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Intraoral human herpes viruses detectable by PCR in majority of patients

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Abstract

Objectives: To identify factors which influence the intraoral prevalence of human herpes viruses using mucosal swabs, saliva samples and qPCR analysis.

Methodology: In this cross-sectional observational study, matched saliva and oral swabs were collected from a total of 115 subjects: 70 immunocompetent subjects with no mucosal abnormalities, 22 with mucosal abnormalities and 23 therapeutically immunocompromised individuals. Extracted DNA was analysed by multiplex qPCR for detection and quantification of human herpes viruses 1-6.

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Results: At least one human herpes virus was detected in 77.1% of immunocompetent individuals with no mucosal abnormalities, with EBV the most commonly detected at 61.4%. HHV-6 was detected in 17.1%, HSV-1 in 4.3% and CMV in 1.1%. Detection was higher in saliva than in oral swabs. There was no detection of HSV-2 or VZV. Neither presence of oral mucosal abnormality nor therapeutic immunocompromise was related to increased detection of human herpes virus.

Conclusion: Commensal detection rates of EBV are high and caution in clinical correlation of positive detection is warranted. Commensal CMV rates are low and detection is likely to be clinically relevant. This study presents a comprehensive commensal detection rate of human herpes viruses 1-6 by qPCR in saliva and swabs.

Introduction

Human herpes viruses have been identified as causative in cases of persistent mucosal ulceration in an immunocompromised host (Casadevall & Pirofski, 2000, Schubert et al., 1993). Human herpes viruses (HHV) can be subdivided into 8 different species; Herpes Simplex 1 (HSV-1), Herpes Simplex 2 (HSV-2), Varicella Zoster Virus (VZV), Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), HHV-6, HHV-7, and Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) (He et al., 2015).

Mycophenolate mofetil (MMF) is a steroid-sparing immunosuppressive agent used worldwide particularly following renal transplant but is now commonly used in the treatment of dermatological disease. Increased risk of opportunistic infection compared to other immunosuppressant has been reported in MMF use (Buell & Koo, 2008). Studies to date suggest a specific risk of CMV, and this may be MMF dose-dependent (Wang et al., 2004). Chronic oral mucosal ulceration can occur for several reasons ranging from trauma, the presence of an oral mucosal dermatoses, malignant processes and microbial infection, particularly viral. Pemphigus Vulgaris (PV) is an example of an immune-related dermatosis associated with chronic intraoral mucosal ulceration requiring therapeutic immunosuppression. Oliveira-Batista et al. found an HHV detection rate of 54.5%-60% of 23 PV patients with ulceration present (Oliveira-Batista et al., 2013). HHV swab detection has been reported in episodic oral ulceration (Brice et al., 2000) but rates of detection in individuals with chronic oral ulceration without systemic immunosuppression has not been reported. Conversely, rates of detection in individuals who are therapeutically immunocompromised due to dermatological disease and who do not have oral mucosal ulceration have not been reported.

Mucosal swabs utilise a rapid and non-invasive sampling technique that is particularly useful in patients experiencing painful oral mucosal ulceration. Individuals who are immunocompetent and used as controls in studies using oral swabs report up to 10% prevalence of HSV-1 (Aggarwal et al., 2014, Marques-Silva et al., 2007), and 0% for CMV has been reported (Correia-Silva Jde et al., 2007, Victoria et al., 2005). Several studies have assessed the presence of herpes viruses in the saliva of immunocompromised patients, particularly in HIV positive patients with regard to an association between herpes viruses and periodontal disease (Slots & Slots, 2011). These studies report commensal HHV detection rates by salivary analysis range from 0% for VZV (Park et al., 2017), 16% for EBV (Ammatuna et al., 1998) and up to 46% for CMV ((Gautheret-Dejean et al., 1997). This contrasts with antibody seroprevalence studies that report rates as high as 80% for HSV-1, 20% for HSV-2, 95% for VZV, 90% for EBV, 70% for CMV and 95-100% for HHV-6 (Cannon et al., 2010, Knox et al., 2011, Smith & Robinson, 2002, Campadelli-Fiume et al., 1999a).

Clinical interpretation of a positive herpes salivary test becomes particularly pertinent for patients with chronic oral ulceration and who are therapeutically immunosuppressed and where an invasive scalpel biopsy that would aid in diagnosis is preferably avoided. There is thus a need to quantify baseline prevalence of HHV in immunocompetent patients without oral mucosal ulceration, immunocompetent participants with chronic oral mucosal abnormality, with individuals on immunosuppression without oral mucosal ulceration, as well as to compare the salivary detection rates between these groups using flocked swabs or whole saliva.

