	130	150mL: 19,5g
White wine	8-13	125	150mL: 19g
Beer	3,5-5,2	49	330mL: 16g
FERNET	39-40	395	100mL: 39g
WHISKY	39-40	400	40mL: 16g

Table accporting availability of beverages in Córdoba City Octuber 2014







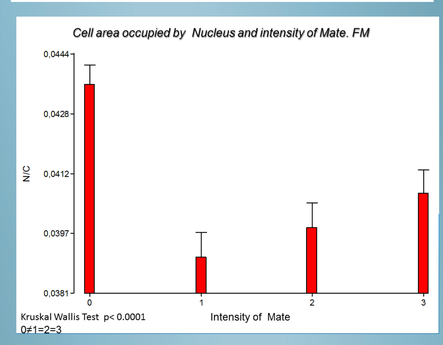
Mate consumption

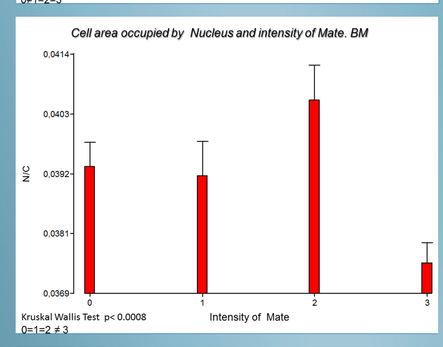
57 (71%) mate consumers
Temperature: hot: 41 (72%),
warm:16

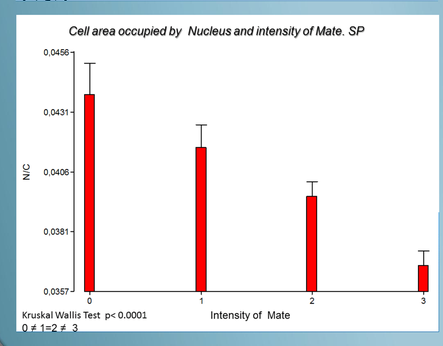
Categorization of Mate consumption

Category (hot mate)	Cc
1	0- 500 cc
2	500- 750 cc
3	> 750 cc

Category (warm)	
1	0-1000 cc
2	1000-1500 cc
3	> 1500 cc

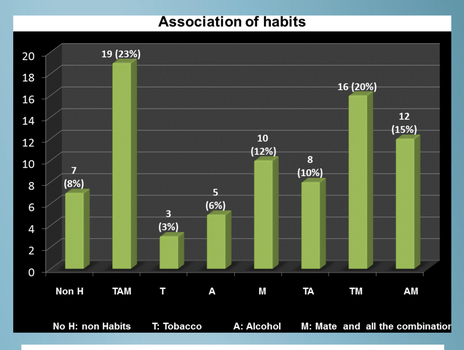


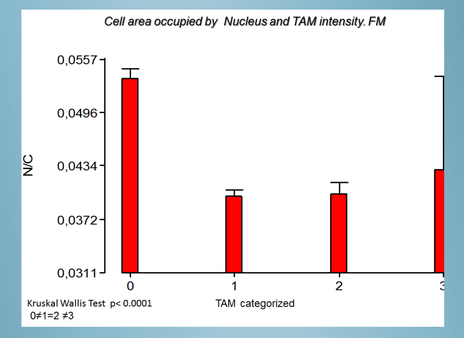


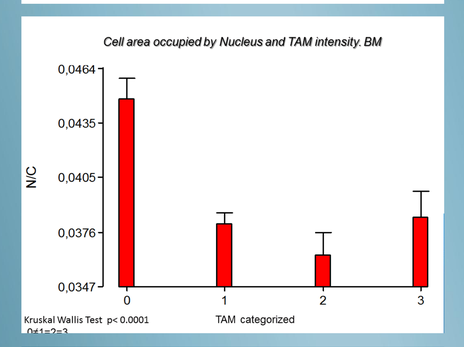


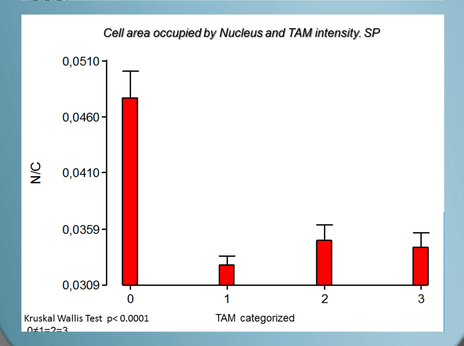
Categorization of TAM

T amount of cig.
T_m= maximum value
T/T_m= Tst
idem for A and M
T_{st}. A_{st}. M_{st}= TAM_{st} (TAM categorized)









CONCLUSION These results indicate that:

* T, A and M consumers, mainly simultaneously, exhibit a decreasing N/C relation of the epithelial cells of the healthy oral mucosa.
* This change is also associated to the amount consumed along life revealing the importance of this registration
* A detailed questionnaire allows to identify patients at risk of malignancy, which should lead to strict clinical controls.
* Professionals have a powerful tool to help patients in controlling habits.
* These findings in people with healthy mucosa contribute to the prevention of oral and oropharyngeal cancers .

ABSTRACT

INTRODUCTION: Some studies show cytological changes indicative of cell damage in exfoliated oral mucosa cells in tobacco smokers (T) and alcohol (A) consumers. There are not publications related to the effects of mate (M), or their combination with the T and A. OBJECTIVE: To study the histo-morphometric changes in cells of the oral mucosa in volunteers who consumed T, A and M. MATERIALS AND METHODS: after signing the informed consent 80 healthy volunteers which completed a medical history detailing the consumption of T (type and amount of cigarettes consumed), alcohol (amount and type of drink) and M (temperature and cc). Samples were obtained with citubrush from three healthy areas: floor of the mouth (A), buccal mucosa (B) and soft palate (C). Image J program was used to determine the cell area, nuclear area and nuclear-cytoplasmic (N / C) ratio was calculated. N/C was correlated with the factors categorized by intensity. Kruskal Wallis test was applied. RESULTS: The cellular area occupied by the nucleus is lower in those who smoke, drink and take hot mate; particularly at locations A and C $p<0.05$. CONCLUSION: The morphological changes observed, establish that the consumption of T, A and M in excess, is able to induce cellular changes that may be associated with an early field cancerization.
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