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Research Article

Micronucleus Frequency in Exfoliated Buccal Cells from Indigenous Coca and Mambe Chewers

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Abstract

Among the Indigenous cultures of Southern of Colombia, the chewing of coca and mambe are a very common and ancient practice in almost all age group. The leaves of several species of the shrub Erythroxylum, popularly known as coca, are the natural source of cocaine. Traditionally, indigenous population has chewed the coca leaves to prevent fatigue during long hours of work. In the practice of chewing, the coca leaves are mixed with limestone powder and maintained between the molars and inner cheeks. This tradition has been associated with adverse effects in oral cavity and upper digestive tract. Effects and the composition of coca have been extensively studied; however, the cytogenetic effects in the oral cavity associated to the habit of chew coca leaves and mambe received less attention. In this context, we used the Micronucleus assay (MN) to compare cytogenetic damage in the cells of oral mucosa of coca and mambe chewers. We observed a significant increase in MN frequency among coca and mambe chewers compared to control group; indicating strong cytogenetic damage associated to the habit of coca and mambe chewing. Together, this study reemphasizes the efficacy of MN assay as method in health hazards monitoring associated by habit and life styles.

ABBREVIATION

MN: Micro Nucleus

INTRODUCTION

In Indigenous cultures of Southern of Colombia, chewing coca and mambe are a very common and ancient practice in almost all age group. Nowadays, the practice is associated as inheriting habits, cultural processes and religious beliefs; in addition, it is associated with extreme poverty and malnutrition conditions. Coca (*Eritroxilum coca*); belongs to the *Eritroxilaceas* family and is a neurotoxic plant which contains the alkaloid cocaine. In humans, cocaine is highly toxic at medium and high doses, highly rewarding and reinforcing at low doses and potentially addictive [1,2]. Traditionally, indigenous population has chewed the coca leaves to prevent fatigue during long hours of work [3]; the activity of chewing coca is called *mambear*; methods are believed to be unchanged since ancient times; however, recent evidences have shown no increase in work capacity after coca chewing, but could possibly delay fatigue during prolonged exercise [4]. In the practice of chewing, the coca leaves are mixed with mambe. The mambe is obtained from limestone which is burning to form a powder. The mixture between coca leaves and limestone powder is carried to the mouth and maintained between the molars and inner cheeks. In the mouth, the alkaline properties of limestone help to liberate the alkaloids presents in plant leaves. Among indigenous population chews coca leaves is a millenary practice; nevertheless, this tradition has been found to be associated with adverse effects in oral cavity and upper digestive tract, including attrition and staining dental, periodontal diseases, lichenoid lesions, oral leukoplakia, submucous fibrosis, squamous cell carcinoma, inflammation and caries [5,6].

In the present study, we used the Micronucleus assay (MN) to evaluate cytogenetic damage (aneugenic and clastogenic) in cells of oral mucosa of coca and mambe chewers. This assay in uncultured exfoliated oral mucosa cells, involving minimally invasive sampling and it has been successfully applied to evaluate inhalation and local exposure to genotoxic agents, impact of nutrition and lifestyle factors [7]. MN is defined as a small

extra nucleus which originates from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division [8].

An increased in MN frequency is related to mutagenic effects and an increased risk for cancer [9,10]. MN frequency in oral cells has been shown to be a prognostic marker for mouth diseases and cancer in a number of studies and seems to be a good candidate for oral cancer bio monitoring [11,12]. The purpose of the present study was to evaluate the MN frequency in buccal cells of coca and mambe chewers.

MATERIALS AND METHODS

Subjects

The subjects involved in this study are farmers and live in a province near Popayán - Colombia. The samples were collected attended the ethical guidelines and with prior consent of subjects. A detailed questionnaire was filled up with information including age, gender, smoking, alcohol consumption, and time and frequency exposure to coca-mambe (Table 1). In this study were excluded individuals with cancer or who suffered chronic disease, as well as individuals who consuming drugs regularity. Study population was comprised of 21 indigenous. The case group (n=11) was composed of individuals whom regularly chew a mixture of coca and mambe, and the control group (n=10) of indigenous from same community. To eliminate factors which could influencing MN count; both, chewers and controls were chosen in a way that they were compared in terms of age, gender (male) and lifestyle.

Micronucleus assay

We used a routine protocol of MN assay as previously described [13] with some modifications [14]. Briefly, buccal cells were obtained by rubbing the inside of the checks with a toothbrush. Cells were collected in a tube containing 3 mL of saline solution (0.9%). After two steps of washes in this solution and followed by centrifugation at 1500 r.p.m for 10 min; the pellet was transferred to an Eppendorf tube with Phosphate Buffered Saline (PBS) at pH. 7.0 And centrifuged at 1500 rpm for 10 min.

Table 1: Characterization of study group.

