ARTÍCULOS ORIGINALES

Micronucleus in exfoliated buccal cells of crack users: systematic review and meta-analysis

Micronúcleos en células bucales exfoliadas en consumidores de crack: una revisión sistemática y meta-análisis

Tavella, Ronan Adler[1]; dos Santos, Marina[1]; da Silveira Tatiane, Brito[1]; Fernandes, Caroline Lopes Feijo[1]; Da Silva Júnior, Flavio Manoel Rodrigues[1*]

[1]Universidade Federal do Rio Grande do Sul.
***f.m.r.silvajunior@gmail.com**

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Abstract. This research aimed to conduct a systematic review and metanalysis to compare the frequency of cell damage in crack users and nonusers, through Micronucleous (MN) test in buccal mucosa cells. A comprehensive search was carried out on MEDLINE via PubMeb, Web of Science, LILACS and the grey literature without restrictions. It was included case-control studies that report the frequency of micronuclei in the oral mucosa of adult crack users and nonusers. A review protocol was registered with PROSPERO (CRD42018115672), and conducted in accordance with the PRISMA guidelines for the report of this systematic review. Furthermore, study quality was evaluated using an adapted Newcastle-Ottawa Scale for cross-sectional studies.The original search yielded 27 references, after eligibility criteriaonly five articles were included. The number of micronuclei was higher in crack users compared to nonusers. Also, secondary outcomes: binucleated cells, nuclear buds, pyknosis, karyorrhexis and karyolysis had higher prevalence in crack users.Crack use is associated with genotoxic and mutagenic effects because there is a higher frequency of micronuclei in exfoliated cells of crack users. In addition, MN test proved to be a goodbiomarker to assess the mutagenic impact of crack use in oral epithelium.

Keywords: Illicit drugs; Crack cocaine; Micronucleus tests;Buccal mucosa cells.

Resumen. Esta investigación tuvo como objetivo realizar una revisión sistemática y un meta-análisis para comparar la frecuencia de daño celular en usuarios de crack y sin crack, a través de la prueba de micronúcleos (MN) en células de la mucosa bucal. Se realizó una búsqueda exhaustiva en MEDLINE a través de PubMeb, Web of Science, LILACS y la literatura gris sin restricciones. Se incluyeron estudios de casos y controles que informaron la frecuencia de micronúcleos en la mucosa oral de usuarios adultos de crack y sin crack. Se registró un protocolo de revisión con PROSPERO (CRD42018115672), y se realizó de acuerdo con las pautas de PRISMA para el informe de esta revisión sistemática. Además, la calidad del estudio se evaluó mediante una escala Newcastle-Ottawa adaptada para estudios transversales. La búsqueda original arrojó 27 referencias, después de los criterios de elegibilidad se incluyeron un total de cinco artículos. El número de micronúcleos fue mayor en los usuarios de crack en comparación con los usuarios sin crack. Además, los resultados secundarios de células binucleadas, yemas nucleares, picnosis, cariorrexis y cariólisis tuvieron una mayor prevalencia en los usuarios de crack. El uso de crack se asocia con efectos genotóxicos y mutagénicos porque hay una mayor frecuencia de micronúcleos en las células exfoliadas de los usuarios de crack. Además, la prueba de MN demostró ser un buen biomarcador para evaluar el impacto mutagénico del uso de crack en el epitelio oral.

Palabras clave: Drogas ilícitas; Crack; Pruebas de micronúcleos; Células de la mucosa bucal.

Introduction

The last World Drug Report has estimated that approximately 1 of every 18 people aged 15–64 years used an illicit drug, corresponding to 5.6 per cent of the global population prevalence. There is a potential supply-driven expansionof drug marketsat the highest levels everrecorded, expanding beyond their usual regions.At the same time, more new psychoactive substancesare being synthesized and more are available tha never, with increasing reports of associated harm and fatalities (UNODC 2018).

World crack cocaine consumption has increased, spreading mainly after the 80's (Balbinot *et al.* 2011). Crack is a substance obtained from cocaine hydrochloride through conversion processes to make it suitable for smoking, is a cheap product, which can reach all social classes (UNODC 2018). Crack is a potent stimulant of the central nervous system with a high potential for addiction (Lima *et al.* 2016). This drug is

rapidly absorbed by mucous membraneswhen smoked, reaching cerebral circulation within 6 to 8 seconds. Another factor responsible for the intense crack action is the fact that the substance is more fat-soluble than the cocaine chloride, allowing quick passage to the central nervous system (Oliveira and Dinis-Oliveira 2018).

