

Pilot study investigating the epidemiology of oral Human Papilloma Viral infection in young adults.

Head and neck squamous cell carcinomas (HNSCC) are the sixth most common type of cancer worldwide, with around 9,000 HNSCC diagnosed within the UK/annum¹. Though around 60-75% of HSNCC are linked to smoking and/or alcohol there is a subset of HNSCC due to oral infection with the Human Papilloma Virus (HPV)².

HPV is the causative agent of cervical cancer³ and oral HPV infection has shown the same risk of developing an oral cancer as a genital HPV associated cancer⁴. Patients presenting at Head and Neck clinics with a HPV-associated HNSCC, particularly in white males under the age of 40, are steadily increasing⁵ and this increase is not fully understood but has been linked to changing life style choices, such as having more oral sex at an earlier age, thereby increasing oral HPV infection⁶.

As yet the frequency of oral HPV infection in healthy individuals is not fully determined², partly due to insufficient reproducible oral HPV screening methodologies and the majority of the research being focused on HPV HNSCC. Furthermore the impact of the HPV vaccination programme on the rates of oral HPV infection has not been investigated. But as females in the UK, who were vaccinated from 2009 onwards become sexually active, these epidemiological studies can now be conducted.

Therefore, this pilot study aimed to implement a reproducible oral screening method to investigate the epidemiology of oral HPV infection in 18-25 years old, who are a high risk group for genital HPV infection, to investigate frequency of infection and to see if any life style choice(s), including HPV vaccination, influenced risk of HPV infection.

Students from a UK university, were asked to complete a questionnaire focused on their life styles. These included drinking and smoking habits, relationship status, sexual orientation, if they performed open mouth kissing and number of sexual partners. Sexually active students also supplied details on their sexual activities (oral, vaginal and anal sex, foreplay and masturbation), frequency of condom use, participation in casual sex, when they last changed their sexual partner and if ever diagnosed with a STI(s). Females were also asked if/when they had received the HPV vaccination. Written consent was obtained before enrolment onto the study.

Participant's also supplied a mouth swab of buccal cells, which was linked to the participant's questionnaire by a unique identification number. Mucosal DNA was extracted from the buccal cells (DNeasy (Qiagen) extraction kit) and DNA viability determined by PCR (polymerase chain reaction) to detect cytochrome B and β -globulin, mitochondrial and chromosomal DNA markers respectively. Viable DNA was then screened by PCR for the detection of the HPV L1 protein, which is highly conserved between different HPV strains. A HPV positive cervical cell line and HPV negative cervical cell line were used as controls (kind gifts by Dr S Roberts, University of Birmingham). Any participants that reproducibly showed HPV L1 detection three times were deemed to have an oral HPV infection.

A total of 124 participants were involved in the study, with 92% being 18-25 years old and the gender divide was 67 males to 57 females, with 86 % White British (data not shown). Most of the cohort drank alcohol at least once a week and around a fifth smoked (Table One). 83% of participants were sexually active and most (91% men, 81% women) were heterosexual (data not shown). The most common number of sexual partners were less than 5, with men overall having more sexual partners than women (Table One). The distribution of sexual activities were similar across both genders (Table One), apart from masturbation in

1 which 80% of men masturbated compared to 38% women. Condom use was similar
2 between genders, as was the number of men or women that had one-night stands (Table
3 One). 70% of females (40 out of 57) had received the HPV vaccination.

4 Laboratory screening of the mouth samples identified that 4% of the total cohort had a
5 detectable oral HPV infection and all infected individuals, 2 females and 3 males, were aged
6 between 18 and 21. This pilot data supports previous oral HPV epidemiological studies within
7 the United States, who demonstrated oral infectivity rates of between 2.5% and 5.4% in 18-
8 24 year olds^{5,6,7}.