	Chewers	Controls	*p*
Age (years)	51.73 ± 11.6	50.60 ± 12.6	0.831[a]
Gender			
Male	11	10	1.00[b]
Female	0	0	
Tobacco consumption	1 (9.1%)	0 (0%)	0.329[b]
Chewing Frequency			
1 time/month	1 (9,1%)		
Several times/week	4 (36.4%)		
All days	6 (54.5%)		
Exposure (years)	33.63 ± 14.85		
MN Frequency	1.83 ± 1.19	0.96 ± 0.21	**0.037**[a]

[a], *t*-student test; [b], chi-square test. Significance difference is despite in bold.

The cytological preparations were made in duplicated (two slides for each sample), fixed in cold methanol: acetic acid (3:1) for 10 min and stained with 1% Giemsa solution (pH 7.4) for 15 min. 1000 cells were scored per subject to find the frequency of MN.

Statistical analysis

The obtained data was expressed as mean ± Standard Deviation (SD), and were analyzed using the SPSS V. 22 software (SPSS Inc., Chicago, IL, USA). Chi-square test and Student's *t*-test were used to evaluate the diversity of demographic variables and MN frequency between cases and controls group. The Kolmogorov-Smirnov test was used to decide if the data are from normal distribution.

RESULTS AND DISCUSSION

The application of the MN assay in uncultured buccal exfoliated cells, started in the 1980s, as a biomarker of genetic damage caused by life-style habits, impact of nutrition, exposure to environmental pollutants, medical procedures, as well as, inherited genetic defects in DNA repair [7]; in addition, a high MN frequency has been detected in cells of subjects affected by cancer-associated congenital syndromes characterized by DNA-repair defects. MN test in buccal cells has been shown to be a prognostic marker for mouth diseases and cancer [11,15]. In addition, increased in MN frequency has been detected in buccal cells of Down syndrome and Alzheimer´s disease patients [16-18]. A growing interest in this assay in the last years was also associated with the follow up of oral cancer and premalignant lesions. MN frequency in buccal cells was shown to be a prognostic marker for mouth diseases and cancer in a number of studies [12,19-22] and seems to be a good candidate for oral cancer bio monitoring [19]. In the present study, the average age of the exposed group was 51.73 years and control group (non-chewers) 50.60 years with no differences detected between groups ($p > 0.05$). Regarding to MN assay, were found a significant increase in MN frequencies in coca and mambe chewers (1.83 ± 1.19) when compared to control group (0.96 ± 0.21; (Table 1)), indicating strong cytogenetic damage associated to the habit of coca and mambe chewing ($p < 0.05$). The exposure to coca and mambe by chewing was of 33.64 ± 14.85 years (Table 1). In line with these results, we can see that more of 50% of the coca and mambe chewers carry out this habit every day (Table 1). In terms of genotoxicity, the results here reported are of interest considering that the high frequency of MN seems to be associated to habit of coca and mambe chewing. However, the findings of the current study do not support the previous research which has shown that Chewing of the leaves did not induce nuclear anomalies reflecting genetic damage such as MN and nuclear buds [23]. The discrepancy between our results and the reported by other authors may be associated to a synergic effect between mambe and leaves of coca. In order to increase the release of alkaloids present in the leaves, in particular cocaine, some chewers use small amount of limestone powder (mambe) which is high alkaline. It is well known that alkaline conditions may create favorable environments for induced genetic instability [24], in addition to mechanisms of acction mediated through the polymerization or depolymerization of microtubules [25]. Chewing coca leaves rarely induces acute effects; nevertheless,

chronic effects are often observed. Through visual exploration of oral cavity, we observed in the case group: dental attrition, periodontal inflammation, staining dental and caries. These results are consistent with previous studies, which have shown that the coca chewing is associated with attrition and staining dental, periodontal diseases, lichenoid lesions, oral leukoplakia, submucous fibrosis, squamous cell carcinoma, inflammation and caries [5,6,26].

In addition, it has been shown that crack cocaine induces significant changes on the oral epithelial cells [27]. Likely, these lesions are associated with extreme heat of the smoke. It is possible that the chemical content of the smoke contributed to lesion development, but the mechanism which these lesions are a result of chemical rather than thermal insult is unknown [28]. In this context, our results of genotoxicity seem to be associated with limestone powder, once low incidences of oral cancer have been reported in traditionally coca chewing countries such as Bolivia and Peru when compared to countries such as Brazil and Argentina, where coca is not used [29]. Chemicals analyses have shown that coca leaves contain β-carotene, vitamin E, trace amounts of vitamin D, polyphenolics and micronutrients such as Zn and Fe [30] which have been reported to regulate and stabilize the genetic material and cancer preventive properties [31,32]. In the context of genotoxicity, the expression of MN have significant biological effects, due to consequent changes in genes expression profiles, dysregulation of several biological and cellular processes, ultimately leading to cell death and disease progression [33]. However studies addressing the interaction between mambe and coca still need further investigation in order to evaluate their contribution to expression of MN.

CONCLUSION

The observations of this study showed that coca and mambe chewing increased the frequency of MN in buccal cells which reflects cell injury as resulted probably of aneugenic and clastogenic events. Consequently, this study reemphasizes the efficacy of MN assay as tool for evaluating the health hazard associated by habit and life styles; however, the limitation of this study was the sample size which could have been larger.

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