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Distribution and factors associated with salivary secretory leukocyte protease inhibitor (SLPI) concentrations

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Abstract

Objectives—This cross-sectional study examined the distribution and correlates of salivary secretory leukocyte protease inhibitor (SLPI) concentrations within a multinational cohort of men.

Methods—Extracellular SLPI was measured in oral gargle cell supernatants of 378 men from three countries using an ELISA-based assay. Risk factor data were collected by a questionnaire. Factors associated with SLPI were assessed using linear and logistic regression for continuous and categorical SLPI, respectively.

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Author Contributions

CMPC and ARG conceived and designed the experiments. MTO and DJI performed the experiments. SR, BNT and CMPC analyzed the data. ARG contributed reagents/materials/analysis tools. SR, CMPC, ARG, LLV, RJS, RCC, EL-P, JS, MQ wrote and revised the paper.

Conflict of Interests

None to declare.

Results—Among men ages 18–73 years, the median SLPI concentration was 492.0 ng/mL (range: 2.3–1919.9). In multivariable modeling, men in Brazil and younger men (18–30 years) were more likely to have higher levels of SLPI, (adjusted odds ratio (aOR) 3.84; 95% confidence interval (CI): 1.94–7.59, and aOR 3.84; 95% CI: 1.98–7.43, respectively). Men with a self-reported STD diagnosis in the past six months were more likely to have higher SLPI levels (aOR 2.98; 95%CI: 1.1–7.83) and men reporting bleeding/swollen gums were less likely to have higher SLPI, (aOR 0.34; 95%CI: 0.15–0.79). Similar results were observed for linear regression models.

Conclusions—SLPI concentrations varied significantly by country and decreased with increasing age. The interaction between SLPI, modifiable factors, and oral infections that influence cancer risk warrants further investigation.

Keywords

salivary secretory leukocyte protease inhibitor; SLPI; immune system protein; saliva; HIM Study

Introduction

Secretory leukocyte protease inhibitor (SLPI) is a positively charged, disulfide bonded protein secreted by serous cells and epithelial cells of mucus membranes (Wahl et al., 1997). SLPI inhibits leukocyte elastase, trypsin, cathepsin G, and chymase and contributes to the immune defense system by regulating inflammation (Sallenave et al., 1994, Wahl et al., 1997, Moreau et al., 2008). High concentrations of SLPI exist in saliva and other secretion such as nasal membranes, breast milk, cervical fluid, and seminal fluids, (Wahl et al., 1997, Lin et al., 2004) and is produced by host inflammatory and immune cells such as macrophages, (Jin et al., 1997, Odaka et al., 2003) neutrophils, (Sallenave et al., 1997) dendritic cells, (Vroeling et al., 2011) and beta cells of the pancreas (Nystrom et al., 1999, Doumas et al., 2005).

In addition to its anti-inflammatory properties, SLPI has many anti-microbial properties. Salivary SLPI appears to block the internalization of viruses, including HIV, (Jana et al., 2005, Ma et al., 2004) herpes simplex virus, (Fakioglu et al., 2008) Epstein Barr virus (Tse et al., 2012), and more recently, human papillomavirus (HPV) (Hoffmann et al., 2013, Woodham et al., 2012). In vitro studies have shown that high concentrations of SLPI protect against HPV infection by blocking viral entry into epithelial cells (Woodham et al., 2012, Hoffmann et al., 2013) and down-regulation of SLPI enhances HPV uptake (Skeate et al., 2016). Moreover, in vivo evidence suggests that HPV DNA is more frequently detected in tissues with low levels of SLPI expression (Nicol et al., 2016).

Salivary SLPI present in the oral immune microenvironment may also protect against the progression (Wen et al., 2011) and metastasis (Cordes et al., 2011) of head and neck squamous cell carcinomas (HNSCC), a subset of which are caused by HPV infection. Oral infection with HPV, particularly HPV16 (Mehanna et al., 2013), causes approximately 70–90% of newly diagnosed oropharyngeal cancers (Young et al., 2015) arising from the squamous epithelial lining of the tonsils, base of tongue, and soft palate. Less often, HPV causes cancer at the oral cavity, nasopharynx, hypopharynx, and larynx (Castellsague et al., 2016). The incidence of HPV-related HNSCC has increased dramatically in the United

States in recent decades and disproportionately affects younger men (Chaturvedi et al., 2011, Young et al., 2015) who report lower consumption of tobacco and alcohol (Gillison et al., 2015). Given the increasing burden of HNSCC, the role of SLPI in HNSCC progression and metastasis, and its potentially important role in protecting against oral viral transmission, it is important to understand the factors that influence levels of salivary SLPI. To this end, we sought to determine the distribution and correlates of salivary SLPI concentrations among men participating in the multinational HPV Infection in Men (HIM) Study cohort.

Materials and Methods

Study Population

This cross-sectional study was nested within the HIM Study, a prospective cohort study of the natural history of HPV infections in men. The study population and methods have been described previously in greater detail (Giuliano et al., 2011). Briefly, the HIM Study enrolled over 4000 men from 2005–2009 in the U.S., Brazil, and Mexico. Participants were eligible to participate in the HIM Study if they were: male, aged 18–70 years at baseline, and reported no previous diagnosis of penile or anal cancer, no previous diagnoses of anogenital warts, and no history of or treatment for sexually transmitted diseases [(STDs) including HIV, chlamydia, gonorrhea, and syphilis]. The oral component of the HIM Study began in 2007, although cell supernatant specimens were not routinely archived until 2012 (Kreimer et al., 2011). Included in this study of salivary SLPI were the first 378 men (stratified by country and age group) whose oral gargle supernatants were archived. None of the men reported being diagnosed with head and neck cancer at enrollment. This study was approved by the Institutional Review Boards of the University of South Florida (Tampa, FL, U.S.), the Ludwig Institute for Cancer Research (São Paulo, Brazil), the Centro de Referencia e Treinamento em Doenças Sexualmente Transmissíveis e AIDS (São Paulo, Brazil), and the Instituto Nacional de Salud Pública de Mexico (Cuernavaca, Mexico). All participants provided written informed consent.