Besides several psychotropic and neurotoxic effects, crack has high potential to generate damage to the user's health (Lima *et al.* 2016). Crack is a major cause of lesions in the upper respiratory emphysema (de Freitas *et al.* 2014; Oliveira and Dinis-Oliveira 2018). Also, there is evidence that crack produces carcinogenic effects (Oliveira and Dinis-Oliveira 2018), cardiovascular disease, neurologicalproblems, and gastrointestinal complications in adults (Butler *et al.* 2017; Antoniazzi *et al.* 2018). This drug appears to be able to induce inflammatory changes in the oral epithelium, increasing periodontal disease (Cury *et al.* 2017) and oral DNA damage such as an increase in keratinization, decrease in the area of nuclei, and increase in the number of nucleolar organizer regions (Antoniazzi *et al.* 2018).

Micronucleus (MN) are extranuclear bodies composed of chromosomes or chromosomal fragments, which are separated from the daughter nuclei during nuclear division (mitosis) (das Graças *et al.* 2014). The presence of MN indicates DNA breaks, that is, clastogenicity or aneuploidy due to disturbances in the mitotic spindle caused by exposure to genotoxic agents (Thomas and Fenech 2011; das Graças *et al.* 2014). The MN test is considered a fast, inexpensive and non-invasive biomarker (Da Silva Pinto *et al.* 2017; da Silva Júnior *et al.* 2018; Aguiar Torres *et al.* 2019). It can be used to prevention and individuals monitoring at risk for cancer (Holland *et al.* 2008; Lorenzoni *et al.* 2017; Espitia-Pérez *et al.* 2018), especially when evaluated in oral exfoliated cells (Bonassi *et al.* 2011), in susceptible population such as chronic users of mutagenic substances as crackcocaine (Lima *et al.* 2016). The cytotoxicity of mutagenic substances has already been associated with MN in oral cells (Webber *et al.* 2016; de Geus *et al.* 2018). However, few studies have assessed the potential effects of crack induce genetic damage and there is no consensus about the effect of crack in oral mucosa and frequency of MN. Thus, due the high prevalence of crack cocaine use and itsassociated serious health outcomes, the purpose of the present study was to conduct a systematic review and meta-analysis to compare the frequency of celldamage in crack users and non-users, through MN test in buccal mucosa cells.

Methods

Protocol and registration

This study protocol were registered at the PROSPERO (international prospective register of systematic reviews) database (CRD42018115672), and conducted in accordance with the PRISMA guidelines for the report of tis systematic review (Moher *et al.* 2009).

Search strategy

The controlled vocabulary (mesh terms) and free keyword in the search strategy was defined based on the following PECO question:

1. Population (P): adults
2. Exposure (E): crack users
3. Comparison (C): non-crack users
4. The outcome (O): frequency of micronuclei
5. Study design (S): cross-sectional studies

To identify trials to be included for this review, we searched on the electronic databases MEDLINE via PubMeb Web of Science, Latin-American and Caribbean Literature on Health Sciences (LILACS), and the grey literature (google-scholar). No restrictions to publication date or languages were performed. The search terms used were: "crack" OR “crack cocaine” and “micronucleus” OR “micronuclei”.

Eligibility criteria

To be included, studies must have been published as an original survey reporting the frequency of micronuclei in users and nonusers of crack in adult patients of any group. The primary outcome of the study was the frequency of micronuclei. It was included only cross-sectional studies in humans. Non-controlled clinical trials, editorial letters, pilot studies, historical reviews, in vitro studies, and descriptive studies, such as case reports and case series, were excluded.

Study selection and data collection process and data extraction

Initially, the articles were selected by title and abstracts. Full-text articles were obtained when the title and abstract had insufficient information to make a clear decision. Subsequently, two reviewers individually classified those that metthe inclusion criteria with approximately 95% agreement. Differences were resolved by discussion and consultation with a third researcher when it was needed. Two

researchers completed data extraction for all studies, one review author checked text entries, and one independent quality control person checked numeric outcome data. To find additional relevant articles, the reference lists of all of the retrieved studies were examined.

