Inflammatory cytologic alterations in the oral epithelium associated with HIV pre-exposure prophylaxis: a preliminary study

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Objective. The objective of this study was to assess inflammatory cytologic alterations in the oral epithelium of patients on human immunodeficiency virus pre-exposure prophylaxis (PrEP).

Material and Methods. Epithelial cells from the buccal mucosa of 30 patients were collected by exfoliative cytology and were evaluated according to inflammatory cellular alterations: karyomegaly, bi- or multinucleation, karyopyknosis, karyorrhexis, perinuclear halo formation, metachromasia, cytoplasmic vacuolization, indistinct cytoplasmic border, keratinization, and atrophy. Epithelial cells were collected initially before PrEP onset (T1) and then after 30 days of PrEP use (T2). Two experienced cytopathologists independently analyzed the slides.

Results. The nonparametric Wilcoxon test showed that there was a statistically significant increase in the number of cells with karyomegaly at T2 compared to T1 ($P = .033$). The other cellular alterations did not present with statistically significant differences between the 2 moments of evaluation ($P > .05$).

Conclusion. The increased number of oral epithelial cells with karyomegaly after 30 days of using PrEP suggests the presence of inflammatory alterations at this site. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:534−539)

In 2018, about 1.7 million new cases of human immunodeficiency virus (HIV) infection were reported, totaling approximately 37.9 million infected people worldwide. Since 1980, 32 million people have died of diseases related to acquired immunodeficiency syndrome (AIDS), mainly tuberculosis.[1] Thus, HIV, which causes AIDS, remains a threat to global health.[2]

To control the disease, prevention efforts have been intensified with an aim to reduce viral transmission. Thus, additional efforts have been made to provide effective and safe prevention tools, including meeting the specific health needs of key populations and allowing access to health and social services.[3] For instance, post-exposure prophylaxis and prevention of mother-to-child transmission have played key roles in reducing HIV incidence in recent years.[4]

Though the development of an HIV vaccine is ongoing, several antiretroviral drugs have been investigated in recent years for effective prevention and treatment of HIV. Among them, the only licensed drug, Truvada, consists of a combination of 2 antiretroviral substances, tenofovir and emtricitabine, and is called HIV pre-exposure prophylaxis (PrEP).[5] It is a protective treatment strategy for people who are not diagnosed with HIV but who are at a substantial risk of contracting HIV.[4] This specific prophylaxis has been shown to be highly effective in key populations, including men who have sex with men (MSM), sex workers, injecting drug users, transgender people, and serodiscordant couples.[3,6-9] In theory, people should take PrEP as long as they are at risk of exposure to HIV.[10]

The first human study, called the Pre-Exposure Prophylaxis Initiative (iPrEx), started in 2010 and used tenofovir disoproxil fumarate and emtricitabine co-formulated as a single once-daily tablet for oral administration. The combined drug provided 44% additional protection among MSM and transsexuals.[6] After several

Statement of Clinical Relevance

The preliminary findings of inflammatory cytologic alterations in the oral epithelium associated with pre-exposure prophylaxis demonstrated in the present study suggest potential oral adverse effects related to this method of preventing human immunodeficiency virus infection.

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studies, in 2012, PrEP received US Food and Drug Administration approval to be used orally, once a day, by high-risk seronegative people.[11,12] Soon after, it was also recommended by the World Health Organization as a preventive option for populations at risk.[13,14]

Several studies have shown that PrEP is effective and safe for HIV prevention.[6,15-18] However, it is necessary to emphasize that it does not offer protection against other sexually transmitted infections (STIs). Prevention of STIs requires medical tests before and during treatment, in addition to recommended combined prevention strategies, such as the use of condoms.[19-21]

The most prevalent initial adverse effects of PrEP are gastrointestinal, such as diarrhea, nausea, vomiting, and abdominal pain, which are usually transient and quickly resolved. On the other hand, PrEP can also cause other effects, such as reduced bone mineral density and altered kidney and liver function, which may take longer period to treat. Therefore, regular monitoring of patients using PrEP is recommended to avoid toxic effects.[4,22-24]