We hypothesized that chronic oral mucosal abnormality of therapeutic immunosuppression is associated with increased detection of HHV above commensal rates. The aims of this research were to

- 1) Determine factors which influence the intraoral prevalence of HHV, measured by mucosal swab and whole saliva samples with qPCR, in non-immunocompromised participants how have no oral mucosal abnormalities.
- 2) Assess the intraoral prevalence of HHV, measured by mucosal swab and whole saliva samples with qPCR, of individuals who are therapeutically immunocompromised for treatment for a dermatosis and individuals with chronic oral mucosal abnormalities.
- 3) Compare detection rates and quantification levels of HHV present in whole saliva with oral mucosal swab samples.

Methodology

This study was approved by the Human Research Ethics Committee of Melbourne Health (HREC/16/MH/265) and the Dental Health Services Victoria Research Review Group (ID: 309).

Participant recruitment

Participants were recruited from June 2017 to March 2018. In this study an individual was defined as immunocompetent if they were not taking any systemic immunosuppressive medications. Immunocompetent participants were recruited from the Royal Dental Hospital of Melbourne (RDHM). Exclusion criteria included participants on systemic immunosuppressants or those on antiviral therapy either at the time or within the 4 weeks prior to recruitment. Therapeutically immunocompromised participants were recruited from the Royal Melbourne Hospital (RMH) Dermatology clinics and included patients on systemic immunosuppressants for their dermatological condition. Patients on active antiviral therapy were excluded.

Data collection

Data collection occurred at a single time point and consisted of a verbal questionnaire to the patient and treating dental practitioner at the time of sample collection and further inspection of clinical records. The presence of caries and periodontitis was determined by the treating dental practitioner according to AAP periodontal classification (Armitage, 1999). Information collected included: age, gender, medical history, dental history, oral hygiene routine, risk related habits and dental health status. The data was initially recorded on a paper collection template and then uploaded to a secure cloud-based clinical data entry system, *mForm*.

Sample collection

Samples taken from participants consisted of an oral mucosal swab and a saliva sample, collected using a standardized technique. First, all patients were asked to expectorate 3 times into a container. Then, an oral mucosal swab was collected using a Copan flocced nylon swab, held and rotated 3 times against the mucosa. If a mucosal abnormality was present, a swab was taken from the site of the ulcer or abnormality. For participants with no ulceration, a control swab was taken from intact mucosa of the left lateral border of the tongue or the left buccal mucosa. The swab was then

immediately placed in 1mL of Copan Universal Transport Medium. All samples were initially stored at -20°C in a secure freezer at RDHM, until transport on dry ice to Genetic Signatures Headquarters (Level 9, Lowy Packer Building, 405 Liverpool Street, Darlinghurst NSW 2010) for eventual analysis.

DNA extraction and quantitative PCR

DNA was extracted from oral swab and saliva samples using a pre-release version of the Genetic Signatures EasyScreen™ Human Herpesvirus Detection Kit (HV001). Samples were prepared using the EasyScreen™ Sample Processing Kit (SP006), and a portion of the resulting elute combined with the supplied PCR reagents in order to amplify human herpesvirus targets. Amplified targets were detected in real-time, using unique fluorophore-labelled probes. Human herpesviruses detected in specific channels included: Herpes-Simplex Virus 1 (HSV-1), Herpes-Simplex Virus 2 (HSV-2), Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Human Herpesvirus 6 and Varicella Zoster Virus (VZV). Signals were measured and distinguished by a HV001 compatible real-time PCR platform (Bio-Rad CFX384™ or Bio-Rad CFX96™). A standard curve of synthetic DNA was used to determine the quantification of viral loads. Detailed PCR methodology can be found in Supplementary Materials.

Data analysis

A clinically significant difference in viral detection was determined as greater than 50% of a test group having detectable HHV compared to a control group. A prevalence of up to 10% was expected within the control group (Aggarwal et al., 2014, Marques-Silva et al., 2007). A power analysis to calculate the number of participants to detect the difference between 0.1 and 0.5 with 80% power and $\alpha = 0.05$ was 19 individuals in each arm.

Categorical variables were assessed using counts and percentage frequencies and compared using chi-squared tests or Fisher's exact test when frequencies were less than 5. Categorical variables included demographic, comorbidity related, HHV exposure-related, habit and oral health related factors. Continuous variables of HHV detection levels were assessed using Student's t-tests or analysis of variance (ANOVA) when variables were normally distributed, or Kruskal-Wallis or Mann-Whitney (rank sum) tests when variables were non-normally distributed following Bartlett's test for constant variance. A p-value of less than 0.05 was considered to indicate statistical significance. Statistical analysis was completed in R 3.5.2.

Results

Patient demographics

A total of 115 participants were recruited from both the Royal Dental Hospital of Melbourne (RDHM) and Dermatology Department at the Royal Melbourne Hospital. There were 70 participants who were immunocompetent with no mucosal abnormalities (Normal group), 23 participants who were therapeutically immunocompromised (TI group) and 22 participants who were immunocompetent with oral mucosal abnormalities (OA group). The mean age for each group was 55.6 years (Normal), 52.5 years (TI), 60.2 years (OA). The male to female ratios were 37:33 (Normal), 14:9 (TI) and 9:13 (OA). There were no statistically significant differences in age or gender between these three groups.