Specimen and Data Collection Procedures

Participants were asked to swish 15 mL of mouthwash (15% alcohol by weight; UP&UP Brand, Target, USA) in their mouths for 15 seconds, gargle in the back of the throat for 15 seconds, and expectorate into a specimen cup. Specimens were stored at 4°C until processing. Within 24 hours of collection, specimens were centrifuged at 3000 x *g* and 4°C for 15 minutes, 1.8 mL of supernatant was decanted into a 2 mL micro-centrifuge tube, and specimens were archived at –80 °C until analysis (Kreimer et al., 2011). All oral gargle specimens were centrifuged at each study site to separate the supernatant from the cell pellet, and all specimens were shipped to a central laboratory (Tampa, FL) for SLPI quantification. The Human SLPI Quantikine ELISA Kit (DP100, R&D Systems, Minneapolis, MN, USA) was used to quantify salivary SLPI. Although this assay was originally designed to measure SLPI in supernatants obtained from cell cultures, plasma, serum, and urine, we previously optimized the assay to measure SLPI concentrations in oral gargle specimens. Details of the optimization process have been published elsewhere (Pierce Campbell et al., 2013).

Statistical Analysis

A series of socio-demographic and behavioral correlates of SLPI were evaluated using data collected by computer-assisted self-interview, including country of residence, age, race, ethnicity, marital status, sexual orientation, education, smoking status, chewing tobacco use, alcohol consumption, tooth-brushing habits, and oral sexual behaviors. Self-reported bleeding gums, tooth loss, as well as gingivitis and STD diagnoses were also evaluated. Since salivary SLPI values were not normally distributed, they were transformed by taking the square root to satisfy the normality assumption for regression (Kolmogorov-Smirnov $P > 0.15$). SLPI values were back transformed to the second power for presentation. Chi-square and Fisher's exact tests were utilized to detect associations between categorical variables and salivary SLPI, with Student's t-tests used for continuous variables. Two regression models were employed: 1) logistic regression with dichotomous salivary SLPI as the outcome, quartile 4 [median: 931.9 ng/mL; range: 726.2–1919.9] versus quartiles 1–3, combined [median: 390.0 ng/mL; range: 2.3–724.5], and 2) linear regression with continuous salivary SLPI concentration as the outcome. Multivariable models utilized backward stepwise selection methods with a significance level of $P=0.1$ for retention in the model. Odds ratios (OR) and 95% confidence intervals (CI) were reported. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Results

Socio-demographic, behavioral, and oral health characteristics of the study population by country of residence are presented in Table 1. Significant differences in all variables were observed by country ($P<0.05$), except for chewing tobacco use and tooth loss.

Among men ages 18–73 years, the median salivary SLPI concentration was 491.9 ng/mL, with an interquartile range of 294.9–724.5 (mean: 476.9; SD: 54.7; range: 2.3–1919.9 ng/mL). Table 2 presents the study population characteristics by salivary SLPI quartiles. SLPI concentration differed significantly by country of residence ($P<0.001$), with a higher proportion of men residing in Brazil having SLPI values in the highest quartile (38.9%) compared to men residing in the U.S. (15.9%) and Mexico (19.8%). Salivary SLPI appeared to decrease significantly with age, with the proportion of men in the youngest age group having high SLPI values (38.1%) more than two-fold higher than the proportion of men in the oldest age group with high SLPI values (16.7%). Tooth loss was significantly associated with salivary SLPI ($P=0.011$), with a higher proportion of men without tooth loss having SLPI values in the highest quartile (26.4%). Also, self-reported history of STDs was significantly associated with salivary SLPI ($P=0.034$), with a higher proportion of men with a history of self-reported STDs having SLPI values in the highest quartiles (50.0%).

Table 3 shows mean salivary SLPI concentration by socio-demographic characteristics. Similar to results shown in Table 2, higher salivary SLPI was significantly associated with residing in Brazil, younger age, and having had oral sex in the past six months.

Table 4 presents unadjusted and adjusted odds ratio (aOR) and 95% confidence interval (95% CI) from the logistic regression model examining the associations between salivary SLPI distribution and participant characteristics (Q4 vs. Q1, Q2, and Q3 combined). In the

unadjusted models, country, age, and self-reported diagnosis of STD in the past six months were significantly associated with SLPI, and in the fully adjusted model, salivary SLPI was significantly independently associated with country, age, bleeding/swollen gums, and STD history. Men in Brazil were significantly more likely to have higher levels of salivary SLPI (aOR 3.84; 95% CI: 1.94–7.59) compared to men residing in the U.S. Compared to older men (45–73 years), younger men (18–30 years) were significantly more likely to have higher levels of salivary SLPI (aOR 3.84; 95% CI: 1.98–7.43). Men with an STD diagnosis in the past six months were also more likely to have higher salivary SLPI (aOR 2.98; 95% CI: 1.14–7.83). However, men who reported bleeding or swollen gums were significantly less likely to have higher levels of salivary SLPI (aOR 0.34; 95% CI: 0.15–0.79)].

