Saliva Contamination by Opportunistic Microorganisms in Drug Addiction Females

Contaminação de Saliva por Microrganismos Oportunistas em Mulheres com Dependência Química

La Presencia en Saliva del Microrganismos Oportunistas en las Mujeres con Adicción a las Drogas

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In recent decades there has been a significant increase in the consumption of legal and illegal drugs, and most of such compounds are able to induce dependence and this increase was observed mainly in females. This drug addiction increases susceptibility to several infectious agents, especially opportunistic microorganisms. The objective of this study was to evaluate the occurrence of opportunistic bacteria and yeasts in the mouth of drug addiction patients and non-addicted patients with different periodontal conditions. The study included 50 addiction patients and 200 non-addiction subjects. Intra and extraoral clinical examinations were performed and saliva samples were transferred to saline solution and the presence of members of the family Enterobacteriaceae, genera Enterococcus and Pseudomonas, as well fungi of the genus Candida was evaluated by culture. Samples were cultivated onto selective and non-selective media under aerobic conditions, at 37^{o}C, for 24 -48 h. Identification of selected microorganisms were carried out through biochemical tests. Chi-square test was used to evaluate the data when three or more categories were involved. Higher detection frequencies of Candida species, family Enterobacteriaceae, E. faecalis, Pseudomonas sp. and P. aeruginosa in addiction patients were verified. It was found that patients addicted to both genders showed a higher occurrence of members of the Enterobacteriaceae, which were also associated with bone loss only in patients with drug addiction.

Keywords: Drugs, Pathogens, Contamination, Prevention.

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INTRODUCTION

The usage of licit and/or illicit drugs has been increasing steadily in recent decades, especially among younger individuals, particularly females. In this condition, users sometimes create a parallel society, with their own codes of conduct and parallel laws. The drugs, legal or not, still compromise the sociability of the individual and present several and extensive social psychological and familiar effects[1]. Moreover, the drug addiction usually presents multiple and deleterious effects and the individual rarely adhere to the consumption of just one or two different compounds. The concomitant use of alcohol, crack, cocaine and tobacco is frequent[2].

The vast majority of psychotropic drugs reduce wound repair and immune reactivity, leading to a decreased resistance to infections, and increasing the occurrence of allergic reactions[3]. In the case of alcohol, its deleterious effects also reach the innate immune system, besides changes in the specific or adaptive immune system[3], affecting dendritic and antigen-presenting cells[4], inducing apoptosis of "natural killer" cells, reducing the activity of cytotoxic immune response and the expression of interleukins[5]. Other

drugs, such as cocaine, still produce significant vasoconstriction, which can lead to tissue necrosis[6], reduced resistance to infection and profound delay in the wound repair,[7] particularly periodontium[8,9].

The stress associated with drug usage as well as the direct effects of the chemical eventually induce hallucinations, syndromes of persecution, severe depression and anxiety[10] and these stressful events are linked to progressive and severe immunosuppression and might create favorable conditions for the transmission and dissemination of opportunistic pathogens in oral cavity[11].

Drug addiction can reduce the motivation to perform daily tasks, such as oral hygiene, which exacerbate the drug effects on oral tissues, cause irritation, insomnia, loss of appetite, and diet changes, generally to reduce sensations associated with xerostomia. In turn, these changes may affect the composition of the oral microbiota[12], although it does not know the extent of these changes, nor the influence of these modifications on the microbial contamination of saliva, which is known to be responsible for transmission and dissemination of microorganisms. Moreover, the oral microbiota has characteristics peculiar to certain ethnic groups and geographic areas[13,14].

Then, considering the relevance of oral and systemic infections in drug addiction patients (AP), and the role of saliva in the process of transmission and dissemination of opportunistic pathogens, the aim of this study was to evaluate the occurrence of the some opportunistic microorganisms in saliva from addicted and non-addicted patients with different periodontal conditions.

MATERIAL AND METHODS

This study was approved by Institutional Review Board of the School of Dentistry of Araçatuba - UNESP (Proc. 1797/09).