Normal Group

Detection of any HHV in any sample occurred in 77.0% of normal participants (**Table 1.**) EBV was detected by swab or saliva in 43 (61.4%), followed by HHV-6 in 12 (17.1%), HSV-1 was detected in 3 (4.3%) of individuals, CMV in 1 (1.1%) of the Normal Group. Neither herpes simplex virus 2 (HSV-2) nor varicella-zoster virus (VZV) were identified from any of the samples collected. Significantly more participants had positive HHV detection in their saliva sample (49/70; 70%) than from their swab sample (32/70; 45.7%) ($p = 0.003$). EBV was most commonly detected in saliva alone or in combination with other HHV (**Figure 1.**) Twenty-five (35.7%) normal participants had detectable EBV in both saliva and swab samples. A further 18 (25.7%) participants had EBV detectable in saliva alone. However, detection by swab was not always accompanied by detection in saliva with the presence of HHV-6 detectable by swab alone (**Table 1**).

There was no evidence of difference between any HHV detection in individuals above and below age 50. However, there was a significantly higher HHV detection in males compared to females (**Table 1.**) However, this difference was not attributable to any single HHV studied with no significant differences in detection of HSV-1 ($p = 0.207$), EBV ($p = 0.388$), CMV ($p = 0.341$) and HHV 6 ($p = 0.092$) between the genders.

The presence or absence of a comorbidity in the Normal group was not associated with a significant difference in HHV detection rate (**Table 2.**) No significant differences in (any) HHV detection

were found between participants with or without cardiovascular diseases, diabetes, respiratory disease or psychological disorder.

History of HHV exposure

No significant differences in detection rate were found between participants with or without a history of chickenpox, cold sores, or antiviral therapy. Additionally, there was no significant association between HHV detection rate and contact as parents or grandparents with children under 10 years of age (**Table 3.**).

Assessment of risk factors

Assessing smoking habits as a factor, current smokers had higher HHV detection rates than past smokers and never-smokers, but these differences were not significant (**Table 3.**). There was no significant difference in the detection rate of HHV between the individuals who reported consuming alcohol compared to those who never consumed alcohol.

Oral health

There was no significant association in the detection of HHV between participants who reported flossing their teeth daily ($p = 0.794$), those who currently used a mouthwash, or those reported consuming sweet foods daily (**Table 3.**). No difference was found in HHV detection between those with more than 20 teeth than those with fewer, or those who reported having a removable appliance or not. There was no difference in HHV detection between those with high or medium caries risk and those who did not, or the presence of active caries, gingivitis, or periodontitis. The presence of dental occlusal attrition (21/33) was associated with lower HHV detection rates compared those without (33/37) ($p = 0.011$), reflected in a significantly reduced detection of HSV-1 ($p = 0.028$) and HHV-6 ($p = 0.025$), but not EBV ($p = 0.388$)

Comparison with individuals with oral abnormalities

The 22 participants who had oral abnormalities included 15 participants with oral lichen planus or an oral lichenoid lesion as defined by the WHO criteria (Van Der Meij & Van Der Waal, 2003), 5 participants with keratosis or leukoplakia, and 2 with fibroepithelial polyps. Sixteen of the 22 (72.7%) participants were positive for (any) HHV detection. There was a significant difference in the detection rate of HSV-1 by either swab or saliva between those with (3/22; 14%) and without oral abnormalities (3/70; 4%) (*chi-square*; $p = 0.032$). However, this was not attributable to the HSV-1 detection in saliva (*chi-square*; $p = 0.211$) nor the control swab (*chi-square*; $p = 0.082$) alone. No difference was found in the quantity of HSV-1 (*t-test* $p=0.820$) or EBV (*t-test*; $p\text{-value} =$

0.090) detected between control swabs and swabs taken from patients with a mucosal abnormality. No difference was found in the quantity of HSV-1 in the saliva from participants with and without oral mucosal abnormality (t-test; $p = 0.995$). There were no significant differences between individuals with and without oral mucosal ulceration in the detection by any sample method of EBV ($p = 0.737$); CMV ($p = 0.573$); and HHV-6 ($p = 0.175$) (Table 4. & 5.).

Comparison with Therapeutically Immunocompromised participants

Of the participants on systemic immunosuppression, 19/23 participants were taking mycophenolate mofetil, 2 participants were taking oral prednisolone, 1 patient was taking azathioprine and one patient was receiving rituximab. Comparing participants on MMF alone, there was no significant association between MMF dosages ($<1g$, $1-2g$, $2-3g$) and HHV detection (*Fisher's exact test* $p = 0.574$). There was also no significant association between duration (<12 months, ≥ 12 months) of MMF therapy and HHV detection (*Fisher's exact test* $p = 0.621$). Individuals in the therapeutically immunocompromised cohort were less likely to have positive HHV detection than either the normal participants, or those with an oral abnormality (Table 4. & 5.).