Results from linear regression (Table 5) were similar to those shown in logistic regression models, with country of residence, age, and bleeding/swollen gums significantly associated with salivary SLPI. In addition, current smoking status was linearly positively associated with salivary SLPI.

Discussion

To our knowledge, this is the first epidemiological study to thoroughly examine extracellular salivary SLPI, a defensin-like immune molecule, its distribution, and associated factors among healthy adult men. Despite emerging evidence of the potential protective role in preventing oral infections such as HIV and HPV, few studies have examined salivary SLPI concentrations in healthy populations. To address this gap, we sought to understand the normal distribution of salivary SLPI among healthy adults living in three different countries to aid in our understanding of the role of SLPI in oral infections. We observed a wide range of salivary SLPI concentrations among participants, with higher levels found among younger men and those residing in Brazil.

Consistent with our previous preliminary findings, (Pierce Campbell et al., 2013) salivary SLPI was inversely associated with age. This finding is consistent with the concept that aging results in decreases in the overall immune response including epithelial and glandular secretions (Larbi et al., 2013, Martin et al., 1996). In a small cross-sectional study (n=45), Shugars et al. reported lower mean salivary SLPI levels among elderly individuals ages 79–89 years compared with those ages 21–51 ($P<0.001$) (Shugars et al., 2001). Lower salivary SLPI concentrations among the elderly may be the result of xerostomia, a condition characterized by dry mouth due to reduced or absence of saliva secretions and a major complaint of the elderly, with 25–65% of elderly individuals affected (Nagler, 2004, Mortazavi et al., 2014). Aging is also associated with a greater likelihood of immune disorders, reduced immune proteins, (Nagler, 2004) chronic oral diseases, and tooth decay; (Mortazavi et al., 2014) all of these in turn may contribute to lower salivary SLPI levels (Ship et al., 2002, Guggenheimer & Moore, 2003).

It is possible that the observed geographical differences observed in salivary SLPI levels reflect differences in dietary, genetic, and environmental factors. In vivo animal models have shown increased SLPI expression in animals fed high fat versus low fat diets (Adapala et al., 2011). Dietary differences across study sites might have influenced SLPI levels in our study.

Genetic diversity among residents of these three countries could be another plausible explanation for our observed differences for SLPI concentrations in these three countries. To the best of our knowledge, no studies have examined SLPI concentrations among healthy, geographically-diverse populations that could be used for comparison of the study results. Also, population-level data is not available on other immune components that are similar in structure and function to SLPI. In the absence of these data, there exists indirect evidence for this proposed explanation in animal models. Gautier et al. reported considerable diversity in gene expression and mRNA transcripts in macrophages obtained from various tissues of the same mouse (Gautier et al., 2012). This suggests that if a great deal of diversity exists within one immune system and macrophage population, diversity in gene expression responsible for immune products such as SLPI among different populations is also highly plausible. Furthermore, higher levels of SLPI were found amongst those residing in Brazil after adjustment for age, education, gum bleeding, and sexual activity in the past six months. Further research is needed to identify the factors that underlie higher salivary SLPI concentrations among Brazilian men.

A recent STD diagnosis was significantly associated with higher salivary SLPI concentrations. To our knowledge, no other previously published studies have examined the association between salivary SLPI and infection with an STD. In one study of vaginal SLPI conducted among women, the authors observed significantly lower vaginal levels of SLPI in women with infections of the lower reproductive tract (STD or bacterial vaginosis) compared to those without infection ($P<.0001$) (Draper et al., 2000). These discrepant findings suggest that SLPI levels may differ based on the bodily fluid tested, type of STD, and population studied. Moreover, these findings may be due to inconsistencies in self-reporting as well as the small number of STD events.

Additionally, oral hygiene appeared to be associated with salivary SLPI. In univariate models, brushing teeth at a frequency of two or more times per day was marginally associated with higher levels of SLPI, and reports of bleeding or swollen gums and lost teeth were associated with lower salivary SLPI levels. The mechanisms by which oral health influences salivary SLPI are unknown; however, long-term and chronic inflammation of oral mucosa and gums, which occurs with gum bleeding, may disrupt the immune response, damage the oral epithelium, and lead to reduced SLPI secretion. Furthermore, overexpression of PAR2, a protein that modulates inflammatory responses in human tissues and plays an important role in the pathogenesis of chronic oral diseases, (Lee et al., 2010, Loubakos et al., 1998) is associated with reduced levels of SLPI (Euzebio Alves et al., 2013, Laugisch et al., 2012). PAR2 is widely expressed in oral epithelial cells, osteoblasts, gingival fibroblasts, and immune cells (Abraham et al., 2000, Loubakos et al., 2001). Oral bacteria, such as *Porphyromonas gingivalis*, can contribute to chronic inflammation (Page, 1991) and overexpression of PAR2, (Loubakos et al., 2001) subsequently reducing salivary SLPI (Euzebio Alves et al., 2013, Laugisch et al., 2012). However, in our study, self-reported gingivitis was marginally associated with higher levels of SLPI in univariate models. We hypothesize that the elevated secretion of SLPI in oral neutrophils and the increased activity of these neutrophils in the setting of acute gingivitis may explain this association (Sallenave et al., 1994, Ear & McDonald, 2008). These findings suggest that salivary SLPI levels may increase in the case of acute infections as a reaction by the oral

epithelium to infection; however, temporality of the association between SLPI and infection remains unknown. Prospective studies of salivary SLPI and acute oral infections are needed to address the issue of temporality.