Quality Assessment

Study quality was evaluated using an adapted Newcastle-Ottawa Scale (NOS) (Wells *et al.* 2012) for cross-sectional studies, which assesses the design and quality of nonrandomized studies and further facilitates the task of incorporating quality assessments into the interpretation of meta-analysis results. The evaluation of each article was given by a star rating from three perspectives: selection (4 criteria), comparability (1 criterion) and exposure (3 criteria). After the sum of the stars received by each article, it is possible to classify them in unsatisfactory (1-4 stars), intermediate (5 stars), good (6-7 stars) andvery goodquality (8-9 stars) studies. Only studies classified as good or very good quality were included in the meta-analysis. It is important to mention that higher quality studies have lower risk of bias, and so on. The NOS was presented in the supplementary material.

Statistical Analysis

When authors evaluated the micronuclei frequency in different regions in the oral cavity or when more than one group of crack users was added, we combined these data to make a single entry in the meta-analysis. In the meta-analysis to compute the overall effect size, it was used fixed effects model due to the number of studies (less than 10 studies). Between-study heterogeneity was examined using Cochran's Q test and I-squared. Publication bias was assessed by visual inspection of funnel plots. Formal statistical assessment of funnel plot asymmetry was done with Egger's regression asymmetry test. The meta-analysis was performed using the software MetaXL 5.3 and the statistical analyses were done by using Stata, version 10. Values of < 0.05 were considered statistically significant.

Results

The flowchart of selected studies was exhibited in *Figure 1*. The original search yielded 27 references. Full-text reports on 8 studies were retrieved for complete review, no articles were excluded after quality assessment as well as no article were included from the reference list. A total of five articles were included in the review.

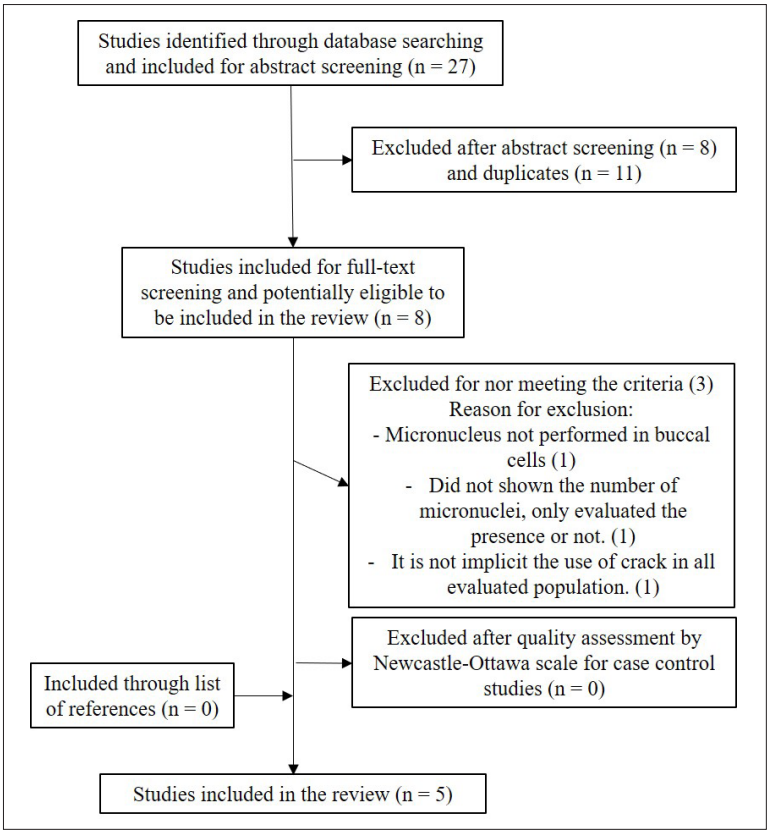

Figure 1. Flow of information through the different phases of a systematic review

The characteristics of the selected studies are shown in *Table 1*. Majority of the studies included in this review used Feulgen staining procedure (Almeida *et al.* 2012; das Graças *et al.* 2014; Webber *et al.* 2015), one study used Giemsa (Antoniazzi *et al.* 2018) and one Feulgen-Rossenbeck (Lima *et al.* 2016). All studies scored 1000 cells and testedbuccal mucosa cells.