1. POPULATION

A total of 50 drug addiction females (aged 27.1 ± 11.3 years) seen at the Hospital "Lar Madre Paulina", as well 200 females (aged 22.4 ± 15.7 years) without history of drug addiction were selected to this study. All patients harbor at least 20 teeth, did not present systemic diseases, which could compromise the collection of clinical samples of saliva, and did not use systemic or topic antimicrobial drugs or receive medical or dental treatments within 6 months prior to the study.

The periodontal evaluations were performed by a single expert, as described by Corraini et al.[15] (2008). According to the criteria of Armitage et al.[16] (2004), drug addiction patients (AP) were considered periodontally healthy (N= 13), gingivitis (N= 19) and periodontitis (N= 18). Among the non-addiction patients (NAP), 89 were periodontally healthy, 61 had gingivitis and 50 had chronic periodontitis.

Drug addiction patients who participated in this study had record about the historical use of licit and illicit drugs and underwent medical assessment on initial 07 days after entering the treatment for detoxification. Patients in the control group, without chemical dependency, were selected through parameterized search program, among the patients at the School of Dentistry of Araçatuba –UNESP, between 2008 and 2011, using the same criteria for inclusion and exclusion

Standardized forms were filled out with information regarding identification, age, ethnic background, systemic health, consumption of licit and illicit drugs, abstinence period, in addition to physical examination. Socioeconomic conditions of the patients were registered through a questionnaire. The psychological and psychiatric conditions were assessed by specialists from the centers participating in the study. The different patterns of drug addiction are presented in Table 1.

2. SALIVA COLLECTION

The collection of saliva specimens was performed from 8:00 to 10:30 hours. All clinical specimens were transported in saline solutions to the laboratory. The samples of saliva were collected

immediately before the clinical examination of dental and periodontal conditions. In order to collect saliva, patients were asked not to drink, eat or perform oral hygiene one hour before the attendance. Saliva collection was carried out by mean of Salivettes devices (Aktiengesellsschaft, Nümbrecht, Germany), which allow to obtain satisfactory amounts of saliva even in patients presenting clinical cases of severe xerostomia.

Drugs & Associations	Frequency N (%)
Alcohol	3 (6.0)
Cocaine	2 (4.0)
Crack	5 (10.0)
Ecstasy	2 (4.0)
Alcohol + cocaine	3 (6.0)
Alcohol + marijuana	2 (4.0)
Alcohol + tobacco	5 (10.0)
Cocaine + tobacco	3 (6.0)
Crack + tobacco	3 (6.0)
Alcohol + tobacco + marijuana	2 (4.0)
Alcohol + crack + tobacco	1 (2.0)
Crack+ marijuana + tobacco	2 (4.0)
Ecstasy + marijuana+ tobacco	1 (2.0)
Alcohol + cocaine + crack + tobacco	2 (4.0)
Alcohol + cocaine + crack + tobacco	3 (6.0)
Alcohol + cocaine + crack + marijuana + tobacco	3 (6.0)
Cocaine + ecstasy + LSD[1] + marijuana + tobacco	1 (2.0)
Cocaine + crack + ecstasy + LSD + tobacco	1 (2.0)
Alcohol + cocaine + crack+ marijuana+ LSD + tobacco	1 (2.0)
Alcohol + marijuana + cocaine + crack+ LSD + tobacco	1 (2.0)
Alcohol + cocaine + crack+ ecstasy + LSD + marijuana + tobacco	3 (6.0)
Cocaine + crack + ecstasy + LSD + marijuana + tobacco + others	1 (2.0)

3. MICROBIAL ISOLATION AND IDENTIFICATION

Specimens were inoculated in peptone water and ethyl violet azide broth (EVA broth, Difco) and incubated 3-7 days at room temperature. From tubes with microbial growth in peptone water, aliquots of 0.1 ml were transferred to Eosin Methylene Blue agar (EMB agar), MacConkey agar, Brilliant Green agar and Brain Heart Infusion agar supplemented with defibrinated horse blood and yeast extract (0.5%), incubated at 37°C, for 24-48 h. Samples were also inoculated onto Sabouraud Dextrose agar with 100 µg/ml of chloramphenicol and incubated at room temperature, for 3-7 days, for yeasts isolation.

