HPV types in oral and oropharyngeal mucosa of patients at Dr George Mukhari Academic Hospital

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Introduction

* Large knowledge gap in Sub-Saharan Africa concerning HPV-associated head and neck Squamous cell carcinoma (SCC).

* Data from Africa is sparse

Blumberg et al., 2015; Warnakulasuria, 2009; Vogt et al., 2013

Introduction

* Very view studies investigated prevalence of HPV-types in specimens of benign and malignant oral lesions in South Africa
* No reliable data describing spectrum of HPV types infecting oral and oropharyngeal mucosae of South African population groups.
* Data on oral/oropharyngeal HPV prevalence in healthy and in HIV-infected children in South Africa is lacking.

Introduction

* Creating a meta-analysis for data published from Africa is challenging:
  * Population groups
  * Specimen types
  * Specimen collection methods
  * Non-standardized analysis methods

Introduction

* Based on current evidence:
  * Oropharyngeal SCC has a strong association with HR-HPV types
  * Oral SCC induced by HPV are rare, and of these, only a limited number are transcriptionally active.
  * Oral and/or oropharyngeal SCC may be associated with a different type of HPV than 16 or 18.

HPV-transmission

* Oral/oropharyngeal HPV infection can be acquired through:
  * Oral-genital contact
  * Mouth-mouth contact
  * Autoinoculation
  * Mother-child (infants)

  * *Fakhry et al., 2006; Coutlee et al., 1997; Fu et al., 2015*

HPV-transmission

* Immunosuppressed people are at significantly greater risk of
  * Acquiring oral HPV infection
  * Experiencing a more aggressive course of the infection
* Immune-responses are low as virus is shielded intracellularly
* E5-ORF product promotes immune evasion
* Active oral HPV infection is transmissible in both Subclinical and Clinical infective stages.

HPV transmission

* Autoinocculation occurs between anatomical sites in mid-adult women.
* Lifetime number of open-mouth kissing partners associated with oral HPV detection.
* Lifetime number of male vaginal sex partners associated with oral HPV detection.
* Oral and fingernail HPV detection had some association with vaginal HPV detection, but perhaps not the main source.

  * *Fu et al., 2015;*

HPV-transmission

* High numbers of vaginal-sex partners, high number of oral sex partners, smoking, alcohol consumption were all shown to be independently significantly associated with positive oral-HPV testing in young, unvaccinated adults.

  * *Dalla Torre et al., 2015*

HPV-transmission

* Smoking has been shown to reduce oncogenic HPV clearance in the mouth, and

* Data on the effects of antiretroviral therapy on HPV clearance is still inconclusive, however, an association exists between development of HPV-associated oral lesions and period of HAART use.