Quantification of HHV levels

Quantification of HHV levels in saliva found that HSV-1 detection levels in saliva were significantly higher than EBV (Tukey's t-test $p < 0.001$) or HHV6 (Tukey's t-test $p = 0.001$) detection levels. HSV-1 detection levels in control swabs were also found to be significantly higher than EBV (Tukey's t-test $p = 0.027$) or HHV-6 (Tukey's t-test $p = 0.024$) (Figure 2.).

Discussion

In the present study, we have shown that HHV detection in swab and saliva samples in immunocompetent individuals with no mucosal abnormalities is common, particularly for EBV. This presents a challenge in discriminating between the presence of active viral replication in contrast to benign commensal viral shedding with these commonly used tests. The fact that HHV DNA was detectable in the majority of participants may be reflective of the life cycle of these commensal viruses and their tissue tropism relevant in the oral cavity.

Factors that were associated with increased HHV detection was male gender and presence of dental attrition. However, there was no association found with older age, smoking or alcohol, medical comorbidities, factors related to virus exposure, oral hygiene or oral health factors including biofilm related diseases caries and periodontitis. Previous reports have suggested association of salivary HSV-1 and EBV (Slots & Slots, 2011) with periodontal disease which was not found in our cohort.

HSV-1, EBV, CMV and HHV6 can be transmitted via saliva, and the presence of VZV in saliva has been documented (Rao et al., 2014). Detection of HHV in saliva was found to be significantly higher than that from a swab, suggesting that saliva samples have superior sensitivity to a swab as a non-invasive sampling method to detect human herpes virus in the oral cavity. However, the fact that positive swab detection did occur in the absence of salivary detection would suggest the relevance of epithelial site collection and that swab collection should not be entirely disregarded.

In addition, the amount of HSV1 viral loads found in saliva and control swab samples were significantly greater, by approximately 2-fold, than any other HHV tested despite high commensal detection rates of EBV. This may indicate that HSV-1 is more likely to play a pathogenic role, however the frequency of shedding, viral load and the relationships with transmission is unknown. It has been suggested that secondary HSV-1 infection only arises in 25–40% of HSV-1-seropositive individuals (Shulman, 2004, Stock et al., 2001) and that shedding in the oral cavity occurs independently of the presentation of herpes labialis (da Silva et al., 2005).

In a study in an Indian population, HSV-2 in 69 %, of children with chemotherapy associated oral mucositis using qPCR compared with 0 % of control participants (Aggarwal et al., 2014). There was no intraoral detection of HSV-2 in any of our cohort, including those with oral abnormalities. There was also no VZV DNA was detected in any of our participants. It is noted that the ophthalmic branch as opposed to branches innervating the oral cavity, is most commonly affected in trigeminal cases (Lopez-Pintor et al., 2009). EBV has dual tropism with B-cells and epithelial cells such as in the oral cavity and genitals (Durzyńska et al., 2011). Our detection rate of EBV in a healthy population is higher than previous reports, which may be reflective of the improved sensitivity of the modern assay we used. CMV associated oral lesions are a rare but serious event in immunocompromised cohorts. We demonstrated a low commensal CMV detection rate by saliva or swab in patients with no abnormalities, and no CMV was detected in OA or TI individuals. Therefore, combined with clinical suspicion, a positive saliva or swab detection by PCR could be clinically significant. Detection of positive CMV by swab or saliva should be followed by viral serum load.

An increased rate of detection between our normal cohort and therapeutically immunocompromised cohort was not observed. In fact, there was decreased detection in our therapeutically immunocompromised cohort. Particularly, our findings regarding HHV-6 were similar to those reported by Gautheret-Dejean et al. that demonstrated a rate of HHV-6 detection significantly higher in HIV-seronegative subjects than in HIV-seropositive (Gautheret-Dejean et al., 1997). Despite that mycophenolate may potentiate the presence of herpes infections (Yun et al., 2019), it also has been found to have antiviral properties, having been shown to have broad activity against influenza viruses (To et al., 2016) as well as potential antiviral activity against herpes viruses (Neyts et al., 1998). Thus, the relationship between mycophenolate and herpes viruses remains unclear.

Our study is limited in its ability to determine the active replication and virulence of detected virus. However, viral DNA PCR detection by swab is a test that is commonly requested by clinicians. In disease, microbiome factors can contribute to reduced and delayed healing despite not being specifically involved in the initial pathological process. Future studies into models of commensal herpes family viral reactivation and interaction, particularly with inflammatory epithelial disease and widely used therapeutics such as corticosteroids and steroid sparing agents, would be of interest.