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11 When comparing what life style choices the HPV infected individuals were undertaking in
12 relation to the rest of the cohort (Table One), the majority of infected individuals smoked with
13 2 out of 3 oral HPV infected males smoking and both infected females. This gave an oral
14 HPV prevalence of infection of 16% of men and 18% of women who smoked, who also had
15 an oral HPV infection (Table One). This study's finding that the majority (80%) of orally HPV
16 infected individuals also smoked, correlates with research conducted in the US who found
17 that smoking was a risk factor for contracting an oral HPV infection⁵. Why smoking may
18 increase the risk of contracting oral HPV is not yet determined, but could be due to the
19 damaging effects smoking has on the mouth epithelial layer thereby allowing HPV entry
20 and/or inducing immunosuppression within smokers allowing a more abundant HPV infection
21 ⁹. Therefore this pilot study highlights that further investigation needs to be undertaken to
22 examine the impact of smoking on contracting oral HPV, and also how this correlates to
23 development of HPV HNSCC.
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27 Examination of how the sexual behaviour of the cohort impacted on oral HPV infection
28 showed that all infected individuals were; sexually active, not in a monogamous relationship
29 (data not shown) and had undertaken more risky sexual behaviour such as; one-night
30 stands, having more than 5 sexual partners and/or not always using a condom (Table One).
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33 A noteworthy finding of this pilot study is though participants are using condoms, they are
34 not being used that frequently (Table One) and only being used for penetrative sex (data not
35 shown) so thereby not offering oral HPV protection. This inconsistent use of condoms when
36 engaging in sexual activities correlates with other research from this research group on oral
37 STI's and how to prevent them (Lees *et al.*, unpublished data) and highlights the requirement
38 for better education about the risk of oral STIs, particularly in relation to HPV and the
39 potential development of HSNCC.
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42 The observation within this study that undertaking more high risk sexual activities appears to
43 lead to increased prevalence of oral HPV infection (Table One) corresponds with previous
44 genital HPV epidemiology studies. It also supports the observations of oral epidemiological
45 HPV studies within the US that sexual behaviour appears to spread oral HPV^{5, 7, 8} with a
46 correlation between the number of sexual partners and increased frequency of HPV
47 infection⁶.
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50 An unexpected finding of this study was that one of the women infected with oral HPV had
51 received vaccination against the high risk strains HPV 16 and HPV 18, as part of the UK
52 HPV vaccination programme (Table One), the other infected woman had not been
53 vaccinated. It is possible that the vaccinated woman may have contracted HPV before she
54 was vaccinated, as she was part of the UK catch up programme which vaccinated girls
55 between 16-18 years old and may have already been sexually active, or she may have a
56 strain that is not prevented by HPV vaccination. It should be noted that 70% of the female
57 cohort said they were vaccinated against HPV and only one woman of this cohort was
58 positive for HPV. This resulted in only 2.5% of the cohort of HPV vaccinated females having
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oral HPV (Table One), compared to 5.8% oral HPV prevalence within non-vaccinated females (data not shown).

This pilot study is one of the first studies conducted within the UK establishing a reproducible oral HPV screening method, to look to quantify the rates of oral HPV infection. Though the number of infected individuals are small the epidemiological findings of oral HPV infection within this UK cohort reflect previous studies conducted within the US, indicating that oral HPV is mainly being spread by sexual practices but smoking can also influence oral HPV infection. This pilot study indicates that more men than women appear to be contracting an oral HPV infection, again confirming previous US studies, and correlates with the fact that a higher frequency of men are developing a HPV HNSCC ². However why more men are contracting oral HPV infections is something yet to be determined and will require further research. Furthermore, the ability of the HPV vaccination to prevent oral HPV infection is a key public health agenda that needs future research, particular in relation to the proposed impact of herd immunity upon males within the UK, who do not receive the HPV vaccination.

Author statement:

This study has been approved by the University of Derby ethical committee. All participants in the study supplied written consent before completing the questionnaire and providing an oral buccal cell sample.

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1: Doobaree I, Landis S, Linklater K, El-Hariry I, Moller H, Tyczynski J. Head and neck cancer in South East England between 1995–1999 and 2000–2004: an estimation of incidence and distribution by site, stage and histological type. *Oral oncology* 2009; **45**(9): 809-814.

2: Dufour X, Beby-Defaux A, Agius G, St Guily JL. HPV and head and neck cancer. *European annals of otorhinolaryngology, head and neck diseases* 2012; **129**(1): 26-31.