A previous study suggested that patients exposed to highly active antiretroviral therapy (HAART), including tenofovir, one of the active drugs of PrEP, exhibit drug-induced molecular and cellular abnormalities in the oral epithelium, altering the cytokeratin expression originated from the oral mucosa,[25] which may be related to an inflammatory process.[26] Specifically, the increased expression of cytokeratin 10 and the decreased expression of cytokeratin 5 and 6 caused by tenofovir suggest that this antiretroviral medication deregulates the growth, differentiation, and proliferation profiles in human gingival tissue, resulting in abnormal epithelial repair and proliferation.[25] Another study showed that tenofovir causes pro-inflammatory effects on the epithelial cells of the female reproductive tract,[27] specifically the ectocervix, which has squamous epithelium similar to that of the oral mucosa. Therefore, we hypothesized that PrEP, which contains the antiretroviral tenofovir medication, could also cause pro-inflammatory effects on the epithelial cells of the oral mucosa.

To the best of our knowledge, there are no scientific studies that have investigated the potential adverse effects of PrEP on the inflammatory cellular response of the oral epithelium. Therefore, the aim of this preliminary study was to assess inflammatory cytologic alterations in the oral epithelium of patients on HIV PrEP.

MATERIAL AND METHODS

This study was conducted in accordance with the Declaration of Helsinki and independently reviewed and approved by the Research Ethics Committee of the Pontifícia Universidade Católica do Paraná, Curitiba, Brazil, under protocol No. 3.227.397 and by the Research Ethics Committee of the Municipal Health Department of Curitiba, Brazil, under protocol No. 3.303.788. All participants involved in this study provided individual informed consent.

The researchers designed an observational, analytic, and prospective study. The population consisted of patients previously selected for HIV PrEP at the Counseling and Advising Center of the Health District Secretary of Curitiba, Brazil South (COA/SMS) from May to December 2019. The inclusion criteria were patients of both sexes, older than 18 years of age, and who had not used PrEP previously. The exclusion criteria were patients with systemic diseases that compromise the salivary flow, patients with a history of head and neck radiotherapy, and patients who had inflammatory or neoplastic oral lesions. Informed consent was obtained from all patients participating in the study. Given that MSM is the key population, which is more exposed to HIV infection,[28-30] the sample size of the present study was calculated based on the number of patients who had started to use PrEP at COA/SMS in 2019 (138), the majority of whom were MSM, and the number of the estimated population of MSM from Curitiba in 2019 (143,049), for a prevalence rate was 0.09647%. Thus, the final sample size was calculated using the proportional sampling method with 95% confidence interval and a margin of error of 1%, resulting in a sample size of 29 patients.

Medical records of the COA/SMS were used to obtain information related to sex, age, cigarette consumption, drug use, reason for consultation (e.g., exposure to risk situations, knowledge of serologic status, prevention, referral from health services), type of exposure (e.g., sexual intercourse, blood transfusion, occupational exposure, needle sharing), and population profile (e.g., MSM, sex workers, heterosexuals, injecting drug users, STI carriers, professional health workers, transsexuals).

Epithelial cells were collected by gently scraping the right and left buccal mucosa with a cytologic brush (Vagispec, Jaraguá do Sul, SC, Brazil) rotating it clockwise 10 times. Then, to collect the cells, the cytologic brush was removed and submerged in a specimen collection vial with a preservative solution (10 mL) identified with the patient code and collection number, based on the methodology previously described.[31] A standard centrifuge tube presented in a volume of 15mL was used to centrifuging the oral smears. All of the steps were repeated for 2 collection times: before the initiation of PrEP (T1) and after 30 days of PrEP use (T2).

The Liqui-PREP System kit (LGM International, Melbourne, FL, USA) was used for liquid-based exfoliative cytology. The samples were obtained and slides were prepared using the kit consisting of the following: Liqui-PREP Specimen Collection Vial (presented in a collection bottle, with 10 mL of preservative solution, which functions to preserve the collected cytologic sample); Liqui-PREP Cellular Base Solution

(presented in 225-mL bottle, a component that encapsulates and adheres processed cells).