In our study, the prevalence of detectable HHV DNA was not impacted by any expected risk factors, such as comorbidities, smoking, microbiome-associated dental disease, presence of ulceration or the presence of therapeutic immunosuppression. Risk factors for viral reactivation, replication and etiological role in mucosal disease appears to be independent of the extensive factors explored here.

Conclusion

This study presents the first comprehensive study of the intraoral commensal detection rates of HHV 1-6 by qPCR in saliva and swabs. Commensal detection rates of EBV are high and caution in clinical interpretation of positive detection is warranted. Commensal CMV rates are low and there was no detection of HSV-2 or VZV, thus detection by swab and saliva of these viruses is likely to be clinically relevant. Therapeutic immunocompromise with the drug MMF was not associated with increased detection rates of HHV in asymptomatic individuals and the relationship of MMF and HHV remains unclear.

Table and Figure Legends

Table Legend

Table 1. Prevalence of oral HHV in relation to gender and age

Table 2. Medical comorbidities and prevalence of oral HHV

Table 3. HHV viral exposure history, habit and oral health factors and oral HHV prevalence

Table 4. Comparison of any HHV detection rates in saliva and swabs of participants

Table 5. Oral prevalence of detected HHV in participants

Figure Legend

Figure 1. HHV is detected in the majority of saliva from 70 normal participants with majority having detectable EBV. Multiple HHV were detected in some individuals.

Figure 2. Comparison of quantified detection levels of CMV, EBV, HHV6 and HSV-1 in saliva and control swab assays. HSV-1 levels were significantly higher than EBV and CMV levels ($p < 0.05$).

Supplementary Table Legend

Table S1: Primer and probe sequences for Genetic Signatures *EasyScreen*TM Human Herpesvirus Detection Kit (HV001).

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Demographic Factor (n of 70)	Any HHV detection (n=54)	Chi-square <i>p-value</i>	HSV-1		EBV		CMV		HHV6		
			Saliva (n=2)	Swab (n=2)	Saliva (n=43)	Swab (n=27)	Saliva (n=1)	Swab (n=1)	Saliva (n=8)	Swab (n=4)	
	n (n/row total %)*										
Gender (n)											
Female (33)	21 (63.6)	*p = 0.011	1 (3.0)	1 (3.0)	18 (54.5)	9 (27.3)	0 (0.0)	0 (0.0)	3 (9.0)	2 (6.0)	
Male (37)	33 (89.1)		1 (2.7)	1 (2.7)	25 (67.6)	18 (48.7)	1 (2.7)	1 (2.7)	5 (13.5)	2 (5.4)	
Age (n)											
<50 (25)	21 (84.0)	p = 0.309	0 (0.0)	1 (4.0)	18 (72.0)	12 (48.0)	0 (0.0)	0 (0.0)	4 (16.0)	0 (0.0)	
50+ (45)	33 (73.3)		2(4.4)	1 (2.2)	25 (55.6)	15 (33.3)	1 (1.4)	1 (2.2)	4 (8.9)	4 (8.9)	

Table 1. Prevalence of oral HHV in relation to gender and age in the Normal Group (n=70)

Table 2. Medical comorbidities and prevalence of oral HHV in the Normal Group (n=70)

Medical Comorbidity (n of 70)	Any HHV detection (n=54)	Chi square p-value	HSV-1		EBV		CMV		HHV6	
			Saliva (n=2)	Swab (n=2)	Saliva (n=43)	Swab (n=27)	Saliva (1)	Swab (1)	Saliva (n=2)	Swab (n=2)
	n (n/row total %)*									
Any co-morbidities										
Yes (49)	35 (71.4)	p = 0.082	2 (4.1)	1 (2.0)	27 (55.1)	19 (38.8)	1 (2.0)	1 (2.0)	4 (8.2)	4 (8.1)
No (21)	19 (90.5)		0 (0.0)	1 (4.8)	16 (76.2)	8 (38.1)	0 (0.0)	0 (0.0)	4 (19.1)	0 (3.5)
Cardiovascular disease										
Yes (11)	7 (68.8)	p = 0.245	1 (9.1)	0 (0.0)	6 (54.6)	4 (36.4)	1 (9.1)	1 (9.1)	1 (9.1)	1 (9.1)
No (59)	47 (75.0)		1 (1.7)	2 (3.4)	37 (62.7)	23 (39.0)	0 (0.0)	0 (0.0)	7 (11.9)	3 (5.1)
Diabetes										
Yes (12)	9 (75.0)	p = 0.846	0 (0.0)	0 (0.0)	6 (50.0)	3 (25.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
No (58)	45 (77.6)		2 (3.5)	2 (3.5)	37 (63.8)	24 (41.4)	1 (1.7)	1 (1.7)	7 (12.1)	4 (6.9)
Respiratory disease										
Yes (19)	16 (84.2)	p = 0.390)	1 (5.3)	1 (5.3)	15 (79.0)	10 (52.6)	1 (5.3)	1 (5.3)	0 (0.0)	2 (10.5)
No (51)	38 (74.5)		1 (2.0)	1 (2.0)	28 (54.9)	17 (33.3)	0 (0.0)	0 (0.0)	8 (15.7)	2 (3.9)
Psychological disease										
Yes (9)	8 (88.9)	p = 0.369	0 (0.0)	0 (0.0)	6 (66.7)	3 (33.3)	0 (0.0)	0 (0.0)	2 (22.2)	1 (11.1)
No (61)	46 (75.4)		2 (3.3)	2 (3.3)	37 (60.1)	24 (39.3)	1 (1.6)	1 (1.6)	6 (9.8)	3 (4.9)