The smallest sample size per group was 10 (Almeida *et al.* 2012) and the highest was 54 (Antoniazzi *et al.* 2018). The mean age of participants ranged from 20.2 to 40.9 for nonusersof crack and 15.5 to 39.6 for crack users (Almeida *et al.* 2012; das Graças *et al.* 2014; Webber *et al.* 2015; Lima *et al.* 2016; Antoniazzi *et al.* 2018). The mean time of crack use ranged from more than 1 year to 6.4 years (Almeida *et al.* 2012; Lima *et al.* 2016; Antoniazzi *et al.* 2018), and two studies did not report this information (das Graças *et al.* 2014; Webber *et al.* 2015). Only, one study did not present significant statistical difference between the number of micronuclei between nonusers of crack compared to users of crack (Webber *et al.* 2015).To obtain the samples, four studies smeared only the mucosa of the cheek (Almeida *et al.* 2012; das Graças *et al.* 2014; Lima *et al.* 2016; Antoniazzi *et al.* 2018) and one smeared the floor of the mouth (Webber *et al.* 2015). All studies analyzed other genotoxic changes in addition to the main outcome (micronuclei).

Table 1. Summary of the studies selected for this systematic review.

Reference	n participants/ group	Age (years)+	Time of Crack use (years)	Micro nuclei Mean± SD
Almeida et al. 2012	10 NC 10 C	20.2 ± 1.0 NC 15.5 ± 1.4 C	3.3 ± 1.4	0.1 ± 0.3 NC 4.3 ± 4.1 C*
das Graças et al. 2014	30 NC 30 C	31.4 ± 9.3 NC 33.6 ± 11.6 C	-	0.7 ± 0.2 NC 1.3 ± 1.4 C*
Weber et al. 2015	34 NC 26 C	40.9 ± 11.0 NC 39.6 ± 12.8 C	-	0.2 ± 0.4 NC 0.4 ± 0.6 C
Lima et al. 2016	15 NC 15 C	30.2 ± 10.65	6.4	0.6 ± 1.6 NC 2.9 ± 3.5 C*
Antoniazzi et al. 2018	54 NC 54 C	22.5 (18.0 - 31.0) NC 25 (18.0 - 32.2) C	> 1.0	3.8 ± 7.6 NC 17.3 ± 21.8 C*

NC non-users of crack; C users of crack;* $p \leq 0.05$. All studies report year in mean± SD (standard deviation) expect + median (25th–75th percentile).

The secondary outcomes are present in *table 2*. Three studies evaluated binucleated cells (Almeida *et al.* 2012; Webber *et al.* 2015; Lima *et al.* 2016), four evaluated nuclear buds (Almeida *et al.* 2012; Webber *et al.* 2015; Lima *et al.* 2016; Antoniazzi *et al.* 2018), three analyzed pyknosis (Almeida *et al.* 2012; das Graças *et al.* 2014; Lima *et al.* 2016), five evaluated karyorrhexis (Almeida *et al.* 2012; das Graças *et al.* 2014; Webber *et al.* 2015; Lima *et al.* 2016; Antoniazzi *et al.* 2018) and three analyzed karyolysis (das Graças *et al.* 2014; Lima *et al.* 2016; Antoniazzi *et al.* 2018). In all cases, except for Webber *et al.* (2015), significant differences were observed between groups. More significant DNA damage was found in the crack users groups (Almeida *et al.* 2012; das Graças *et al.* 2014; Lima *et al.* 2016; Antoniazzi *et al.* 2018).

The quality assessment of selected studies was presented in *Table 3*. In summary, four studies were considered of very good quality (Webber *et al.* 2015; das Graças *et al.* 2014; Lima *et al.* 2016; Antoniazzi *et al.* 2018) and one of good quality (Almeida *et al.* 2012), having low risk of bias, according to the quality assessment components of the Newcastle-Ottawa Scale (Wells 2012). Therefore, all the studies met the best requirement features for inclusion in the meta-analysis of the frequency of micronuclei in crack users.