Biological material was processed on glass slides in the Laboratory of Cytology of the School of Life Sciences, Pontifícia Universidade Católica do Paraná, using the Liqui-PREP kit according to the manufacturer's instructions. The cells were fixed using 95% alcohol for 10 min, washed with tap water for 3 min, and treated with Papanicolaou stain (Newprov Laboratory Products, Pinhais, Paraná, Brazil). Cover slips were sealed by applying 2 drops of Entellan (Merck KGaA, Darmstadt, Germany and/or its affiliates) varnish.[31]

All glass slides were coded, masking the identification data. Cytologic evaluation was performed independently in a blinded fashion by 2 experienced cytopathologists (J. C.M. and M.I.A.) after interobserver calibration. For smear analysis, a binocular microscope equiped with planachromatic objective lens (Nikon, Tokyo, Japan) was used, with 10- and 40-fold objectives and 10-fold eyepiece.

The entire slide was analyzed to determine the percentage of cells with the following alterations: karyomegaly, bi- or multinucleation, karyopyknosis, karyorrhexis, perinuclear halo formation, metachromasia, cytoplasmic vacuolization, indistinct cytoplasmic border, keratinization, and atrophy. To assess the presence of each inflammatory cell alteration and for further statistical analysis, the following scores were used:

1. Absent.
2. Rare: The alteration was detected in <25% of the cells.
3. Discrete: The alteration was detected in 25%-50% of the cells.
4. Moderate: The alteration was detected in 50%-75% of the cells.
5. Severe: The alteration was detected in 75%-100% of the cells.

The data obtained were tabulated and all statistical analyses were carried out using SPSS version 24.0 (IBM Software, New York, NY, USA).

The statistical test used for interobserver analysis was the nonparametric Wilcoxon test, which confirmed that there were no statistical differences in the median score of each cellular alteration between the 2 observers, for all variables, at both T1 and T2 ($P > .05$). Of note, there was a discreet departure in alignment of the cytologic class ratings between the observers but without statistical differences. Thus, the results from the comparison of each cellular alteration investigated in the present study between T1 and T2 would be the same, regardless of the observers' scores. Thereafter, statistically significant differences for all variables were determined using the abovementioned scores (0, 1, 2, 3, 4) between T1 and T2, applying the same nonparametric Wilcoxon test, with a significance level of 5%.

RESULTS

The initial sample size consisted of 47 patients selected for PrEP, who were examined consecutively at the COA. Of them, 17 were excluded from the study because they discontinued the medication. The final sample consisted of 30 patients.

Of the 30 patients, 93.4% ($n = 28$) were male (mean age = 28.75 [±7.10] years; range, 19-44 years) and 6.6% ($n = 2$) were female (mean age 49.50 [±20.51] years; range, 35-64 years). The majority of patients reported being nonsmokers (53.3%; $n = 16$) and consuming alcohol (43.3%; $n = 13$). Of the 30 patients, 56.7% sought care with interest in PrEP because of exposure to risk ($n = 17$). All patients (100%; $n = 30$) were exposed to HIV through sexual intercourse, of which 26 patients (86.8%) were MSM (Table I).

Of all inflammatory cell alterations analyzed, there was a statistically significant increase in the number of cells with karyomegaly at T2, after 30 days of using PrEP, compared to the initial time T1 ($P = .033$). The other cellular alterations did not show statistically significant differences between the 2 moments of evaluation ($P > .05$; Table II).