Smoking is known to have direct effects on oral immune status (Lee et al., 2012). The higher salivary SLPI concentrations among current smokers observed in this study are consistent with findings from other studies that have reported increased SLPI secretions among smokers (Meyer et al., 2014, Bouloukaki et al., 2011). Smoking has been shown to enhance the expression of the signal transducer and activator of transcription (STAT1 protein), which, in turn, leads to increased SLPI expression (Meyer et al., 2014).

The major strengths of this study include the multinational population including men with a wide range of ages, and prior optimization of the SLPI quantification assay for use with oral gargle specimens. However, a few limitations should be considered. Oral specimens and survey data were collected concurrently, limiting our ability to establish temporal associations between SLPI and potential correlates. In addition, oral health measures and prior STD diagnoses were self-reported which may have resulted in non-differential misclassification. Although the same clinical and laboratory protocols were used at all HIM Study sites to ensure that samples were collected, processed, and stored in a comparable manner, we cannot rule out the potential influence of geographical variation on differences in SLPI concentrations. Moreover, participants were not specifically asked to abstain from eating or drinking prior to oral collection; however, most participants would have abstained for at least 30 minutes while they responded to the questionnaire. Lastly, oral specimens were not collected at the same time each day due to logistical constraints. While we recognize that diurnal variation in salivary SLPI concentrations may exist, as with the hormone cortisol, there is no published research to support this hypothesis. Nevertheless, the results from this study provide the first examination of salivary SLPI and its correlates among healthy adult men, which is an important first step in further assessing the potential role of salivary SLPI and its interaction with other biomarkers for oral infections in future.

Conclusions

Salivary SLPI may reduce the risk of transmission of a variety of oral infections through its antimicrobial and anti-inflammatory characteristics; however, its concentration appears to vary widely across populations and with age, along with a variety of behavioral characteristics, including smoking and oral hygiene habits. Further investigations are required to determine factors that may increase or decrease SLPI concentrations and evaluate the impact of extracellular SLPI levels on the subsequent risk of acquiring oral infections, such as HPV, in a longitudinal study.

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Socio-demographic, behavioral, and oral health characteristics of men in the study population by country

Table 1

Characteristics	Entire Population (N=378)	U.S. (N=126)	Brazil (N=126)	Mexico (N=126)	P-value ^d
	n (%)	n (%)	n (%)	n (%)	
Age, years					
18–30	126 (33.3)	42 (33.3)	42 (33.3)	42 (33.3)	0.999
31–44	126 (33.3)	42 (33.3)	42 (33.3)	42 (33.3)	
45–73	126 (33.3)	42 (33.3)	42 (33.3)	42 (33.3)	
Race					
White	161 (42.6)	82 (65.1)	68 (54.0)	11 (8.7)	<0.001 [*]
Black	76 (20.1)	29 (23.0)	47 (37.3)	0 (0.0)	
Asian	9 (2.4)	8 (6.4)	1 (0.8)	0 (0.0)	
American Indian/ Pacific Islander	5 (1.3)	0 (0.0)	5 (4.0)	0 (0.0)	
Mixed race/other	127 (33.6)	7 (5.6)	5 (4.0)	115 (91.3)	
Ethnicity					
Hispanic	168 (44.4)	20 (15.9)	37 (29.4)	111 (88.1)	<0.001 [*]
Non-Hispanic	209 (52.3)	106 (84.1)	89 (70.6)	14 (11.1)	
Missing	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.8)	
Marital status					
Single or never married	116 (30.7)	52 (41.3)	38 (30.2)	26 (20.6)	0.001 [*]
Married	158 (41.8)	41 (32.5)	45 (35.7)	72 (57.1)	
Cohabiting	55 (14.6)	14 (11.1)	22 (17.5)	19 (15.1)	
Divorced/separated/widowed	47 (12.4)	19 (15.1)	20 (15.9)	8 (6.3)	
Missing	2 (0.5)	0 (0.0)	1 (0.8)	1 (0.8)	
Sexual orientation^b					
MSW	314 (83.1)	108 (85.7)	92 (73.0)	114 (90.5)	0.002 [*]
MSM	48 (12.7)	12 (9.5)	25 (19.8)	11 (8.7)	
MSWM	12 (3.2)	3 (2.4)	8 (6.4)	1 (0.8)	
Missing	4 (1.0)	3 (2.4)	1 (0.8)	0 (0.0)	