From tubes containing EVA broth, aliquots of 0.1 mL were transferred to Bile Esculin agar and incubated in aerobiosis for 48 h. at 37°C, in order to isolate enteric bacteria and pseudomonads[17].

The yeast identification was performed by carbon and nitrogen assimilation tests, fermentation of carbohydrates, germ tube formation (at 37°C and at 39°C), colonial morphology on CHROMagar *Candida* (MastDiagnostica, Paris, France), and growth at 37°C and 42°C. In the carbohydrate assimilation tests, the inoculum cultivated overnight in yeast peptone dextrose broth, was adjusted to 5 McFarland standard, centrifuged for 5 min. at 2000.g and the pellets were 3 times washed and added to 1 mL of sterile Yeast Nitrogen Base (YNB, Difco) and 20 mL of bacteriological agar (Bacto Agar, Difco), mixed and plated in 15 cm Petri dishes. After solidification of the medium, discs with 2% carbohydrates were plated and incubated for 96 h. at 30°C. The presence of microbial growth around paper discs containing carbohydrates was registered daily. The *Candida* isolates with inconclusive identification to species level were submitted to DNA extraction and amplification following the methodology previously described, using PCR with specific primer pairs.

Bacterial species were identified using the following methods: colonial characteristics, Gram-staining, growth at 10% sodium chloride, production of gas from glucose, and biochemical tests using the API-20E commercial kit (BioMérieux SA, Marcy-l'Etoile, France). Some bacterial isolates were identified using

the BBL Crystal Enteric/Nonfermenter system (Becton Dickinson Microbiology Systems, Cockeysville, MD), following recommendations of the manufacturer[17].

STATISTICAL ANALYSIS

The Chi-square test was used to assess the significance of associations between microbial parameters, clinical results and drug addiction patterns. The prevalence of the target organism in the different groups was also evaluated using analysis of variance for repeated measures. In all analyses, the significance level was 5%.

RESULTS

The salivary contamination by *Candida* sp. (Chi-square test, p = 0.012), *C. albicans* (Chi-square test, p = 0.007), family *Enterobacteriaceae* (Chi-square test, p = 0.021), *E. faecalis* (Chi-square test, p = 0.025), *Pseudomonas* sp. (Chi-square test, p = 0.019) and *P. aeruginosa* (Chi-square test, p = 0.021) was significantly higher than observed in non-addiction patients.

Citrobacter freundii (AP, 6.0 %; NAP, 1.0%), *Enterobacter cloacae* (AP, 8.0%; NAP, 1.3%), *E. intermedius* (AP, 4.0%; NAP, 1.3%), *E. sakazakii* (AP, 4.0%; NAP, 2.8%), *Enterobacter* sp. (AP, 2.0%; NAP, 3.3%), *Escherichia coli* (AP, 4.0%; NAP, 2.3%), *Klebsiella oxytoca* (AP, 4.0%; NAP, 1.0%), *K. pneumoniae* (AP, 2.0%, NAP, 2.8%), *Morganella morganii* (AP 2.0%, NAP, 1.8%), *Pantoea* sp. (AP, 0.0%, NAP, 1.0%), *Proteus mirabilis* (AP, 4.0%, NAP, 0.5%), *P. vulgaris* (AP, 2.0%, NAP, 0.5%), *Proteus* sp. (addiction patients, 0.0%; NAP, 1.0%), *Providencia alcalifaciens* (AP, 0.0%; NAP, 1.5%), *Providencia* sp. (AP, 2.0%; NAP, 0.5%), and *Serratia marcescens* (AP, 1.0%; NAP, 0.0%) were the members of family *Enterobacteriaceae* cultivated. In drug addiction patients it was also observed correlation between the presence of members of the *Enterobacteriaceae* and bone/connective tissue attachment loss (Chi-square, p = 0.028), and this correlation was not observed in non-addiction patients.