Table I. Demographic and epidemiological characteris-tics of the sample size

Gender	*Frequency (N=30)*	*Percentage (%)*
Male	28	93.4
Female	2	6.6
Smokers		
Yes	11	36.7
No	16	53.3
Not informed	3	10
Drugs		
Alcohol	13	43.3
Marijuana	4	13.3
Cocaine (snorted)	3	10
Amphetamine	2	6.6
Cocaine (injected)	0	0
Crack	0	0
Heroin	0	0
Others	1	3.3
Reason for consultation		
Exposure to risk situation	17	56.7
Knowledge of serological status	6	20
Prevention	7	23.3
Type of exposure		
Sexual intercourse	30	100
Population profile		
Men who have sex with men	26	86.8
Sex worker	2	6.6
Heterosexual	2	6.6

Table II. Comparison of cellular alterations in the oral epithelium of patients using PrEP, before (T1) and after 30 days of PrEP use (T2), Curitiba, Paraná, Brazil, 2020

Cellular alteration	*N*	*Average*	*SD*	*Minimum*	*Maximum*	*P value*
Karyomegaly (T1)	30	0.030	0.183	0	1	.033*
Karyomegaly (T2)	30	0.230	0.430	0	1	
Bi-multinucleation (T1)	30	0.070	0.254	0	1	.157
Bi-multinucleation (T2)	30	0.000	—	0	0	
Karyopyknosis (T1)	30	0.000	—	0	0	>.99
Karyopyknosis (T2)	30	0.000	—	0	0	
Karyorrhexis (T1)	30	0.000	—	0	0	>.99
Karyorrhexis (T2)	30	0.000	—	0	0	
Perinuclear halo (T1)	30	0.300	0.466	0	1	.796
Perinuclear halo (T2)	30	0.270	0.521	0	2	
Metachromasia (T1)	30	0.070	0.254	0	1	.157
Metachromasia (T2)	30	0.000	—	0	0	
Vacuolization (T1)	30	0.070	0.254	0	1	.563
Vacuolization (T2)	30	0.030	0.183	0	1	
Erasure border (T1)	30	0.000	—	0	0	>.99
Erasure border (T2)	30	0.000	—	0	0	
Keratinization (T1)	30	0.300	0.466	0	1	.438
Keratinization (T2)	30	0.400	0.563	0	2	
Atrophy (T1)	30	0.000	—	0	0	>.99
Atrophy (T2)	30	0.000	—	0	0	

PrEP, pre-exposure prophylaxis.
*$P < .05$: statistically significant differences between the 2 moments of evaluation.

DISCUSSION

In this study, a statistically significant increase in the number of cells with karyomegaly was observed in the oral epithelium after 30 days of PrEP. The inflammatory cellular alteration known as karyomegaly represents abnormally enlarged nuclei,[32] characterizing, in this case, a reaction of the oral epithelium. In this context, Biswas et al.[27] argued that the pro-inflammatory effects of tenofovir on epithelial cells and fibroblasts of the female reproductive tract may compromise the effectiveness of the HIV drug. Epithelial cells and fibroblasts are not productively infected with the virus, but they likely play an important role in the infection process through secretion of pro-inflammatory cytokines, including the macrophage inflammatory protein 3 α and interleukin 8. The secreted cytokines have complex implications in the acquisition of HIV, because they can increase susceptibility to the virus.[27] Although the results of the present study are preliminary and were observed in epithelial cells of the oral mucosa, the presence of karyomegaly in these cells after 30 days of using PrEP suggests a potential pro-inflammatory effect of tenofovir.

Both antiretrovirals, tenofovir and emtricitabine, are nucleoside reverse transcriptase inhibitor, which work by inhibiting the transformation of HIV RNA into DNA and delaying or stopping viral replication.[30] After oral ingestion, tenofovir and emtricitabine are promptly detectable in plasma and a single dose of the 2 associated antiretroviral drugs provides rapid and high blood levels.[33] The concentration and pharmacokinetics of antiretrovirals vary according to the type of mucosa.[11,34] In one study, the concentration of tenofovir in the rectal mucosa was higher than the concentration of emtricitabine, whereas the concentration of emtricitabine in vaginal tissue was higher than the concentration of tenofovir.[35] Another study showed that emtricitabine diffuses relatively well in saliva, whereas diffusion of tenofovir is poor.[36] Therefore, tenofovir should be used in combination with emtricitabine to inhibit viral replication and prevent viral transmission through oral sex.[35,36]

A preclinical study in mice confirmed the presence of the drug 4′-ethynyl-2-fluoro-2′-deoxyadenosine, a reverse transcriptase inhibitor that prevents HIV vaginal and oral transmission, in several regions and organs. The authors confirmed the systemic distribution after a single oral administration, showing high concentrations of the drug in the female reproductive tract and in saliva.[37] Thus, theoretically, epithelial cells of the oral mucosa could be exposed to possible adverse effects of PrEP.