Characteristics	Entire Population (N=378)	U.S. (N=126)	Brazil (N=126)	Mexico (N=126)	P-value ^d
	n (%)	n (%)	n (%)	n (%)	
Education, years					
12	158 (41.8)	21 (16.7)	48 (38.1)	89 (70.6)	<0.001 [*]
13–15	87 (23.0)	35 (27.8)	41 (32.5)	11 (8.7)	
16	133 (35.2)	70 (55.6)	37 (29.4)	26 (20.6)	
Smoking status					
Current	82 (21.7)	20 (15.9)	22 (17.5)	40 (31.7)	0.010 [*]
Former	43 (11.4)	19 (15.1)	12 (9.5)	12 (9.5)	
Never	253 (66.9)	87 (69.0)	92 (73.0)	74 (58.7)	
Chewing tobacco use					
Everyday	3 (0.8)	2 (1.6)	1 (0.8)	0 (0.0)	0.860
Sometimes	6 (1.6)	2 (1.6)	2 (1.6)	2 (1.6)	
Not at all	369 (97.6)	122 (96.8)	123 (97.6)	124 (98.4)	
Alcohol, no. drinks/month					
0	111 (29.4)	39 (31.0)	36 (28.6)	36 (28.6)	0.046 [*]
1–30	144 (38.1)	42 (33.3)	41 (32.5)	61 (48.4)	
31–60	49 (13.0)	20 (15.9)	16 (12.7)	13 (10.3)	
61	74 (19.6)	25 (19.8)	33 (26.2)	16 (12.7)	
Brushing teeth, no. times/day					
1	63 (16.7)	37 (29.4)	16 (12.7)	10 (7.9)	0.047 [*]
2	198 (52.4)	78 (61.9)	43 (34.1)	77 (61.1)	
3	116 (30.7)	11 (8.7)	66 (52.4)	39 (31.0)	
Missing	1 (0.3)	0 (0.0)	1 (0.8)	0 (0.0)	
Gingivitis^c					
Yes	72 (19.0)	27 (21.4)	31 (24.6)	14 (11.1)	0.017 [*]
No	306 (81.0)	99 (78.6)	95 (75.4)	112 (88.9)	
Bleeding/swollen gums					
Yes	57 (15.1)	9 (7.1)	24 (19.0)	24 (19.0)	0.010 [*]

Characteristics	Entire Population (N=378)	U.S. (N=126)	Brazil (N=126)	Mexico (N=126)	P-value ^d
	n (%)	n (%)	n (%)	n (%)	
No	321 (84.9)	117 (92.9)	102 (81.0)	102 (81.0)	
Tooth loss, no. teeth extracted					
0	273 (72.2)	93 (73.8)	87 (69.0)	93 (73.8)	0.052
1–3	58 (15.3)	20 (15.9)	17 (13.5)	21 (16.7)	
4	40 (10.6)	13 (10.3)	20 (15.9)	7 (5.6)	
Missing	7 (1.9)	0 (0.0)	2 (1.6)	5 (4.0)	
STD diagnosis in past 6 months^c					
Yes	24 (6.3)	1 (0.8)	8 (6.3)	15 (11.9)	<0.001 [*]
No	345 (91.3)	125 (99.2)	111 (88.1)	109 (86.5)	
Don't know	9 (2.4)	0 (0.0)	7 (5.6)	2 (1.6)	
Ever had oral sex with a woman/man					
Yes	332 (87.8)	116 (92.1)	117 (92.9)	99 (78.6)	0.002 [*]
No	41 (10.8)	8 (6.3)	8 (6.3)	25 (19.8)	
Missing	5 (1.3)	2 (1.6)	1 (0.8)	2 (1.6)	
Had oral sex in past 6 months					
Yes	227 (60.1)	70 (55.6)	91 (72.2)	66 (52.4)	0.047 [*]
No	148 (39.2)	55 (43.7)	34 (27.0)	59 (46.8)	
Missing	3 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)	

^a Chi-square and Fisher's exact tests were used to calculate p values.

^b MSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.

^c Self-reported diagnosis.

^{*} Significant P-value.

Table 2

Participant characteristics by SLPI distribution (quartiles).^a

Characteristics	Quartiles of SLPI								P-value ^b
	Q1		Q2		Q3		Q4		
	N	(%)	N	(%)	N	(%)	N	(%)	
Country									
USA	42	(33.3)	40	(31.7)	24	(19.0)	20	(15.9)	<0.001 *
Brazil	17	(13.5)	21	(16.7)	39	(31.0)	49	(38.9)	
Mexico	35	(27.8)	34	(27.0)	32	(25.4)	25	(19.8)	
Age, years									
18–30	24	(19.0)	28	(22.2)	26	(20.6)	48	(38.1)	0.009 *
31–44	28	(22.2)	34	(27.0)	39	(31.0)	25	(19.8)	
45–73	42	(33.3)	33	(26.2)	30	(23.8)	21	(16.7)	
Race									
White	35	(21.7)	41	(25.5)	41	(25.5)	44	(27.3)	0.695
Black	18	(23.7)	18	(23.7)	20	(26.3)	20	(26.3)	
Asian	5	(55.6)	3	(33.3)	0	(0.0)	1	(11.1)	
American Indian/Other Pacific Islanders	2	(40.0)	1	(20.0)	1	(20.0)	1	(20.0)	
Mixed	34	(26.8)	32	(25.2)	33	(26.0)	28	(22.0)	
Ethnicity									
Hispanic	46	(27.4)	39	(23.2)	39	(23.2)	44	(26.2)	0.625
Non-Hispanic	48	(23.0)	56	(26.8)	55	(26.3)	50	(23.9)	
Missing	0	(0.0)	0	(0.0)	1	(100.0)	0	(0.0)	
Marital status									
Single or never married	23	(19.8)	30	(25.9)	25	(21.6)	38	(32.8)	0.220
Married	45	(28.5)	35	(22.2)	43	(27.2)	35	(22.2)	
Cohabiting	12	(21.8)	16	(29.1)	15	(27.3)	12	(21.8)	
Divorced/separated/widowed	14	(29.8)	14	(29.8)	10	(21.3)	9	(19.1)	
Missing	0	(0.0)	0	(0.0)	2	(100.0)	0	(0.0)	