Table 2. Data from secondary outcomes

Reference	Binucleation	Nuclear Buds (Broken eggs)	Pyknosis	Karyorrhexis	Karyolysis
Almeida et al. 2012	10.2 ± 9.6 NC 15.9 ± 20.85 C	0.5 ± 1.2 NC 1.5 ± 2.3 C	0.2 ± 0.6 NC 17.5 ± 13.8 C*	34.4 ± 24.1 NC 347.9 ± 276.3 C *	-
das Graças et al. 2014	-	-	91 ± 48 NC 88 ± 40 C	10 ± 9 NC 11 ± 10 C	19 ± 17 NC 31 ± 23 C*
Weber et al. 2015	1.2 ± 0.2 NC 1.3 ± 0.3 C	0.7 ± 1.6 NC 1.6 ± 3.0 C	-	1.7 ± 2.9 NC 4.7 ± 8.4 C	-
Lima et al. 2016	9.3 ± 6.8 NC 11.1 ± 6.5 C	5.0 ± 5.8 NC 4.1 ± 3.4 C	10.8 ± 6.8 NC 13.1 ± 8.8 C	24.9 ± 24.0 NC 54.1 ± 38.6 C*	8.3 ± 9.3 NC 26.7 ± 39.6 C
Antoniazzi et al. 2018	-	0.02 ± 0.1 NC 2.1 ± 11.3 C	-	10.11 ± 24.04 NC 30.39 ± 27.64 C*	9.46 ± 12.9 NC 12.4 ± 22.8 C*

NC nonusers of crack; C users of crack;* $p \leq 0.05$.
All studies report year in mean± SD (standard deviation)

Table 3. Assessment of the quality of case control studies included in the meta-analysis using the Newcastle-Ottawa Scale.

Reference	Selection 1 2 3 4	Comparability	Exposure 1 2	Total	Final Rating
Almeida et al. 2012	* * *	*	* * *	7/9	Good quality
das Graças et al. 2014	* * * *	* *	* * *	9/9	Very good quality
Weber et al. 2015	* * * *	* *	* * *	9/9	Very good quality
Lima et al. 2016	* * * *	* *	* * *	9/9	Very good quality
Antoniazzi et al. 2018	* * * *	* *	* * *	9/9	Very good quality

The standardized mean difference of the frequency of micronuclei between groups was -0.72, with a 95% confidence interval of -0.96 to -0.48 ($p < 0.001$). Based on these studies, a significant statistical difference between the groups could be identified (*Figure 2*). Data were homogeneous (chi-square test, $p < 0.52$; $I2 = 0\%$), which means that all studies included in the analysis share a common effect size.

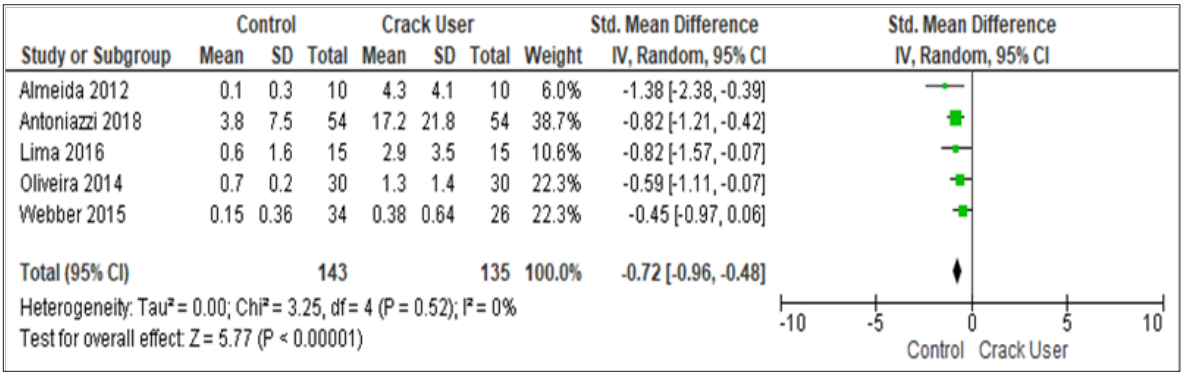

Figure 2. Forest plot of the frequency of micronuclei for crack users vs non-users.