- Gaester et al., 2014; Giuliano et al., 2012; Anaya-Saavedra et al., 2013

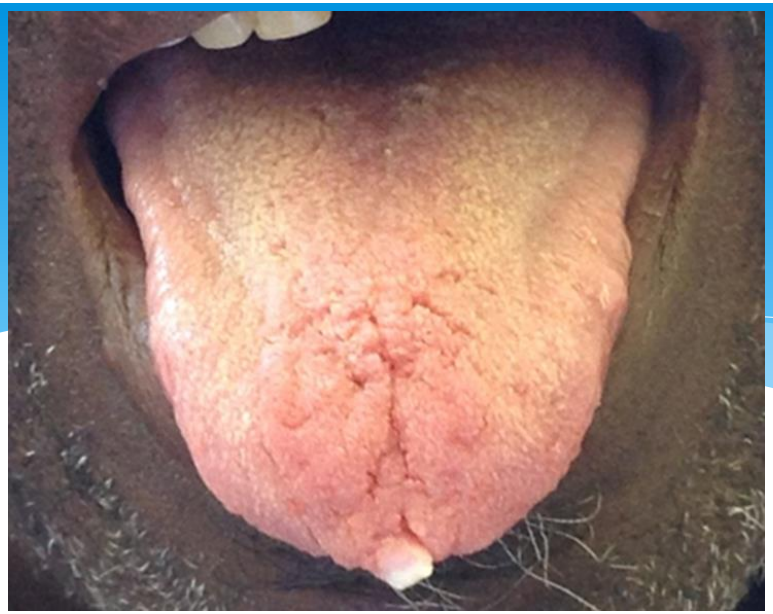
Oral HPV-associated lesions

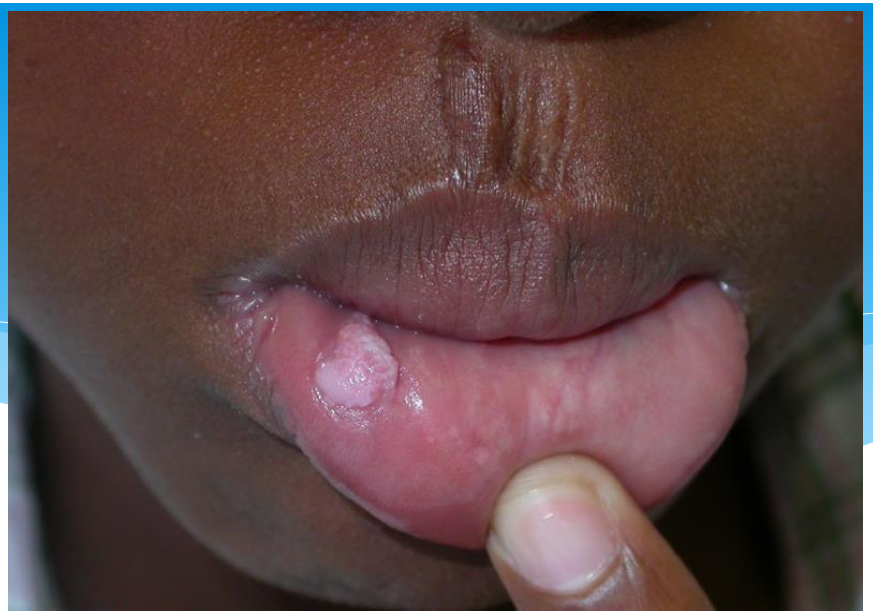
* Manifestations of oral HPV infection:
  * Transitory (Subclinical) up to 50% of cases
  * Spontaneous regression in up to 30% of cases
  * Persistent infection
* Clinical manifestations vary with the anatomic site affected and with the genotype of HPV

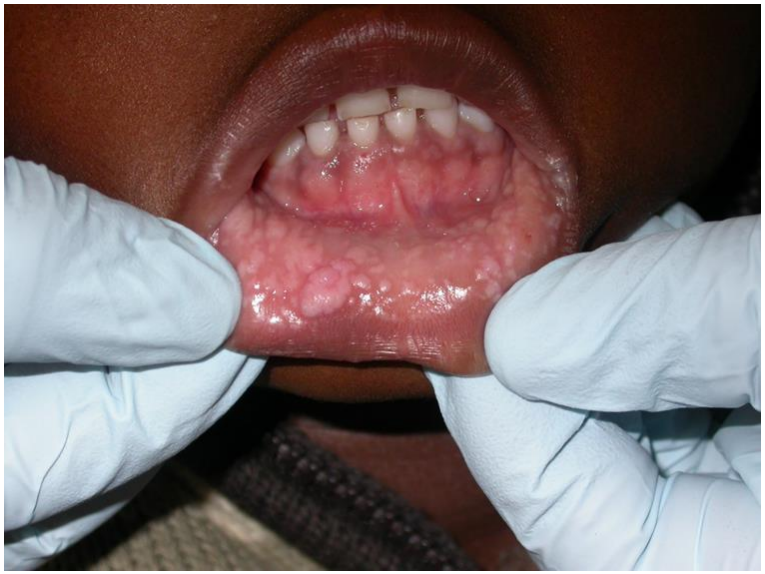
HPV Types and association to lesions:

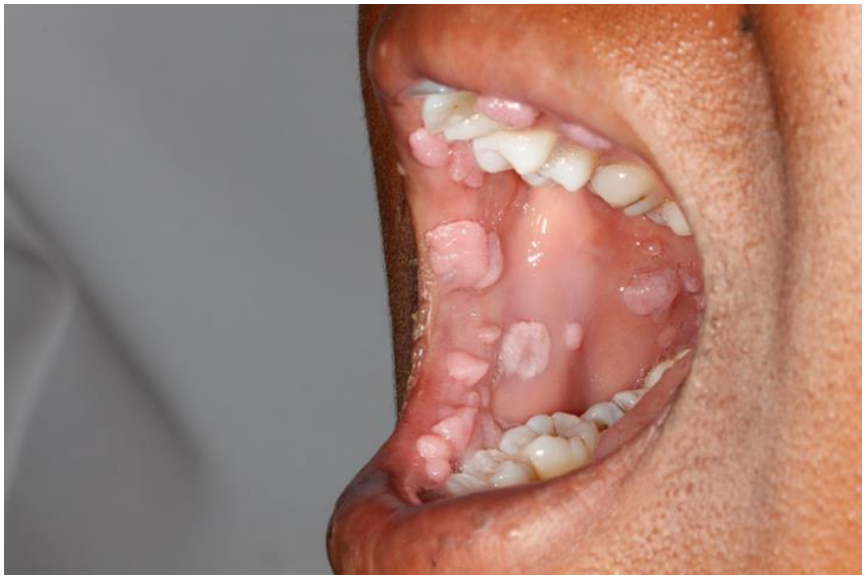
Benign oral lesions

* *Oral squamous cell papilloma* HPV types 2, 4, 6, 11 and 40
* *Veruca vulgaris* HPV types 1, 2, 4, 7 and 57
* *Condyloma acuminatum* HPV types 2, 6, 11 (and less frequently HPV types 16, 18, 31, 33 and 35
* *Focal epithelial hyperplasia* (Heck disease) HPV types 13 and 32









Courtesy Drs R Chandran and A Masilana, Dept Periodontology and Oral Medicine

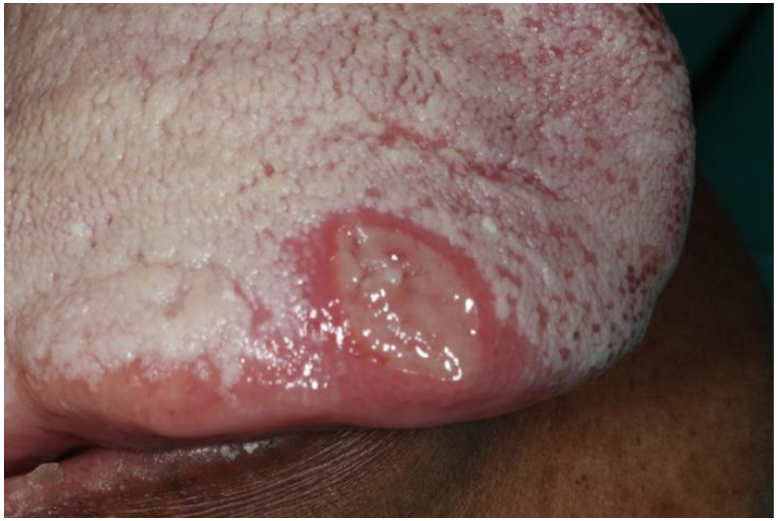
HPV Types and association to lesions:

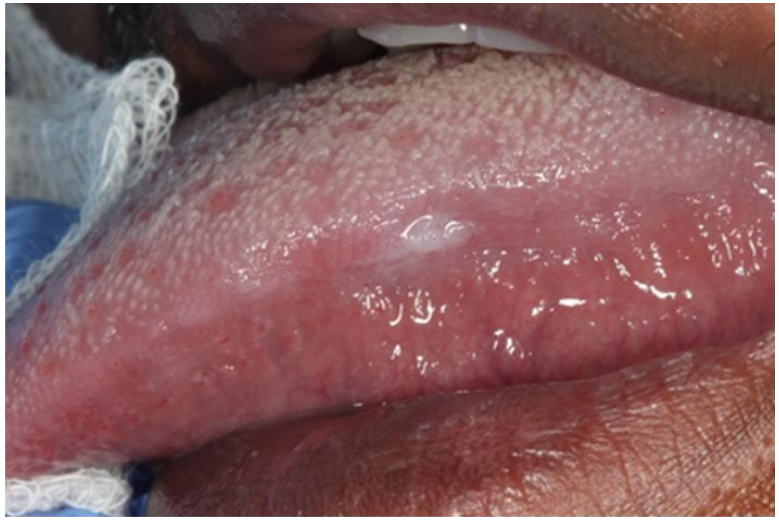
Potentially malignant oral lesions:

* Leukoplakia HPV types 6, 11, 16 and 18
* Erythroplakia HPV types 6, 11, 18, 31 and 33

Oral and oropharyngeal squamous cell carcinoma HPV types 16 and 18 mostly

Recurrent respiratory papillomatosis HPV types 6 and 11





Courtesy Drs R Chandran and A Masilana, Dept Periodontology and Oral Medicine

Oral and Oropharyngeal SCC

* Head and neck cancer incidence is decreasing worldwide, but oropharyngeal SCC appears to be increasing – *Marur et al., 2010; Blumberg et al., 2015*
* Only one study from South Africa (Africa) supports this trend – *Ayo-Yusuf et al., 2013*
* These cases trending mainly in younger, non-smokers that may not have any other risk factors – *Gillison et al., 2000; Blumberg et al., 2015*

Oropharyngeal HPV-associated cancer

* Associated with HPV 16, 18, 31 and 33 in studies from industrialised countries
* HPV-oropharyngeal SCC described as a distinct entity in 2000 – *Gillison et al., 2000*
* HR-HPV is on a trend to become the dominant aetiologic factor for OP-SCC in most Western countries – *Chaturvedi et al., 2011*
* Oral HPV prevalence is higher in patients with tonsillar and base of tongue SCC – *Nordfors et al., 2014*

* Profile of head and neck HPV infection is yet to be determined for Africa – *Labouba et al., 2015*

Country Data

Gabon - *Labouba et al., 2015*:

* Head and neck SCC is increasing in Gabon.
  * But, tobacco and alcohol consumption epidemiology is poorly documented and,
  * No cause/effect link has been studied between HPV and head and neck cancer in Middle Africa

Senegal - *Ndiaye et al., 2013*:

* 117 H&N cases including 41 oral and 7 oropharyngeal
* Out of these, HPV-35 detected in one gingival SCC
* None showed $p16^{INK4a}$ positivity

Country Data

* Mozambique - *Blumberg et al., 2015*:
  * HPV-16 presence in oral and oropharyngeal SCC
    * All oropharyngeal specimens (n=22) were p16 negative
    * Two oral-tongue (n=29) specimens were p16 positive
    * All specimens negative for E6/E7 PCR

* South Africa - *Paquette et al., 2013*:
  * 72% of oropharyngeal SCC were HPV-16 positive
  * 11.8% of oropharyngeal SCC positive for HPV-31 alone

Literature review

* Van Rensburg et al., 1995 - S Afr Med J
* Van Rensburg et al., 1996 - Anticancer Res
* Boy et al., 2006 - J Oral Pathol Med
* Richter et al., 2008 - J Oral Pathol Med
* Marais et al., 2008 - J Med Virol
* Paquette et al., 2013 - Head & Neck Pathol
* Vogt et al., 2013 - Front Oncol
* Mbulawa et al., 2014 - J Infect Dis
* Davidson et al., 2014 - S Afr Med J

Literature review

* Van Rensburg et al., 1995
  * 66 cases of Oral Squamous Cell Carcinoma
  * Targeted HPV 6, 11, 16 and 18

* Van Rensburg et al., 1996
  * 146 cases of Oral Squamous Cell Carcinoma
  * Targeted HPV 6, 11, 16 and 18

It could be that oral or oropharyngeal Squamous Cell Carcinoma is associated with a different type of HPV.

Literature review

* Boy et al., 2007
  * 59 patients with Oral Squamous Cell Carcinoma
  * Only investigated HPV 16 and 18
  * 7 were RT-PCR positive for HPV 16, none for HPV 18
  * All negative on ISH for both HPV 16 and HPV 18
- Results were contrasting to a meta-analysis by Miller and Johnstone (2001)

By definition, the sites investigated by these three studies included the oropharynx.