Characteristics	Quartiles of SLPI								P-value ^b
	Q1		Q2		Q3		Q4		
	N	(%)	N	(%)	N	(%)	N	(%)	
Sexual orientation ^c									
MSW	80	(25.5)	82	(26.1)	77	(24.5)	75	(23.9)	0.358
MSM	11	(22.9)	7	(14.6)	17	(35.4)	13	(27.1)	
MSWM	3	(25.0)	4	(33.3)	1	(8.3)	4	(33.3)	
Missing	0	(0.0)	2	(50.0)	0	(0.0)	2	(50.0)	
Education, years									
12	36	(22.8)	39	(24.7)	42	(26.6)	41	(25.9)	0.156
13–15	21	(24.1)	19	(21.8)	17	(19.5)	30	(34.5)	
16	37	(27.8)	37	(27.8)	36	(27.1)	23	(17.3)	
Smoking status									
Current	15	(18.3)	23	(28.0)	19	(23.2)	25	(30.5)	0.417
Former	11	(25.6)	7	(16.3)	12	(27.9)	13	(30.2)	
Never	68	(26.9)	65	(25.7)	64	(25.3)	56	(22.1)	
Chewing tobacco use									
Everyday	2	(66.7)	0	(0.0)	0	(0.0)	1	(33.3)	
Sometimes	3	(50.0)	0	(0.0)	3	(50.0)	0	(0.0)	
Not at all	89	(24.1)	95	(25.7)	92	(24.9)	93	(25.2)	
Alcohol, no. drinks/month									
0	27	(24.3)	29	(26.1)	34	(30.6)	21	(18.9)	0.413
1–30	39	(27.1)	32	(22.2)	36	(25.0)	37	(25.7)	
31–60	12	(24.5)	15	(30.6)	6	(12.2)	16	(32.7)	
61	16	(21.6)	19	(25.7)	19	(25.7)	20	(27.0)	
Brushing teeth, no. times/day									
1	21	(33.3)	13	(20.6)	16	(25.4)	13	(20.6)	0.571
2	48	(24.2)	52	(26.3)	51	(25.8)	47	(23.7)	
3	25	(21.6)	30	(25.9)	27	(23.3)	34	(29.3)	
Missing	0	(0.0)	0	(0.0)	1	(100.0)	0	(0.0)	

Characteristics	Quartiles of SLPI								P-value ^b
	Q1		Q2		Q3		Q4		
	N	(%)	N	(%)	N	(%)	N	(%)	
Gingivitis^d									
Yes	13	(18.1)	20	(27.8)	22	(30.6)	17	(23.6)	0.391
No	81	(26.5)	75	(24.5)	73	(23.9)	77	(25.2)	
Bleeding/swollen gums									
Yes	21	(36.8)	11	(19.3)	16	(28.1)	9	(15.8)	0.065
No	73	(22.7)	84	(26.2)	79	(24.6)	85	(26.5)	
Tooth loss, no. of teeth extracted									
0	69	(25.3)	68	(24.9)	64	(23.4)	72	(26.4)	0.011 [*]
1–3	19	(32.8)	14	(24.1)	11	(19.0)	14	(24.1)	
4	2	(5.0)	12	(30.0)	18	(45.0)	8	(20.0)	
Missing	4	(57.1)	1	(14.3)	2	(28.6)	0	(0.0)	
STD diagnosis in past 6 months^d									
Yes	4	(16.7)	5	(20.8)	3	(12.5)	12	(50.0)	0.034
No	89	(25.8)	88	(25.5)	87	(25.2)	81	(23.5)	
Don't know	1	(11.1)	2	(22.2)	5	(55.6)	1	(11.1)	
Ever had oral sex with a woman/man									
Yes	81	(24.4)	86	(25.9)	81	(24.4)	84	(25.3)	0.346
No	12	(29.3)	8	(19.5)	14	(34.1)	7	(17.1)	
Missing	1	(20.0)	1	(20.0)	0	(0.0)	3	(60.0)	
Had oral sex in past 6 months									
Yes	48	(21.1)	54	(23.8)	60	(26.4)	65	(28.6)	0.078
No	45	(30.4)	40	(27.0)	34	(23.0)	29	(19.6)	
Missing	1	(33.3)	1	(33.3)	1	(33.3)	0	(0.0)	

^a Square root of SLPI was used to obtain quartiles.

^b Chi-square and Fisher's exact tests were used to calculate *P*-values.

^c MSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.

Self-reported diagnosis,
p
* Significant *P*-value (*P*<0.05).

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Table 3

Participant characteristics by SLP concentration (ng/mL).^{a, b}

Characteristics	Mean	SD	Median	IQR	P-value ^c
Country					
USA	378.30	59.29	386.12	(207.38 – 612.49)	<0.001 *
Brazil	617.03	46.92	625.50	(425.03 – 926.98)	
Mexico	450.71	43.69	459.67	(279.39 – 638.24)	
Age, years					
18–30	579.36	53.14	586.12	(321.01 – 928.77)	<0.001 *
31–44	455.82	44.09	500.86	(308.65 – 697.07)	
45–73	403.61	59.44	439.74	(228.23 – 601.55)	
Race					
White	504.00	58.52	504.00	(304.84 – 767.54)	0.232
Black	468.29	64.16	526.24	(317.02 – 777.59)	
Asian	282.24	51.12	241.49	(199.51 – 435.99)	
American Indian/Pacific Islander	428.49	62.73	425.18	(220.32 – 689.70)	
Mixed race/other	465.70	43.56	480.49	(281.36 – 701.04)	
Ethnicity					
Hispanic	469.16	55.80	488.41	(269.64 – 739.18)	0.861
Non-Hispanic	482.68	54.46	492.40	(312.25 – 716.03)	
Missing	605.65	-	605.65	(605.72 – 605.72)	
Marital status					
Single or never married	543.82	61.31	523.04	(314.99 – 878.20)	0.055
Married	448.59	53.29	490.62	(246.51 – 689.70)	
Cohabiting	484.00	41.99	488.41	(361.17 – 720.57)	
Divorced/separated/widowed	401.60	53.14	435.97	(236.12 – 609.34)	
Missing	651.27	1.64	651.27	(605.72 – 698.33)	
Sexual orientation^d					
MSW	470.02	54.02	484.44	(282.86 – 716.12)	0.764