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Table 3. HHV viral exposure history, habit and oral health factors and oral HHV prevalence in the Normal Group

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Viral exposure history and habit factors (n of 70)	Any HHV detection (n=54)	Chi square p-value	HSV-1		EBV			CMV		HHV6	
			Saliva (n=2)	Swab (n=2)	Saliva (n=43)	Swab (n=27)	Saliva (n=1)	Swab (n=1)	Saliva (n=2)	Swab (n=2)	
n (n/row total%)											
History of chicken pox											
Yes (38)	28 (73.7)	p = 0.565	0 (0.0)	0 (0.0)	21 (55.3)	14 (36.8)	0 (0.0)	0 (0.0)	4 (10.5)	1 (2.6)	
No (25)	20 (80.0)		2 (8.0)	2 (8.0)	17 (68.0)	9 (36.0)	0 (0.0)	0 (0.0)	3 (12.0)	3 (12)	
Unsure (7)	6 (85.7)		0 (0.0)	0 (0.0)	5 (71.4)	4 (57.1)	1 (14.3)	1 (14.3)	1 (14.3)	0 (0.0)	
History of cold sores											
Yes (32)	24 (75.0)	p = 0.638	1(3.1)	1 (3.1)	16 (50.0)	11 (34.4)	1 (3.1)	1 (3.1)	5 (15.6)	2 (6.3)	
No (30)	24 (75.0)		1 (3.3)	1 (3.3)	21 (70.0)	13 (43.3)	0 (0.0)	0 (0.0)	3 (10.0)	2 (6.7)	
Unsure (8)	6 (75.0)		0 (0.0)	0 (0.0)	6 (75.0)	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Contact with children (age <10 yrs)											
Yes (26)	19 (73.0)	p = 0.604	0 (0.0)	1 (3.9)	16 (61.5)	8 (30.8)	1 (3.9)	1 (3.9)	2 (7.7)	3 (11.5)	
No (42)	33 (78.6)		2 (4.8)	1 (2.4)	25 (59.5)	18 (42.9)	0 (0.0)	0 (0.0)	6 (14.3)	1 (2.4)	
Unsure (2)	2 (100.0)		0 (0.0)	0 (0.0)	2(100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Antiviral ever											
Yes (8)	5 (62.5)	p = 0.325	0 (0.0)	0 (0.0)	4 (50.0)	4 (50.0)	1 (12.5)	1 (12.5)	0 (0.0)	1 (12.5)	
No (51)	40 (78.4)		1 (2.0)	1 (2.0)	32 (62.8)	19 (37.3)	0 (0.0)	0 (0.0)	5 (9.8)	3 (5.9)	
Unsure (11)	9 (81.3)		1 (9.0)	1 (9.1)	7 (63.6)	4 (36.4)	0 (0.0)	0 (0.0)	3 (27.3)	0 (0.0)	
Smoking status											
Current (19)	17 (89.5)	p = 0.318	0 (0.0)	0 (0.0)	13 (68.4)	8 (42.1)	0 (0.0)	0 (0.0)	6 (31.6)	1 (5.3)	
Past (23)	17 (73.9)		2 (8.7)	1 (4.4)	12 (52.1)	8 (34.5)	1 (4.4)	1 (4.4)	1 (4.4)	1 (4.4)	
Never (28)	20 (71.4)		0 (0.0)	1 (3.6)	18 (64.3)	11 (39.3)	0 (0.0)	0 (0.0)	1 (3.6)	2 (7.1)	
Alcohol ever											
Ever (50)	38 (76.0)	p = 0.719	2(4.0)	1 (2.0)	29 (58.0)	21 (42.0)	1 (2.0)	1 (2.0)	6 (12.0)	4 (8.0)	
Never (20)	16 (80.0)		0 (0.0)	1 (5.0)	14 (70.0)	6 (30.0)	0 (0.0)	0 (0.0)	2 (10.0)	0 (0.0)	
Current Mouthwash use											
Yes (23)	18 (78.3)	p = 0.876	1 (4.4)	1 (4.4)	15 (65.2)	10 (43.5)	0 (0.0)	0 (0.0)	2 (8.7)	1 (4.4)	
No (47)	36 (76.6)		1 (2.1)	1 (2.1)	28 (59.6)	17 (36.2)	1 (2.1)	1 (2.1)	6 (12.7)	3 (6.4)	