Literature review

* Richter et al., 2008
  * 30 women, HIV positive, prior to HAART
  * Oral sites *scraped*: buccal mucosa and lateral borders of the tongue

  * However: HPV types investigated –

HR: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82

P-HR: 26, 53, 66

LR: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108

Literature review

* Richter et al., 2008 continued…
  * Oral HPV types identified:

    HPV – 45, 59, 62, 72, 81, 84

  * 2/30 had multiple oral HPV types
  * 6/30 had concurrent oral/genital HPV types, but only 3 corresponded…

* Marais et al., 2008 identified oral infection with HPV in 45.5% of HIV + and 25% of HIV- women with confirmed cervical disease.
  * Most commonly identified HPV - 33, 11 and 72

Literature review

* Paquette et al., 2013
  * HPV 16, 18, 31, 33, 35, 39, 45, 52, 58, 59 and 68
  * 37/55 Oropharyngeal Squamous Cell Carcinoma FFPE tissue specimens were HPV positive
    * HPV 16 AND 31 – 32%
    * HPV 16 – 32%
    * HPV 31 – 24%
    * HPV 16 and 18 – 8%
    * HPV 18 – 4%
  * Contrast to Boy et al., (2007) and Blumberg et al., 2015

Literature review

* Vogt et al., 2013
  * 34 Couples' oral and genital HPV prevalence
  * Investigated 37 types including oncogenic types:

    16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82
  * Described as 'oral' but because a gargle and rinse technique was used, the wash is representative of oral and oropharyngeal
  * 3 couples had concordant oral-genital HPV infection which supports the oral-sex transmission route.
  * Detected: HPV – 62, 72, 35, 52, 33, 58, 16, 74, 66
  * (4% oncogenic types)

Literature review

* Mbulawa et al., 2014
  * 221 Heterosexual couples – brush collection buccal
  * 6.8% of women and 13.5% of men – oral HPV positive
  * 13.5% of all participants had multiple oral HPV types
  * Most commonly identified types:

    HPV – 72, 55, 62, 61
  * Other HPV types in the mouth:

    HPV – 52, 84, 81, 11, 31, 69, 51, 81, 89, 53, 59, 42, 35, 33, 58, 16

Literature review

* Davidson et al., 2014
  * 125 Male factory workers
  * HPV types investigated:

HR: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82

P-HR: 26, 53, 66

LR: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108

  * 7 (5.6%) tested positive for oral HPV infection with one having HPV 71 and 72 co-infection
  * Two participants had a HR-HPV-type each (16 and 68)

Practice of Oral Sex

* When interpreting data on oral sex practice, the definition of Oral Sex needs to be deliberately constructed.
* US studies show rates of oral sex practice between 20-78% of young adults.
* In China 6.9% was recently reported.
* Increased prevalence of oropharyngeal cancer is thought to be related to increased oral sex by males on females.

Ma et al., 2013; Gillison et al., 2012; Ompad et al., 2006

Practice of Oral Sex

* Contrary to other reports *Young et al., 2015* state that less than 1% of persons that report to engage in oral sex practice have positive oral HPV tests and that HPV cannot be spread through kissing.

* Yet, *Fu et al., 2015* demonstrates a significant association between open-mouth kissing and HPV-positive tests.

Practice of Oral Sex

* Number of oral sex and vaginal sex partners is a risk factor for oropharyngeal SCC – *D'Souza et al., 2007*
* Oral sex practice significantly correlates to positive oral-HPV testing (Brush specimens) – *Dalla Torre et al., 2015*
* Data on oral sex practice and related behaviour in South Africa is sparse – *Blumberg et al., 2015*

Practice of Oral Sex

Reports of Oral sex practice in South African oral/oropharyngeal HPV studies

Study	N=	Women	Men	Population
Vogt et al., 2013	68	82%	84%	Heterosexual couples
Davidson et al., 2014	125	N/A	40.80%	Male factory workers
Mbulawa et al., 2014	442	8.70%	6.20%	Heterosexual couples
Wood et al., 2014*	514	16.2%	32%	Dental clinic attendees