Discussion

Mutagenic and genotoxic effects of crack has been a topic of interest to scientific community and public health, due to drug abuse increases deleterious effects on genetic material, such as genotoxic and carcinogenic products resulting from pyrolysis of substances present in crack smoke (Nakahara and Ishigami 1991). Crack use was associated with a range of health outcomes in humans (Butler *et al.* 2017). Beyond that, according to Yujra *et al.* (2015) in their study evaluating genomic damage in multiple organs of mice following acute exposure to crack cocaine, this substance induced DNA damage in peripheral blood and brain cells by single cell gel (comet) assay data. The results of our systematic review and meta-analysis point that there is a higher frequency of MN in oral mucosa cells in crack users when compared to non-users, statistically significant, demonstrating the potentially mutagenicity of this drug (Almeida *et al.* 2012; das Graças *et al.* 2014; Webber *et al.* 2015; Lima *et al.* 2016; Antoniazzi *et al.* 2018). Moreover, the detection of an elevated frequency of micronuclei in some tissues and/or organs indicates increased risk of cancer (Butler *et al.* 2017).

It is important to highlight that literature report differences in MN assay methodology, which might influence the results found in this study. Some protocols Feulgen (Almeida *et al.* 2012; das Graças *et al.* 2014; Webber *et al.* 2015; Lima *et al.* 2016) and others used Giemsa (Antoniazzi *et al.* 2018) as staining method. A review proposed by Nersesyan *et al.* (2006) indicated that nuclear anomalies, like the MN, might be misinterpreted with non-specific DNA dyes, such as Giemsa, and lead to false positives. However, in the results found in our review, the staining method used by the different authors showed no significant difference. Similar results were observed in the review developed by De Geus *et al.* (2018) with cigarette smokers.

Even though this review included databases that have article repositories from all around the world, all selected articles were developed in Brazil. This is probably due to Brazil being considered the world's largest market for crack cocaine (Laranjeira *et al.* 2014; UNODC 2018). The results of this systematic review corroborate to the data from the Brazilian National Survey on the use of Crack, since it reports thatthe majority of crack users are young adults - with an average age of 30.28 years and that approximately 1/3 of them is concentrated in the age group of 18 to 24 years. Also, it was observed high mean time of crack use contradicting the commonly reported that crack users would have survival less than 3 years of consumption (Bastos and Bertoni 2014). Furthermore, of the five selected studies, four were conducted with crack users who were in chemical dependency treatment programs and one used an interview with users who lived on the street.

Beyond MN assay, evaluated studies have addressed cytotoxicity characteristics that wereusually related to anomalies that reflect acute cell death (Nersesyan *et al.* 2013). Among these changes are: binucleation (cells that contain two nuclei); nuclear buds (MN-like bodies attached to the nucleus by a thin nucleoplasmic connection); pycnosis (cells containing dark, dense small nuclei without noticeable chromatin); karyorrhexis (cells that exhibit particle chromatin condensation showing nucleus fragmentation into intact small round or oval bodies); and karyolysis (absence of nucleus in cells) (Lima *et al.* 2016). Although these parameters are approached associated with MN, only the results of pycnosis, karyorrhexis and karyolysis showed statistical difference between users and nonusers in the studies. This may indicate that crack consumption leads to inflammatory changes and these parameters may serve as bioindicators that precede carcinogenesis (Nersesyan *et al.* 2013; Lima *et al.* 2016). However, it is important to note that the presence of MN in the oral mucosa cells of crack users may also be related to the products generated during pyrolysis and also the exposure of the mucosa to high temperatures, as pointed out by De Geus *et al.* (2018) in their systematic review on MN in cigarette smokers.

The present review highlights the very low heterogeneity degree between studies, which provide results that are considered more consistent. The homogeneity of the results may be due all studies evaluated MN in the same cell type. In addition, all studies were from Brazil, which may be related with the high prevalence of crack addiction in the country. According to Abdalla *et al.* (2014) 370,000 Brazilians regularly used crack and similar for at least six months in 2012, corresponding to 0.8% of the country's capitals population. In Brazil, approximately 2 million of people (aged 14 years and older) smoked cocaine (crack) at least once in their lifetime (1.5% among adults, 0.8% among adolescents) and one in one hundred adults used crack in the past year (Abdalla

et al. 2014). As observed, there is a gap in the literature on this thematic elsewhere in world. Thus, there is the possibility that many drug users susceptible to DNA damage may have different outcomes than those of the studies included in this review and, therefore, require further research in different populations.

Conclusion

Summary, the micronuclei frequency was higher in users of crack compared to non-users, as well as, secondary outcomes such as binucleated cells, nuclear buds, pyknosis, karyorrhexis and karyolysis. Therefore, there is a negative impact of crack use in oral mucosa exfoliated cells and MN assay proved to be a good biomarker to assess the mutagenic impact of crack use in oral epithelium.