Sweet foods										
Daily (33)	24 (72.7)	p = 0.205	1 (3.0)	0 (0.0)	17 (51.5)	11 (33.3)	0 (0.0)	0 (0.0)	6 (18.2)	1 (3.0)
Not daily (35)	29 (82.9)		1 (2.9)	2 (5.7)	25 (71.4)	16 (45.7)	1 (2.9)	1 (2.9)	2 (5.7)	3 (8.6)
Unknown (2)	1 (50.0)		0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dentate (20+ Teeth)										
20+ teeth (47)	36 (76.6)	p = 0.876	1 (2.1)	2 (4.3)	31 (66.0)	19 (40.4)	0 (0.0)	0 (0.0)	3 (6.4)	3 (6.4)
<20 teeth (16)	12 (75.0)		1 (6.3)	0 (0.0)	9 (56.3)	7 (43.8)	1 (6.3)	1 (6.3)	2(12.5)	1 (6.3)
Unknown (7)	6 (85.7)		0 (0.0)	0 (0.0)	3 (42.9)	1 (14.3)	0 (0.0)	0 (0.0)	3 (42.9)	0 (0.0)
Removable Prosthesis										
Yes (23)	17 (73.9)	p = 0.653	0 (0.0)	0 (0.0)	14 (60.9)	8 (34.8)	1 (4.4)	1 (4.5)	2 (8.7)	2 (8.7)
No (34)	28 (82.4)		2 (5.9)	2 (5.9)	24 (70.6)	16 (47.1)	0 (0.00)	0 (0.0)	3 (8.8)	2 (5.9)
Unknown (13)	9 (69.2)		0 (0.0)	0 (0.0)	5 (38.5)	3 (23.1)	0 (0.0)	0 (0.0)	3 (23.1)	0 (0.0)
Caries risk										
High or Moderate (29)	23 (81.8)	p = 0.716	2 (6.9)	2 (6.9)	20 (69.0)	10 (34.5)	0 (0.0)	0 (0.0)	2 (6.9)	4 (13.8)
Low (19)	13 (79.3)		0 (0.0)	0 (0.0)	10 (52.6)	10 (52.6)	0 (0.0)	0 (0.0)	2 (10.5)	0 (0.0)
Unknown (22)	18 (68.4)		0 (0.0)	0 (0.0)	13 (59.0)	7 (31.8)	1 (4.6)	1 (4.6)	4 (18.2)	0 (0.0)
Active caries										
Yes (27)	12 (77.8)	p = 0.920	1 (3.7)	1 (3.7)	18 (66.7)	8 (29.6)	0 (0.0)	0 (0.0)	2 (7.4)	4 (14.8)
No (19)	14 (73.7)		1 (5.3)	1 (5.3)	13 (68.4)	11 (57.9)	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)
Unknown (24)	19 (79.2)		0 (0.0)	0 (0.0)	12 (50.0)	8 (33.)	1 (4.1)	1 (4.2)	5 (20.8)	0 (0.0)
Gingivitis										
Yes (27)	19 (70.4)	p = 0.285	2 (7.4)	1 (3.7)	16 (59.3)	11 (40.7)	0 (0.0)	0 (0.0)	1 (3.7)	3 (11.1)
No (11)	8 (72.7)		0 (0.0)	0 (0.0)	7 (63.6)	5 (45.5)	0 (0.0)	0 (0.0)	1 (9.0)	0 (0.0)
Unknown (32)	27 (84.4)		0 (0.0)	1 (3.1)	20 (62.5)	11 (34.4)	1 (3.1)	1 (3.1)	6 (18.8)	1 (3.1)
Periodontal disease										
Active (23)	18 (78.3)	p = 0.876	1 (4.3)	2 (8.7)	15 (65.2)	9 (39.1)	0 (0.0)	0 (0.0)	2 (8.7)	2 (8.7)
No or Past (stable) (22)	15 (68.2)		1 (4.6)	0 (0.0)	14 (63.6)	10 (45.5)	0 (0.0)	0 (0.0)	1 (4.6)	0 (0.0)
Past (stable) (25)	21 (84.0)		0 (0.0)	0 (0.0)	14 (56.0)	8 (32.0)	1 (4.0)	1 (4.0)	5 (20.0)	2 (8.0)

Table 4. Comparison of any HHV detection rates in saliva and swabs of participants

	Normal	TI	OA	Normal vs TI	Normal vs OA
Detection of any HHV	n = 70	n= 23	n = 22		
	n(%)		Chi-square p-value		
Saliva	49 (70)	10 (43)	15(68)	*0.023	0.872
Swab	32 (46)	5 (22)	9 (41)	*0.042	vs ulcer swab 0.692
Both	28 (40)	5 (22)	9 (41)	0.111	0.871

Abbreviations: HHV (Human herpes virus), OA (Oral Abnormality), TI (therapeutically immunocompromised)