The commonly cultivated fungi belonged to *C. albicans* (AP, 28.0%; NAP, 17.0%), *C. tropicalis* species (AP, 12.0%; NAP, 4.0%), which were associated with alcohol consumption (Chi-square, p = 0.018) and high plaque index (Chi-square, p = 0.03),

The associations between salivary presence of targeted microorganisms and the different patterns of drug use and dependence proved to be complex and due to the several schemes of illicit drug use, it was not possible to determine the influence of a singular compound and the presence of a particular microorganism or clinical parameter. However, tobacco favorably affected the distribution of the family *Enterobacteriaceae* (Chi-square test, p = 0.013).

The occurrence of family *Enterobacteriaceae*, *Pseudomonas* sp. and *P. aeruginosa* evidenced correlation with the presence of removable oral prosthetic devices, poor oral hygiene (presence / absence of biofilm), as well as tobacco, cocaine, crack and alcohol (Chi-square, p = 0.023 at p = 0.001). The saliva contamination by *Pseudomonadaceae* and *Enterobacteriaceae* was associated with plaque index, when individuals with higher biofilm accumulation were more likely to be colonized by these microorganisms (Chi-square, P = 0.029). The presence of such opportunistic microorganisms was significantly associated with drug addiction, particularly alcohol, crack and tobacco.

DISCUSSION

Saliva is used in the evaluation of caries risk, but in this situation the main microorganisms detected, the cariogenic streptococci, are part of supragingival microbiota and eventually have easy access to the salivary flow, so that the composition of saliva is mainly composed by members of the microbiota of the supragingival biofilm. However, there are few reports

about the presence of opportunistic microorganisms in saliva and their relevance to oral ecology and health conditions[18], which contrasts with the relevance of saliva as vehicle of transmission pathogenic microorganisms of medical and dental relevance[19].

This lack of knowledge about presence of opportunistic pathogens in the saliva and its correlation with periodontal status of the patients[19] is aggravated when it appears that the vast majority of drug addiction patients present moderate to severe xerostomia, depending on the effect of these agents on the nervous system, particularly as tobacco, alcohol and other psychoactive compounds, which could affect the presence of different microorganisms. Some of these agents, such as alcohol, are considered recreational but presents a large scope of side effects on social cohesion, and their economic and health effects on the general population are becoming pervasive, with serious consequences on public health and social life[1].

The complications produced by licit or illicit drugs are exacerbated by the fact that most drug users employ more than one compound, concomitantly, as observed in this study and also reported by Guindalini et al.[2] (2006). Table 1 evidenced that just 24% of addiction females used a single compound, whereas 32%, 12% and 10% declared to be dependent of two, three, four and five drugs, respectively, which were used concomitantly, in combinations or not.

The prognostic of oral infections and their treatment might be influenced by drug addiction, reducing the social integration of the individual and producing hallucinations, persecution syndromes, severe psychological depression and anxiety, which complicate everyday activities such as their own personal hygiene[10], which is associated with stress, immunosuppression and xerostomia, creating favorable conditions for the establishment of opportunistic microorganisms[9,11,20].

Most opportunistic microorganisms which showed higher occurrence in drug addiction females may be associated with periodontal inflammation, such as members of the families *Enterobacteriaceae* and *Pseudomonadaceae*[21,22] but are not considered as resident in oral environment. These aspects are relevant because in samples of patients with drug addiction it was observed higher incidence of gingivitis and severe periodontitis compared to non-addicted individuals, although the hygiene standards of both groups have been shown to be similar. However, socioeconomic conditions of the population may have minimized the differences in dental and periodontal conditions between groups, since addicted individuals showed better social status, with greater exposure to dental and medical treatment and prevention, than non-addicted females.

The higher detection frequencies of opportunistic species of families Enterobacteriaceae and Pseudomonadaceae in drug addiction females must be correlated with progressive deterioration of their periodontal conditions, particularly with the establishment and progression of inflammatory reactions, since the vast majority of these microorganisms are not part of the oral microbiota[17,23]. It is possible that xerostomia, reducing mechanical and chemical effects of salivation, may create favorable conditions to the maintenance of such pathogens in oral cavity, and these effects on oral microbiota seems to be more pronounced in adolescents and young adults[24].