Authors Disclosures

Contributors

Key search parameters, including search strategy, screening and data extraction were designed by FMRSJ, MS, RAT, TBS and CLFF. Screening, data extraction, and summarizing was performed primarily by FMRSJ, MS and RAT with validation performed by two independent reviewers. Statistical analyses were performed by FMRSJ and CLF with input from RAT. Newcastle-Ottawa quality assessment scale adapted for cross-sectional studies was performed by RAT and validated by FMRSJ. Data interpretation, manuscript drafting and revising was conducted jointly by all authors, led by FMRSJ. The corresponding author (FMRSJ) had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

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References

Abdalla RR, Madruga CS, Ribeiro M, Pinsky I, Caetano R, Laranjeira R. 2014. Addictive Behaviors Prevalence of Cocaine Use in Brazil : Data from the II Brazilian National Alcohol and Drugs Survey (BNADS). Addict. Behav. 39: 297–301.

Aguiar Torres L., dos Santos Rodrigues A, Linhares D, Camarinho R, Nunes Páscoa Soares Rego ZM, Ventura Garcia P. 2019. Buccal epithelial cell micronuclei: Sensitive, non-invasive biomarkers of occupational exposure to low doses of ionizing radiation. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 838: 54–58.

Almeida TC, Stefanon EBC, Rech VC, Sagrillo MR, Bohrer PL. 2012. Analysis of oral mucosa of users of crack through micronucleus technique. Clin. Lab. 58(11): 1269.

Antoniazzi RP, Lago FB, Jardim LC, Sagrillo MR, Ferrazzo KL, Feldens CA. 2018. Impact of crack cocaine use on the occurrence of oral lesions and micronuclei. Int. J. Oral Maxillofac. Surg.47(7): 888-895.

Balbinot AD, Alves SGL, Amaral Junior FA, Araujo RB. 2011. Associação entre fissura e perfil antropométrico em dependentes de crack Relation between craving and anthropometric profile in crack dependents. J. Bras. Psiquiatr. 60: 205–209.

Bastos FI, Bertoni N. 2014. Pesquisa Nacional sobre o uso de crack: quem são os usuários de crack e/ou similares do Brasil? quantos são nas capitais brasileiras[Internet]. Rio de Janeiro: ICICT; [citado el IT IS NECESSARY TO CITE THE DATE OF VIEW]. Available at: https://www.arca.fiocruz.br/handle/icict/10019

Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, Holland N, Kirsh-Volders M, Knasmueller S, Zeiger E. 2011. The Human MicroNucleus project on eXfoLiated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. Mutation Research/ Reviews in Mutation Research. 728(3): 88-97.

Butler AJ, Rehm J, Fischer B. 2017. Health outcomes associated with crack-cocaine use: Systematic review and meta-analyses. Drug Alcohol Depend.180: 401-416.

Cury PR, Oliveira MGA, Andrade KM, De Freitas MDS, De Santos JN. 2017. Dental health status in crack / cocaine-addicted men : a cross-sectional study. Environ. Sci. Pollut. Res. 24(8): 7585-7590.

da Silva Júnior FMR, Tavella RA, Fernandes CLF, Soares MCF, de Almeida KA, Garcia EM,

da Silva Pinto EA, Baisch ALM.2018. Genotoxicity in Brazilian coal miners and its associated factors. Hum. Exp. Toxicol. 37: 891–900.

Da Silva Pinto EA, Garcia EM, de Almeida KA, Fernandes CFL, Tavella RA, Soares MCF, Baisch PRM, Muccillo-Baisch AL, da Silva Júnior FMR. 2017. Genotoxicity in adult residents in mineral coal region—a cross-sectional study. Environ. Sci. Pollut. Res. 24: 16806–16814.

das Graças AOM, dos Santos JN, Cury PR, da Silva VHP, Oliveira NRC, da Costa Padovani R, Tucci AM, Ribeiro DA. 2014. Cytogenetic biomonitoring of oral mucosa cells of crack cocaine users. Environ. Sci. Pollut. Res. 21(8): 5760-5764.

de Freitas TAB, Palazzo RP, de Andrade FM, Reichert CL, Pechansky F, Kessler F, de Farias CB, de Andrade GG, Leistner-Segal,S, Maluf SW. 2014. Genomic instability in human lymphocytes from male users of Crack Cocaine. Int. J. Environ. Res. Public Health. 11: 10003–10015.

de Geus JL, Wambier LM, Bortoluzzi MC, Loguercio AD, Kossatz S, Reis A. 2018. Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review and meta-analysis. Clin. Oral Investig. 1: 1–11.