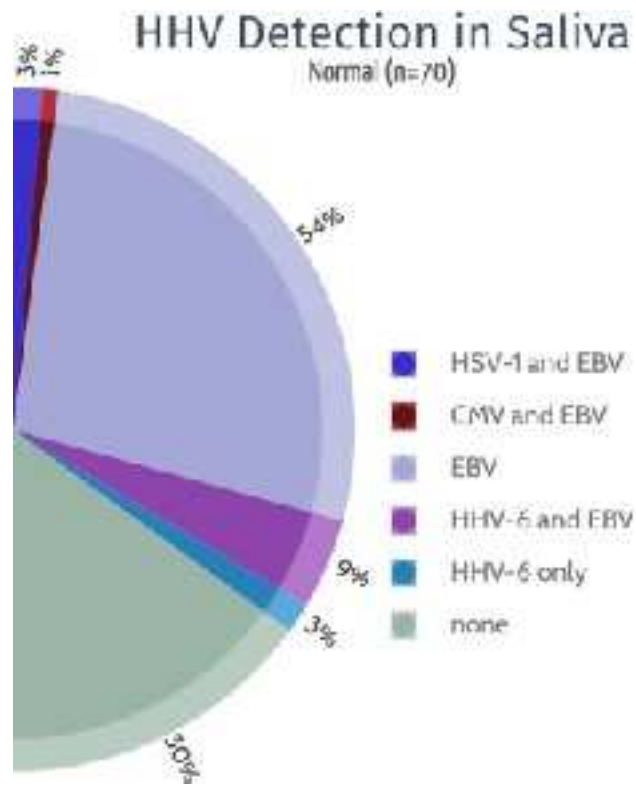
Table 5. Oral prevalence of detected HHV in participants

HHV of positive sample collection		Total Participants (n=115)		
		OA (22)	Normal (70)	TI (23)
		n (n/22 %)	n (n/70 %)	n (n/23 %)
HSV-1	Saliva	2 (9.1)	2 (2.9)	1 (4.3)
	Control swab *	2 (9.1)	2 (2.9)	1 (4.3)
	Ulcer swab ‡	2 (9.1)	n/a	n/a
EBV	Saliva	15 (68.2)	42 (60.0)	10 (43.5)
	Control swab	9 (40.9)	27 (38.6)	5 (21.7)
	Ulcer swab	8 (36.4)	n/a	n/a
CMV	Saliva	0 (0.0)	1 (1.4)	0 (0.0)
	Control swab	0 (0.0)	1 (1.4)	0 (0.0)
	Ulcer swab	0 (0.0)	n/a	n/a
HHV6	Saliva	0 (0.0)	8 (11.4)	0 (0.0)
	Control swab	0 (0.0)	4 (5.7)	0 (0.0)
	Ulcer swab	1 (4.5)	n/a	n/a

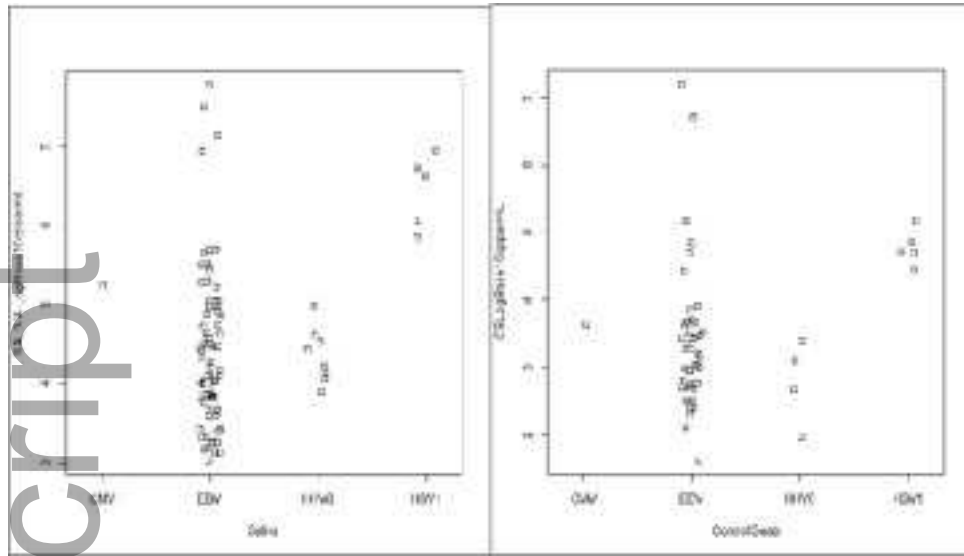
Abbreviations: HHV (Human herpes virus), OA (Oral Abnormality), TI (therapeutically immunocompromised)

* Control swab was taken from intact mucosa.

‡ Ulcer swab was taken from the site of ulcer or abnormality.



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odi_13523_f2.png