Studies have associated the prolonged use of antiretrovirals with xerostomia and reduced salivary flow, but it is not yet known whether the concentration of tenofovir and emtricitabine in saliva can also cause this alteration, which consequently would affect the epithelial cells of the oral mucosa.[38-40] Thus, the increase in the number of cells with karyomegaly may possibly be associated with an inflammatory response of the oral epithelium due to the action of the medication and also with the potential reduction in salivary flow. This, in turn, can predispose the oral mucous membrane to physical and chemical injury due to the absence of

saliva, which, among other functions, acts as a protective barrier of oral tissues.

The study by Nittayananta et al.[41] determined the oral expression of cytokeratins (CKs) in individuals undergoing HAART. The prolonged use of HAART significantly affected the expression of CK13 in the basal layer, and the expression of CK1, which is usually expressed in the skin, was also observed in the normal oral mucosa of HIV-infected individuals undergoing HAART. This may suggest that the oral epithelial cells are at an early stage of abnormal differentiation. The patients in that study were administered azidothymidine, which belongs to the same class of antiretrovirals as tenofovir and emtricitabine.[41] In this context, the presence of an inflammatory process in the oral epithelium could be associated with the increased expression of CK10 as well as with the decreased expression of CK5 and CK6, caused by tenofovir, predisposing this site to oral complications.[25] Thus, we suggest that the use of PrEP could also cause inflammatory epithelial alterations, such as karyomegaly. Furthermore, the presence of cytokeratin can be used as a biomarker for early diagnosis and monitoring of oral lesions, and because tenofovir has a proliferative effect that increases the level of cytokeratin, we hypothesized that PrEP may also cause increased keratinization of oral epithelial cells. In the present study, although there was no statistically significant difference between T1 and T2, the presence of keratinized cells was remarkable. Of the 30 patients, 30% had keratinized cells at T1, before starting treatment (n = 9), and 36.6% had keratinized cells at T2, after 30 days of PrEP (n = 11). Of the 11 patients who presented keratinized cells at T2, 36.3% already had keratinized cells at T1 (n = 4), and 63.6% presented keratinized cells only at T2 (n = 7). Because of the exposure of oral mucosa to the chemicals in cigarettes, keratinization is frequently related to smoking. However, of the 7 patients who presented keratinized cells only in T2, only 28.5% were smokers who did not report an increase in frequency of cigarette consumption (n = 2). Therefore, this type of cellular alteration may be potentially related to the use of PrEP. However, studies with larger sample sizes covering diverse populations and longer exposure times are needed to confirm the same.

In the present study, the sample size consisted of 86.8% MSM (n = 26), which represents the profile of patients in most studies on PrEP because they are at an increased risk of HIV exposure.[6,28,42,43] Brazil is the only country in Latin America where PrEP is available through the public health service.[44] However, the candidates are submitted to a very strict medical evaluation in order to become eligible for PrEP. Specifically, the Brazilian city where the present study was performed, Curitiba, has only one PrEP dispenser center (COA/SMS) where PrEP is offered for free. Consequently, our preliminary cytopathological findings cannot be extrapolated to the worldwide key populations or even to the whole Brazilian population. Of note, considering the reasonably short time of exposure corresponding to the first 30 days of PrEP and the fact that this exposure is limited to one class of drugs, our study demonstrated that early inflammatory alterations of oral epithelium could already be present. Moreover, treatment with tenofovir can deregulate the cell proliferation and differentiation pathways even when applied in low concentrations for short periods, as previously reported.[25] The preliminary findings and originality of this study considering the large number of people who take PrEP to prevent HIV infection are also of note. However, long-term follow-up studies of patients undergoing PrEP are recommended.

CONCLUSION

The increased number of oral epithelial cells with karyomegaly after 30 days of using PrEP suggests the presence of inflammatory alterations at this site.

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