3: Termine N, Giovannelli L, Matranga D, Caleca MP, Bellavia C, Perino A, Campisi G. Oral human papillomavirus infection in women with cervical HPV infection: new data from an Italian cohort and a meta-analysis of the literature. *Oral oncology* 2011; **47**(4): 244-250.

4: Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA. The biology and life-cycle of human papillomaviruses. *Vaccine* 2012; **30**: F55-F70.

5: Gillison ML, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L, Chaturvedi, AK Prevalence of oral HPV infection in the United States, 2009-2010. *Jama* 2012; **307**(7): 693-703.

6: D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviours associated with prevalent oral human papillomavirus infection. *Journal of Infectious Diseases*, 2009; **199**(9):1263-1269.

7: Pickard RK, Xiao W, Broutian TR, He X, Gillison ML. The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18–30 years. *Sexually transmitted diseases* 2012; **39**(7): 559-566.

8: Edelstein ZR, Schwartz SM, Hawes S, Hughes JP, Feng Q, Stern ME, O'Reilly S, Shu-Kuang L, Long F, Koutsky LA. Rates and Determinants of Oral Human Papillomavirus (HPV) Infection in Young Men. *Sexually transmitted diseases* 2012; **39**(11): 860.

9: Bagaitkar J, Demuth DR, Scott DA. Tobacco use increases susceptibility to bacterial infection. *Tob Induc Dis* 2008; **4**(1):12.

Life style choice	Total number of males (number of HPV infected males)	Oral HPV prevalence in males	Total number of females (number of HPV infected females)	Oral HPV prevalence in females
Consumes Alcohol	62 (3)	4.8 %	54 (2)	3.7%
Smokes	12 (2)	16.6%	11 (2)	18.2%
Open mouth kisses	58 (3)	5.1%	52 (2)	3.8%
<5 sexual partners	25 (1)	4%	25 (0)	0%
5-10 sexual partners	9 (0)	0%	15 (1)	6.7%
11-20 sexual partners	15 (2)	13.3%	3 (1)	33.3%
21-40 sexual partners	5 (0)	0%	5 (0)	0%
Never had one-night stand	20 (0)	0%	22 (0)	0%
Infrequent one-night stands	13 (2)	15.3%	11 (1)	9%
Frequent one-night stands	8 (1)	12.5%	7 (0)	0%
Previous one-night stands (over 6 months ago)	16 (0)	0%	8 (1)	12.5%
Always use condom	11 (0)	0%	9 (0)	0%
Mostly use condom	14 (2)	14.3%	10 (1)	10%
Sometimes use condom	10 (1)	10%	8 (1)	12.5%
Never use condom	21 (0)	0%	20 (0)	0%
Has vaginal sex	56 (3)	5.3%	47 (2)	4.2%
Has oral sex	54 (3)	5.5%	46 (2)	4.3%
Has anal sex	15 (0)	0%	12 (0)	0%
Performs foreplay	54 (3)	5.5%	40 (2)	5%
Masturbates	54 (3)	5.5%	22 (1)	4.5%
Diagnosed with STI	6 (0)	0%	1 (0)	0%
Received HPV vaccination	N/A	N/A	40 (1)	2.5%

Table One: The number of study participants undertaking identified risks for contracting genital and oral HPV infection. The data is divided into males and females, and show the number of participants for each gender that answered yes to undertaking that particular life style choice when completing the questionnaire (column 2 and 4). In brackets is the number of HPV infected individuals, from the same cohort, that undertook the same life style choice. In columns 3 and 5 the percentage prevalence of oral HPV infection within the male or female cohort is calculated. This percentage prevalence of oral HPV infection was derived by dividing the number of infected individuals, males or females, by the total number of respective males or females in the study who performed the same life style choice. N =124, 67 males: 57 females

Highlights of: Pilot study investigating the epidemiology of oral Human Papilloma Viral infection in young adults.

- UK based study investigating the rates of oral HPV infection in young adults
- An oral HPV infectivity rate of 4% was found within the cohort
- Particular life style choices, such as sexual behaviour and smoking, appeared to influence the rates of HPV infection