The presence of *Candida* species in oral microbiota is influenced by several factors, such as immunosuppression and xerostomia[17,25,26], but the effect of drug addiction on their occurrence in the oral cavity, particularly in saliva, remains unclear. These yeasts are part of the oral microbiota, being recovered from 10% to 80% of healthy individuals, but in patients with immune impairment or in the presence of other predisposing factors as diabetes, these opportunistic pathogens can proliferate and produce severe infections, especially in patients with a history of alcoholism and smoking, and deficient cellular immune response[25,26].

Thus, it is possible that the high frequency of fungi detection in drug addiction female with chemical dependency may reflect the direct effects of the drug on the mucosa and, quite possibly, on the cellular immune response associated with mucosal surfaces. Moreover, the occurrence of opportunistic candidiasis in patients with impairment of immune system and other predisposing conditions is quite frequent and sometimes a matter of time[25], and stand out among the dependent carriers of HIV infection. In general, these disorders involve *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *Candida krusei*, with frequent association between two or more species[25,27], as was also observed in this study. Some data about symptomatic HIV-positive patients, many of whom were drug users, suggested that the presence of C. *albicans* may be related to necrosis of the gingival tissue[17], which was not observed in this study.

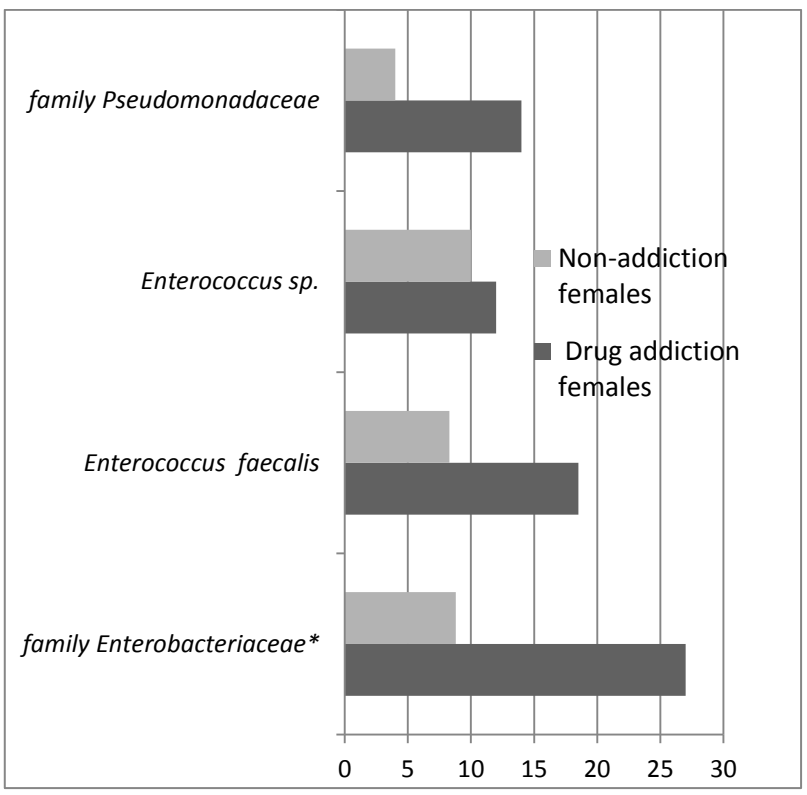

Figure 1. Occurrence (%) of selected opportunistic pathogens in saliva samples from addiction and non-addiction females.

The poor hygiene standards observed in this investigation, in addiction and non-addiction patients, may have facilitated the contamination of oral cavity by of enteric organisms and pseudomonads[21], which are also often associated with opportunistic infections,[26,28] particularly in addiction patients[29]. These microorganisms are more tolerant to adverse environmental conditions and their presence in the oral cavity may be increased in patients with xerostomia and pronounced immunosuppression[26,28].

