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Cytogenetic changes in oral mucosa cells from individuals submitted to oral human immunodeficiency virus pre-exposure prophylaxis use

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate cytogenetic changes in individuals submitted to oral human immunodeficiency virus pre-exposure prophylaxis use through the micronucleus test in oral mucosa.

METHODS: This study consisted of 37 individuals, of whom 17 comprised the pre-exposure prophylaxis group and 20 comprised the control group. A total of 2,000 cells per slide were analyzed for the determination of micronuclei, binucleation, nuclear buds, and cytotoxicity parameters: pyknosis, karyolysis, and karyorrhexis (KR), in a double-blind manner. The repair index was also evaluated in this setting.

RESULTS: In the mutagenicity parameters, the pre-exposure prophylaxis group showed increased frequencies of micronuclei (p=0.0001), binucleation (p=0.001), and nuclear buds (p=0.07). Regarding the cytotoxicity parameters, there was an increase with a statistical difference (p≤0.05) in the karyorrhexis frequency (p=0.001). Additionally, the repair system efficiency decreased in the pre-exposure prophylaxis group.

CONCLUSION: These results indicate that individuals undergoing pre-exposure prophylaxis use have geno- and cytotoxicity in oral mucosal cells.

KEYWORDS: Anti-retroviral agents. DNA damage. Micronucleus tests. Mouth mucosa.

INTRODUCTION

The different existing methods to avoid contamination by the human immunodeficiency virus (HIV) have not yet been enough to eradicate the disease. Since its form of transmission was discovered, through secretions, such as vaginal secretions, sperm, blood, and breast milk, the incessant recommendations before the use of mechanical barrier (condoms), the non-sharing of needles, the decrease of high-risk behaviors, especially for alcohol and drug users, regular testing for HIV, prompt treatment of other sexually transmitted infections, and prevention of transmission by HIV-positive individuals with regular use of antiretroviral therapy (ART) have been part of the strategy to minimize the spread of AIDS[1].

Since 2016, the World Health Organization (WHO) has published a guide of recommendations closely related to AIDS and has oral pre-exposure prophylaxis (PrEP) as part of the combined prevention strategy (biomedical and behavioral) to HIV infection for people at high risk[2]. PrEP consists of the continuous use of antiretroviral drugs in HIV-negative people to reduce the risk of acquiring HIV infection[1]. The medication initially offered included oral tenofovir, either alone or in combination with emtricitabine, both being nucleoside reverse transcriptase inhibitors. In 2021, the use of the vaginal ring with dapivirine was another option for women at risk, and in 2022, the injectable use of long-acting cabotegravir was recently added to the prophylactic medications[3]. Several studies have demonstrated that the treatment with PrEP reduces HIV infection[4,5]. For example, Tsai et al.[6] studied the use of the antiretroviral tenofovir in monkeys (*Macaca fascicularis*) inoculated with HIV and observed that when treated in the first 24 h after infection for 28 days, they did not show viral replication after interruption of treatment. Grant et al.[7], in their study on 2,499 participants from 6 countries, observed a 44% decrease in HIV infection in individuals who made the prophylactic use of tenofovir associated with emtricitabine. In the same year, Abdool Karim et al.[8] observed that the use of 1% tenofovir vaginal gel reduced HIV infection between 39 and 54% in women.

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Genotoxicity assays are widely used to identify the chemical compounds that would be able to induce DNA damage. To evaluate this effect, the micronucleus assay is suitable for this purpose as it is simple and low cost with reproducible results[9]. The assay allows analyzing DNA alterations in exfoliated cells of the oral mucosa in a minimally invasive way, where it is possible to verify nuclear alterations such as the presence of micronuclei (MN), binucleation (BN), and nuclear buds (NB) as indicators of genetic damage. Also, cytotoxicity through the phases of cell death, karyorrhexis (KR), pyknosis (PK), and karyolysis (KL) was evaluated in these individuals. The biological significance of the micronucleus lies in exposure to chemical agents, chronicle diseases, and aging[9].

In this context, this study aims to evaluate possible cytogenetic changes due to the continuous use of PrEP, which are not assessed in routine tests adopted in clinical practice by micronucleus assay. To the best of our knowledge, this approach has not been made so far. Certainly, such data will provide insights for better understanding regarding the safety of PrEP use.

METHODS

Casuistics

The study was approved by the Institutional Human Ethics Committee from the Universidade Federal de São Paulo (UNIFESP), under protocol 0485/2019. All participants received a detailed explanation about the project, and the participants signed an informed consent form.