Characteristics	Mean	SD	Median	IQR	P-value ^c
MSM	513.48	61.62	566.44	(342.33 – 761.23)	
MSWM	483.56	64.32	453.69	(288.52 – 857.19)	
Missing	586.61	27.67	605.65	(393.56 – 817.68)	
Education, years					
12	487.53	52.27	500.42	(308.65 – 733.47)	0.216
13–15	515.29	66.42	525.33	(294.94 – 865.27)	
16	440.58	49.56	458.82	(246.51 – 638.04)	
Smoking status					
Current	541.49	44.76	507.60	(316.07 – 803.42)	0.070
Former	513.48	65.61	565.49	(246.51 – 773.07)	
Never	450.71	55.35	480.49	(266.22 – 696.92)	
Chewing tobacco use					
Everyday	290.02	230.43	236.24	(7.53 – 1087.43)	0.445
Sometimes	404.41	43.43	392.44	(207.38 – 704.1)	
Not at all	479.61	54.02	492.40	(300.68 – 726.24)	
Alcohol, no. drinks/month					
0	436.81	57.30	488.41	(300.24 – 678.56)	0.431
1–30	484.88	56.10	494.17	(254.34 – 752.91)	
31–60	501.76	60.06	400.00	(294.94 – 768.62)	
61	507.15	44.76	517.56	(316.07 – 772.84)	
Brushing teeth, no. times/day					
1	433.47	49.84	470.46	(212.12 – 704.75)	0.409
2	470.46	57.00	483.12	(300.68 – 716.03)	
3	510.76	53.58	519.84	(317.02 – 780.20)	
Missing	698.54	-	698.54	(698.33 – 698.33)	
Gingivitis^e					
Yes	507.15	42.51	525.78	(360.85 – 704.50)	0.384
No	470.02	57.61	487.97	(266.22 – 726.24)	
Bleeding/swollen gums					

Characteristics	Mean	SD	Median	IQR	P-value ^c
Yes	407.23	48.44	454.12	(224.24 – 629.99)	0.066
No	489.74	55.50	496.84	(304.79 – 753.89)	
Tooth loss, no. teeth extracted					
0	476.99	58.52	490.18	(288.4 – 753.89)	0.105
1–3	436.81	52.42	459.24	(222.52 – 704.75)	
4	571.21	31.25	548.03	(386.07 – 679.05)	
Missing	316.13	30.58	281.23	(142.72 – 549.58)	
STD diagnosis in past 6 months^e					
Yes	598.78	62.57	653.31	(451.53 – 878.16)	0.173
No	467.86	54.91	483.12	(281.36 – 710.54)	
Don't know	523.95	15.92	605.65	(401.83 – 620.14)	
Ever had oral sex with a woman/man					
Yes	482.68	54.61	490.62	(299.00 – 729.08)	0.472
No	423.54	54.02	492.40	(246.51 – 667.07)	
Missing	547.09	75.34	772.84	(333.41 – 863.79)	
Had oral sex in past 6 months					
Yes	520.75	52.71	529.46	(316.07 – 782.37)	0.002 [*]
No	419.43	53.44	437.65	(246.28 – 676.80)	
Missing	207.36	111.51	343.36	(5.78 – 495.97)	

^aSquare root of SLPI was used to obtain mean, median, standard deviation (SD), range and P-value.

^bMean, median, SD and range presented in the table are back transformed by taking 2nd power of the value.

^cIndependent sample t-test and F test were used to calculate P-values based on square root transformed SLPI.

^dMSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.

^eSelf-reported diagnosis.

SD=standard deviation, IQR=interquartile range.

^{*} Significant P-value.

Table 4

Association between SLPI distribution and participant characteristics - unadjusted and adjusted logistic regression models.^{a, b}

Characteristic	OR (95% CI)	aOR (95% CI)
	Q4 vs.Q1–Q3	Q4 vs.Q1–Q3
Country		
USA	1.00	1.00
Brazil	3.37 (1.86 – 6.13)	3.84 (1.94 –7.59)
Mexico	1.31 (0.69 – 2.51)	1.15 (0.52 –2.54)
Age, years		
18– 30	3.08 (1.70 – 5.55)	3.84 (1.98 –7.43)
31– 44	1.24 (0.65 – 2.35)	1.16 (0.59 –2.32)
45– 73	1.00	1.00
Race		
White	1.00	“__”
Black	0.95 (0.51 – 1.76)	“__”
Asian/American Indian/Pacific Islanders	0.44 (0.10 –2.06)	“__”
Mixed race/other	0.75 (0.44 – 1.3)	“__”
Ethnicity		
Hispanic	1.00	“__”
Non-Hispanic	0.89 (0.55 – 1.42)	“__”
Marital status		
Single or never married	1.71 (1.00 – 2.94)	“__”
Married	1.00	“__”
Other	0.91 (0.50 – 1.68)	“__”
Sexual orientation^c		
MSW	1.00	“__”
MSM	1.18 (0.60 – 2.35)	“__”
MSMW	1.59 (0.47 – 5.44)	“__”
Education, years		
12	1.00	1.00
13–15	1.5 (0.85 – 2.65)	1.12 (0.56 –2.24)
16	0.6 (0.34 – 1.06)	0.51 (0.26 – 1.02)
Smoking status		
Current	1.54 (0.88 – 2.69)	“__”
Former	1.52 (0.75 – 3.12)	“__”
Never	1.00	“__”
Chewing tobacco use		
Yes	0.37 (0.05 – 3.01)	“__”