Espitia-Pérez L, da Silva J, Espitia-Pérez P, Brango H, Salcedo-Arteaga S, Hoyos-Giraldo LS, de Souza CT, Dias JF, Agudelo-Castañeda D, Valdés Toscano A, Gómez-Pérez M, Henriques JAP. 2018. Cytogenetic instability in populations with residential proximity to open-pit coal mine in Northern Colombia in relation to PM10 and PM2.5 levels. Ecotoxicol. Environ. Saf. 148: 453–466.

Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, ZeigerE, Knasmueller S, Fenech M. 2008. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps.Mutat. Res. - Rev. Mutat. Res. 659(1-2): 93-108.

Laranjeira Ro, Madruga CS, Pinsky I, Caetano R, Mitsuhiro SS. 2014. II LENAD Segundo Levantamento Nacional de Álcool e Drogas. São Paulo: Instituto Nacional de Ciência e Tecnologia para Políticas Públicas de Álcool e Outras Drogas, UNIFESP.

Lima RC de, Ferraz P, Chaiben CL, Fernandes Â, Machado MÂN, Azevedo-Alanis LR, Lima AAS de. 2016. Genotoxic and Cytotoxic Potential of Smoke Crack Cocaine on the Epithelium of the Human Oral Mucosa. J. Dent. Indones. 33: 33–39.

Lorenzoni DC, Pinheiro LP, Nasciment HS, Menegar-do CS, Silva RG, Bautz WG, Henriques JF, Almeida-Coburn KL, da Gama-de-Souza LN. 2017. Could formaldehyde induce mutagenic and cytotoxic effects in buccal epithelial cells during anatomy classes? Med. Oral Patol. Oral Cir. Bucal.22(1): e58.

Moher D, Liberati A, Tetzlaff J, Altman DG. 2009.Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. Annals of internal medicine. 151(4): 264-269.

Nakahara Y, Ishigami A. 1991. Inhalation efficiency of free-base cocaine by pyrolysis of “crack” and cocaine hydrochloride. J. Anal. Toxicol. 15: 105–109.

Nersesyan A, Kundi M, Atefie K, Schulte-Hermann R, Knasmüller S. 2006. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiol. Biomarkers Prev. 15(10): 1835-1840.

Nersesyan A, Kundi M, Krupitza G, Barcelos G, Mišík M, Wultsch G, *et al.* 2013. Induction of nuclear anomalies in exfoliated buccal cells of coca chewers: Results of a field study. Arch Toxicol. 87(3): 529-534.

Oliveira NG, Dinis-Oliveira RJ. 2018. Drugs of abuse from a different toxicological perspective: an updated review of cocaine genotoxicity. Arch. Toxicol. 92(10): 2987-3006.

Thomas P, Fenech M. 2011. Buccal micronucleus cytome assay. In: Totowa NJ, ed. DNA Damage Detection In Situ, Ex Vivo, and In Vivo. Humana Press. 235-248.

UNODC, United Nations Office on Drugs and Crime. 2018. World Drug Report 2018. United Nations publication, Viena (Austria).

Webber LP, Pellicioli ACA, Magnusson AS, Danilevicz CK, Bueno CC, Filho MSA, Rados PV, Carrard VC. 2015. Human & experimental toxicology. 35(2): 184-193.

Webber LP, Pellicioli ACA, Magnusson AS, Danilevicz CK, Bueno CC, Sant'Ana Filho M, Rados PV, Carrard VC. 2016. Nuclear changes in oral mucosa of alcoholics and crack cocaine users. Hum. Exp. Toxicol. 35: 184–193.

Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. 2012. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. European journal of epidemiology. 25(9): 603-605.

Yujra VQ, Moretti EG, Claudio SR, Silva MJD, Oliveira FD, Oshima CTF, Ribeiro DA. 2016. Genotoxicity and mutagenicity induced by acute crack cocain exposure en mice. Drug and Chemical Toxicology. 39(4): 388-391.