This study consisted of 37 individuals, of whom 17 comprised the PrEP group and 20 comprised the control group. A single examiner, a dentist, performed the collection, staining, and examination of the unidentified samples. A total of 17 volunteers from the PrEP group aged between 19 and 50 years were recruited under regular monitoring in the Specialized Care Service in the city of Santos, SP, Brazil. Exclusion criteria were the absence of infectious diseases, oral lesions, and exposure to radiographic or tomographic exams in 15 days prior to sample collection. The control group was randomly recruited by a direct approach in public places in the city of Santos, SP, Brazil. Notably, 20 people were recruited, with the exclusion criteria similar to the PrEP group.

Cytogenetic assay

The oral mucosa MN test followed the protocol described by Belien et al.[9]. With the help of a wooden spatula previously moistened with saline solution, a gentle scraping was performed on the inner portion of the jugal mucosa on both sides. The stain used was Feulgen/Fast Green. The correct identification of metanuclear changes was proposed by Bolognesi et al.[10]. For this, the following criteria were established for the correct identification of cytogenetic changes. MN: (1) intact main nucleus and cytoplasm; (2) diameter one-third of the main nucleus; (3) similar stain and texture of the main nucleus; and (4) MN in the same focus as that of the main nucleus. KR: The nucleus may also exhibit extensive fragmentation indicative of advanced nuclear fragmentation. BN: Two main nuclei within a single cell and the nuclei are of similar size and staining intensity. NB: The main nucleus has a sharp constriction forming a bud of nuclear material being attached to the main nucleus by a narrow or wide nucleoplasmic bridge. PK: The nucleus is small and shrunken with a diameter that is approximately one-third of that in a fully differentiated cell being uniformly and intensely stained. KL: They do not have a DNA-containing nucleus or other structures that stain with Feulgen.

The repair index (RI), proposed by Ramirez and Saldanha[11], represented by the formula, RI=(KL+KR)/(MN+NB), was also evaluated in this setting.

Statistical analysis

All data were submitted for normalization using the Kolmogorov-Smirnov test. After that, non-parametric data were confirmed by all data collected in this setting. The nonparametric Mann-Whitney test was used to evaluate the metanuclear alterations and DNA RI between the control and experimental groups. The statistical significance level was set at 5%. The statistical analysis was conducted by the BioStat software (version 5.0, Maringá, Brazil).

RESULTS

All participants in the PrEP group were males and reported eating well, including fruits and vegetables; five participants used vitamin supplements, nine reported using mouthwash, the majority (15 people) reported taking alcoholic beverages, and five were smokers (less than 20 cigarettes/day). The minimum time of treatment with Truvada® was 1 month of continuous use, and the maximum time was 13 months. One participant was diabetic and hypertensive using metformin, and the other was hypertensive using valsartan. In the control group, all volunteers were also males and the age ranged from 20 to 51 years. A total of 5 volunteers were smokers, 10 reported using mouthwash, and none was taking any medication. The demographic characteristics are shown in Table 1.

The PrEP group showed an increase with a statistical difference compared with the control group for all mutagenicity

parameters: the frequency of MN (p=0.001), BN (p=0.001), and NB (p=0.078), according to Table 2. In cytotoxicity parameters, there was a statistical difference in the frequency of KR (p=0.001). In other parameters evaluated in this setting, KL (p=0.57) and PK (p=0.8) did not show significant differences (p>0.05) between groups according to the results presented in Table 3. The RI index is shown in Table 2 and the findings suggest the lower repair capacity in the PrEP group in oral mucosa cells when compared with the control group.

DISCUSSION

According to the UNAIDS (the Joint United Nations Programme on HIV/AIDS) report, it was estimated that more than 1.6 million people worldwide would have received oral PrEP by the year 2021[12]. The goal set for 2025 is 10 million people to use this HIV prophylaxis[12]. Initially concentrated in high-income countries, a substantial increase has been observed in underdeveloped countries in the past 2 years. The rate of HIV infections worldwide has shown a steady decline, but in the last 5 years, this has been associated with the COVID-19 pandemic, as well as with the lack of prevention programs that especially reach the most vulnerable groups of people, as they account for more than half of new infections worldwide[13].

Table 1. General characteristics of study participants.

Parameters	Control group (n=20)	PrEP group (n=17)
Mean age	35.2±9.6	34.6±9.7
Gender	M/20	M/17
Time of treatment	–	6.4±4.2
Tobacco smoking	5	5
Mouthrinse	9	10
Illicit drugs	5	5
Vitamins supplement	4	5
Chronicle diseases	–	3

Table 2. Mean+SD frequency of cytogenetic changes related to mutagenicity in individuals submitted to pre-exposure prophylaxis use.

Groups	MN	BN	NB	DNA repair index
Control	0.35±0.6	0.3±0.5	0.05±0.2	198.5±103.8
PrEP	2.35±1.6*	4.3±2.8*	0.7±0.9*	104.6+75.5*

*p≤0.05; MN: micronucleus; BN: binucleation; NB: nuclear bud.

Table 3. Mean+SD frequency of cytogenetic changes related to cytotoxicity in individuals submitted to pre-exposure prophylaxis use.