Characteristic	OR (95% CI)	aOR (95% CI)
	Q4 vs.Q1–Q3	Q4 vs.Q1–Q3
No	1.00	“—”
Alcohol, no. drinks/month		
0	1.00	“—”
1–30	1.48 (0.81 – 2.71)	“—”
31–60	2.08 (0.97 – 4.46)	“—”
61	1.59 (0.79 – 3.19)	“—”
Brushing teeth, no. times/day		
1	1.00	“—”
2	1.2 (0.60 – 2.39)	“—”
3	1.59 (0.77 – 3.31)	“—”
Gingivitis^d		
Yes	0.92 (0.50 – 1.68)	“—”
No	1.00	“—”
Bleeding/swollen gums		
Yes	0.52 (0.24 – 1.11)	0.34 (0.15 –0.79)
No	1.00	1.00
Tooth loss		
Yes	0.81 (0.47 – 1.39)	“—”
No	1.00	“—”
STD diagnosis in past 6 months^d		
Yes	3.26 (1.41 – 7.53)	2.98 (1.14 –7.83) 1.00
No	1.00	
Ever had oral sex with a woman/man		
Yes	1.65 (0.70 – 3.85)	“—”
No	1.00	“—”
Had oral sex in past 6 months		
Yes	1.65 (1.00 – 2.71)	“—”
No	1.00	“—”

^aThe square root of SLPI was used as the outcome for regression models.

^bThe final adjusted model was obtained using backward stepwise selection methods with a significance level of P -value=0.1 for retention in the model.

^cMSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.

^dSelf-reported diagnosis.

Q=quartile; OR=odds ratio; aOR=adjusted odds ratio; CI=confidence interval.

“—”Variables not included in the final adjusted model.

Table 5

Association between SLPI concentration and participant characteristics - unadjusted and adjusted linear regression models.^{a, b}

Characteristics	Unadjusted Models		Adjusted	Model
	Beta	P-value	Beta	P-value
Country				
U.S.	Reference		Reference	
Brazil	5.39	<0.001	5.77	<0.001
Mexico	1.78	0.047	2.25	0.012
Age, years				
18– 30	3.98	<0.001	4.74	<0.001
31– 44	1.26	0.168	1.34	0.127
45– 73	Reference		Reference	
Race				
White	Reference		Reference	
Black	−0.81	0.430	“__”	“__”
Asian/American Indian/ Pacific Islanders	−4.25	0.040	“__”	“__”
Mixed race/other	−0.89	0.310	“__”	“__”
Ethnicity				
Hispanic	Reference		Reference	
Non-Hispanic	0.31	0.690	“__”	“__”
Marital status				
Single or never married	2.12	0.020	“__”	“__”
Married	Reference		Reference	
Other	−0.09	0.930	“__”	“__”
Sexual orientation^c				
MSW	Reference		Reference	
MSM	0.91	0.430	“__”	“__”
MSMW	0.33	0.880	“__”	“__”
Education				
12	Reference		Reference	
13–15	0.62	0.530	“__”	“__”
16	−1.04	0.240	“__”	“__”
Smoking status				
Current	2.10	0.030	1.96	0.029
Former	1.36	0.270	0.92	0.423
Never	Reference		Reference	
Chewing tobacco use				
Yes	−2.80	0.270	“__”	“__”

Characteristics	Unadjusted Models		Adjusted	Model
	Beta	P-value	Beta	P-value
No	Reference		Reference	
Alcohol, no. drinks/month				
0	Reference		Reference	
1–30	1.26	0.190	“—”	“—”
31–60	1.64	0.200	“—”	“—”
61	1.69	0.140	“—”	“—”
Brushing teeth, no. times/day				
1	Reference		Reference	
2	0.97	0.370	“—”	“—”
3	1.90	0.110	“—”	“—”
Gingivitis^d				
Yes	0.83	0.400	“—”	“—”
No	Reference		Reference	
Bleeding/swollen gums				
Yes	−1.90	0.080	−3.39	0.001
No	Reference		Reference	
Tooth loss				
Yes	0.34	0.700	1.66	0.053
No	Reference		Reference	
STD diagnosis in the past 6 months^d				
Yes	2.89	0.070	“—”	“—”
No	Reference		Reference	
Ever had oral sex with a woman/man				
Yes	1.37	0.270	“—”	“—”
No	Reference		Reference	
Had oral sex in the past 6 months				
Yes	2.48	0.020	“—”	“—”
No	Reference		Reference	

^a Square root of SLPI was used as outcome for regression models.

^b Final adjusted model was obtained using backward stepwise selection method with significance level for retention in model of P -value=0.1.

^c MSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.

^d Self-reported diagnosis.

“—”, Variables not included in the final adjusted model.

Note: Beta represents change in SLPI concentration i.e. compared to US, men in Brazil had 5.39 units increased concentration of the salivary SLPI in the unadjusted model (P -value <.001), and 5.77 units while adjusting for other covariates in the model (P -value <.001).