Diz Dios et al.[30] (1993) isolated enteric bacteria from 68% of HIV-positive patients presenting drug addiction, especially cocaine, and these data are similar to those presented in Figure 1. The pseudomonads can be transmitted between different individuals by saliva or direct contact and from external environments, such as in dental clinics or hospital effluents, frequently associated with severe infections.[31]

CONCLUSION

The results of the present investigation evidenced that saliva from addiction females present higher occurrence of some opportunistic pathogens, such as oral yeasts, enteric microorganisms and pseudomonads, than non-addiction patients. These microbial species may be associated with hygiene and health.

RESUMO

Nas últimas décadas, tem havido um aumento significativo no consumo de drogas lícitas e ilícitas, sendo que a maioria desses agentes é capaz de induzir a ocorrência dependência e este aumento foi observado principalmente em mulheres. Esta dependência de drogas aumenta a susceptibilidade a vários agentes infecciosos, especialmente microrganismos oportunistas. O objetivo deste estudo foi avaliar a ocorrência de bactérias oportunistas e leveduras na boca de pacientes com dependência de drogas e pacientes não dependentes com diferentes condições periodontais. O estudo incluiu 50 pacientes com dependência química e 200 indivíduos sem dependência. Exames clínicos intra e extra-oral foram realizados e as amostras de saliva foram transferidas para solução salina e a presença dos membros da família Enterobacteriaceae, gêneros Enterococcus e Pseudomonas, bem como fungos do gênero Candida foi avaliada por cultura. As amostras foram cultivadas em meios seletivos e não-seletivos em condições aeróbias, a 37^{o}C, por 24-48 h. Identificação de microrganismos selecionados foram realizados por meio de testes bioquímicos. Teste do qui-quadrado foi utilizado para avaliar os dados quando três ou mais categorias estavam envolvidas. Foram observadas freqüências mais elevadas de detecção do gênero Candida, da família Enterobacteriaceae, bem como E. faecalis, Pseudomonas sp. e P. aeruginosa. Verificou-se que os pacientes

dependentes, de ambos os sexos, mostraram uma maior ocorrência de membros da família Enterobacteriaceae, e nesses pacientes esses patógenos mostraram relação com perda óssea.
Palavras Chave: Drogas, Patógenos, Contaminação, Prevenção.

RESUMEN

En las últimas décadas se ha producido un aumento significativo en el consumo de drogas legales e ilegales, y la mayoría de estos compuestos son capaces de inducir dependencia y se observa este aumento sobre todo en las mujeres. Esta adicción a las drogas aumenta la susceptibilidad a varios agentes infecciosos, especialmente microrganismos oportunistas. El objetivo de este estudio fue evaluar la presencia de bacterias entéricas oportunistas en la boca de los pacientes con adicción a las drogas y los pacientes no adictos con diferentes condiciones periodontales. El estudio incluyó a 50 pacientes con adicción a las drogas y 200 sujetos sin la adicción a las drogas. Exámenes clínicos intra y extraorales se realizaron, muestras de saliva se transfirieron a salina y la presencia de los miembros de la familia Enterobacteriaceae, géneros Enterococcus y Pseudomonas, así como los hongos del género Candida se evaluó por la cultura. Las muestras se cultivaron en medios selectivos y no selectivos en condiciones aeróbicas, a 37ºC, durante 24-48h. Identificación de microorganismos seleccionados se llevaron a cabo a través de pruebas bioquímicas. Prueba de Chi-cuadrado se utilizó para evaluar los datos cuando se trataba de tres o más categorías. Mayores frecuencias de detección de especies de Candida, familia Enterobacteriaceae, E. faecalis, Pseudomonas sp. y P. aeruginosa en pacientes con adicción fueran observadas. Se encontró que los pacientes adictos mostraron una mayor incidencia de la familia Enterobacteriaceae, que también se asocia con la pérdida de hueso sólo en pacientes con adicción a las drogas.
Palabras clave: Drogas, Patógenos, Contaminación, Prevención.

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