Groups	Normal cells	KL	KR	PK
Control	1,664.1±48.3	195.7±56	27.2±6.5	112.5±42.5
PrEP	1,617.7±85	203.2±66.4	48.9±21.2*	122.7±59.5

*p≤0.05; PK: pyknosis; KL: karyolysis; KR: karyorrhexis.

The success of prophylactic treatment is widely documented as lowering the risk of contracting HIV by 90%, provided by good adherence to treatment[13]. Two dosing regimens are proposed, daily and continuous use of one tablet, or on-demand use, which consists of using 2 tablets between 2 and 24 h before exposure, 1 tablet 24 h after the first dose, and 1 more tablet 48 h after the first dose, totaling 4 tablets, with good efficacy[14]. However, some adverse effects such as nausea, headache, flatulence, stool softening/diarrhea, and edema can be reported and can be treated symptomatically.

The association between tenofovir and emtricitabine has been described for PrEP use, but also severe cases depicted by lactic acidosis and hepatomegaly with steatosis and some rare fatalities, especially in women, obese people, and people who take this drug combination for prolonged use[15]. Tenofovir fumarate presents a potential risk of renal toxicity, and its prolonged use can lead to progressive loss of renal function, acute renal failure, and Fanconi syndrome. According to Jotwani et al.[16], subclinical changes in renal tubular function have been observed in people taking PrEP, warranting further study. Tenofovir fumarate is also related to decreased bone mineral density[17,18], although no increase in the number of fractures is documented. According to Havens et al.[19], in a study on 15–22-year-olds, they showed bone loss after continuous PrEP use for 48 weeks, and with its discontinuation, there was partial or complete improvement after 48 months.

Regarding the geno- or cytotoxicity induced by these drugs, the results were largely obtained through experimental studies. Wu et al.[20] observed the genotoxicity of several antiretroviral drugs. Tenofovir was related to the presence of hepatocellular adenomas, carcinomas, and lung adenomas in rats. Emtricitabine showed no changes for genotoxicity and induction of carcinogenesis. Moraes Filho et al.[21,22] used the test for somatic mutation and recombination detection comet assay in *Drosophila melanogaster* and micronucleus assay in bone marrow cells. Tenofovir promoted DNA damage by inducing mutational and/or recombination events, although it did not produce toxic effects.

Recently, Gutiérrez-Sevilla et al.[23] evaluated genomic instability, through the buccal mucosa micronucleus test, of people with HIV on different types of ARTs and also without medication, and increased BN cells and NB were detected in these individuals. However, there are no studies evaluating the cytogenetic changes in HIV-uninfected individuals undergoing PrEP use. In this study, we evaluated HIV-free individuals taking antiretroviral drugs as a preventive measure against HIV

infection (PrEP). Mutagenicity, an irreversible cell damage factor, was evaluated by cell nucleus alteration events such as BN, MN, and NB. For this, we used the micronucleus test in exfoliated cells of the oral mucosa, as this methodology has demonstrated a direct correlation with the micronucleus test in lymphocytes, with the advantage of being a minimally invasive, low-cost method that allows the evaluation of DNA injury. It has versatility and can be employed for various risk factors, such as environmental, nutritional, radioactive, licit, or illicit drug use, among others[24,25]. Our results revealed that all parameters closely related to mutagenicity showed a statistically significant increase compared with the control group. Cytotoxicity was assessed by cell death parameters, in its distinct phases: PK, KR, and KL. KR, a less frequent event to be observed, as it represents a transition between the initial and final phases of cell death, showed a statistically significant increase when compared with the control group, suggesting that the cell damage may be leading to more cell death events, even if not represented by PK and KL. Taken together, these results indicate that PrEP is capable of inducing genotoxicity and apoptosis in oral mucosal cells. The RI also showed a decrease in the PrEP group in buccal mucosal cells. These results are completely new and, therefore, difficult to discuss at the present time. Anyway, we can infer that the ability to repair oral mucosa cells may be reduced in volunteers submitted to PrEP, favoring the processes of genotoxicity and cell death. However, further studies are needed to accurately assess this condition properly.

CONCLUSION

These results indicate that individuals undergoing PrEP use have geno- and cytotoxicity in oral mucosal cells. As PrEP plays a pivotal role in controlling HIV infection, especially in high-risk populations, further studies are needed to elucidate what tissues and organs are more vulnerable to PrEP, in addition to oral mucosa in humans. Certainly, such data will establish correctly and unequivocally the real risks of PrEP use in order to avoid danger to people.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The project was approved by the Research Ethics Committee of the Universidade Federal de São Paulo (UNIFESP), Protocol number #3.461.911.

AUTHORS' CONTRIBUTIONS

MESA: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MMC**: Conceptualization, Writing – original draft, Writing – review & editing. **DAR**: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **CMCBM**: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **DVS**: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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