*under consideration for publication

Specimen collection

Specimen collection to determine HPV presence

* Oral rinse suitable for screening studies – *Lawton et al., 1992*
* Oral rinse provides best representation of HPV infection in the mouth. Superior to brush – *Steinau et al., 2012*
* Oral Rinse samples are suitable for HPV detection and screening – *Dang et al., 2015*

Specimen collection to determine HPV presence

* Biopsy specimen more accurate than brush specimen for Oral SCC – *Termine et al., 2015*

* No difference between brush and biopsy, BUT brush had larger number of inadequate samples – *Marques et al., 2015*

Study components

AIMS

* To describe the sensitivity and specificity of the APTIMA® HPV assay on the Panther platform and the LUMINEX® genotyping systems for use in the detection of HPV in oral wash, brush and FFPE tissue specimens.

* To determine the prevalence of, and to genotype HPV in the oral and oropharyngeal mucosae of the HIV-seropositive participants and in the dental hospital attendees of all ages.

AIMS

* To compare and to report on relationships between oropharyngeal HPV-infection, benign and malignant oral lesions and age, gender, HIV-serostatus, smoking and self-reported sexual practices in all participant groups.

* To characterize and describe, as a case-control study, the HPV-prevalence in benign and malignant oral and oropharyngeal FFPE tissue specimens.

Study population*

* Prevalence of oral and oropharyngeal human papillomavirus types in patients attending:
  * The Sefako Makgatho Health Sciences University Oral Health Centre, and
  * The Dr George Mukhari Academic tertiary hospital HIV clinic, South Africa

* The population groups:
  * General population attending a dental clinic
  * HIV seropositive individuals on HAART
  * Children and Adults

*Min 120 HPV positive

Questionnaire and Wash

* An self-administered questionnaire will be completed prior to oropharyngeal wash specimen collection.

* Participants will be asked to rinse for 15 seconds and then to gargle for 15 seconds with 7ml of a commercially available, saline mouthwash, and then spit the contents into a Thinprep® vial containing Preservcyt® solution. The specimens will be stored at 4°C until transportation to the Virology laboratory.

Methodology

* Brush sample collection will be done in the left soft palate, the right tonsillar fauces and the left floor of the mouth of each participant using the Rovers® Orcellex® brush system.

* The brush will then be vigorously agitated in a Thinprep® vial containing Preservcyt® as a liquid-based preparation and stored at room temperature until transportation to the laboratory.

Methodology

* Tonsillar tissue removed during routine tonsillectomy will serve as control for the study of the FFPE tissue specimens.

* Benign and malignant HPV-associated oral and oropharyngeal FFPE tissue specimens will be prepared for DNA extraction, amplification, and genotyping.

Methodology

* HPV detection by APTIMA® system using oral wash samples - Although the APTIMA® assay cannot perform genotyping, the presence of HR HPV mRNA of proteins E6/E7 as expressed during viral replication is detected in this fully automated assay.

Methodology

* DNA will be extracted using an automated Biomérieux system from the oral and oropharyngeal wash, brush and FFPE tissue specimens however, tissue specimens will undergo pre-lysis step before being placed on the automated Biomérieux system for complete extraction. Specimens extracted with this platform will be subjected to polymerase chain reactions (PCR) and genotyping using Luminex® system.

Statistics

* Data will be analysed through X^2 tests and Z-tests for proportions, using a 5% significance level throughout.
* All data will also be descriptively presented.
* Logistic regression analyses – multivariate analysis will be applied to the effect of each of age, adult/child, gender, smoking status, alcohol use and oral sexual practices, and its interaction with the variable in question, on the presence/absence of HPV, and on HPV type.

Closing remarks

* HPV-status of a lesion may influence the diagnosis, treatment and follow-up – *Mirghani et al., 2015*
* Increase in HPV-associated oropharyngeal cancer might influence vaccination policies for males
* This knowledge can be further applied to investigate the proposed chemical/HPV combined pathway of carcinogenesis.

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