

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



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Background

- ▶ 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
 - HIV seropositive individuals on HAART
 - Children and Adults

Problems

- ▶ No reliable data describing spectrum of HPV types infecting oral and oropharyngeal mucosae of South African population groups.
- ▶ Very few studies investigated prevalence of HPV types in specimens of benign and malignant oral lesions in South Africa

Problems

- ▶ Data on oral and oropharyngeal HPV prevalence in healthy and in HIV–infected children in South Africa
- ▶ Data on oral sex practice of South Africans is sparse

Research Questions

- ▶ What is the prevalence of oral and oropharyngeal HPV infection in a general South African cohort?
- ▶ What is the prevalence of oral and oropharyngeal HPV infection in an HIV–seropositive South African cohort?

Research Questions

- ▶ Which HPV types are the most prevalent in HPV-associated benign and malignant oral and oropharyngeal lesions?
- ▶ Which HPV types are the most prevalent in the oral and oropharyngeal mucosae of the HIV-seropositive participants?
 - Which HPV types affect which anatomical regions of the mouth and oropharynx?

Research Questions

- ▶ What is the prevalence of oral sexual practice in South African population groups?

Objectives

- ▶ To describe cell morphology of the oral and oropharyngeal mucosae of South African population groups (cytology).
- ▶ To determine the *prevalence* of HPV types in different oral and oropharyngeal mucosal sites of South African population groups.

Objectives

- ▶ To determine the prevalent HPV types detected in oral and oropharyngeal benign and malignant FFPE tissue specimens.
- ▶ To investigate the relationship of the benign and malignant FFPE tissue specimen anatomical site to the diagnosis of the lesion and to the type of HPV associated with the lesions.

Objectives

- ▶ To investigate the association between the HPV types in benign and malignant FFPE tissue specimens with the site of occurrence, age, ethnicity and gender.

Objectives

- ▶ To investigate the association between the HPV types in oral and oropharyngeal mucosae and age, gender, HIV–serostatus, ethnicity, use of HAART, CD4+ T–cell counts and viral load, smoking habits, site of HPV infection and self–reported sexual practices of the study participants.

Method

- ▶ Cross-sectional and exploratory, descriptive
- ▶ Randomized sampling at sites
- ▶ Tissue specimens: benign and malignant 1996–2004, and 2012–2014
- ▶ Ethical clearance prior to data collection

Method

- ▶ Self-administered questionnaire
- ▶ Oral/oropharyngeal wash specimen collection:
 - Rinse gargle spit into fixative
- ▶ Brush specimen collection: tonsillar, soft palate, lateral border/base of tongue.
- ▶ Tissue specimen collection (Formalin-fixed, paraffin-embedded)

Method

- ▶ HPV–detection by APTIMA®
 - Presence/absence of HPV–mRNA
 - 20ml oral wash for APTIMA assay on Panther system
 - Internal HPV control will be used

- ▶ DNA extraction:
 - Automated Biomérieux system

Method

- ▶ PCR and genotyping
 - Multiplex HPV–type specific PCR (Luminex®), and sequencing to be done at Inqaba Biotechnological industries.
 - E7–targeting primers for initial genotyping of HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a,b, 70, 73, 82.
 - Positive and negative controls will be included
 - 50 probes to be developed for HR and LR HPV types

Data analysis

- ▶ Descriptive statistics
- ▶ Z-test for proportions
- ▶ Logistic regression – multivariate analysis

Important

- ▶ Wash specimens versus swabbing or scraping
- ▶ Self-reporting of HIV-serostatus in a general population –piloting

Practice of Oral Sex

- ▶ Ano-genital / genital-genital and genital-oral transmission has been described.
- ▶ Receptive oral sex is a significant factor for oral HPV.
- ▶ Oral HPV infection acts as a reservoir (carrier)
 - -active expression?
 - -age of infection?
- ▶ Data on Oral Sex practice in South Africa is limited.

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

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

Background

- ▶ Defining the oropharynx:
 - The anatomical area that includes the posterior third of the tongue and the palatine and pharyngeal tonsils. This area is inferiorly limited by the epiglottis and superiorly by the soft palate.
 - (*Paquette et al., 2013*)

Background

- ▶ HPV is a known aetiologic factor for benign oral/oropharyngeal lesions:
 - Verruca Vulgaris
 - Oral Squamous Papilloma
 - Condyloma Accuminatum
 - Focal Epithelial Hyperplasia (Heck disease)

- ▶ HPV has a strong association with oropharyngeal malignancy:
 - Squamous Cell Carcinoma
 - (Some attribute 30% of oral SCC's to HPV)

Background

Oral Squamous Papilloma

Verruca Vulgaris HPV 2, 4, 40



Background

Condyloma Accuminatum



Courtesy Dr A Masilana, Dept Periodontology and Oral Medicine

Background

Focal epithelial hyperplasia



HPV types 13, 32, 1, 6, 11, and rarely 16

Background

Focal epithelial hyperplasia



Courtesy Dr R Chandran, Dept Periodontology and Oral Medicine

Background

Miscellaneous forms



Courtesy Dr A Masilana, Dept Periodontology and Oral Medicine

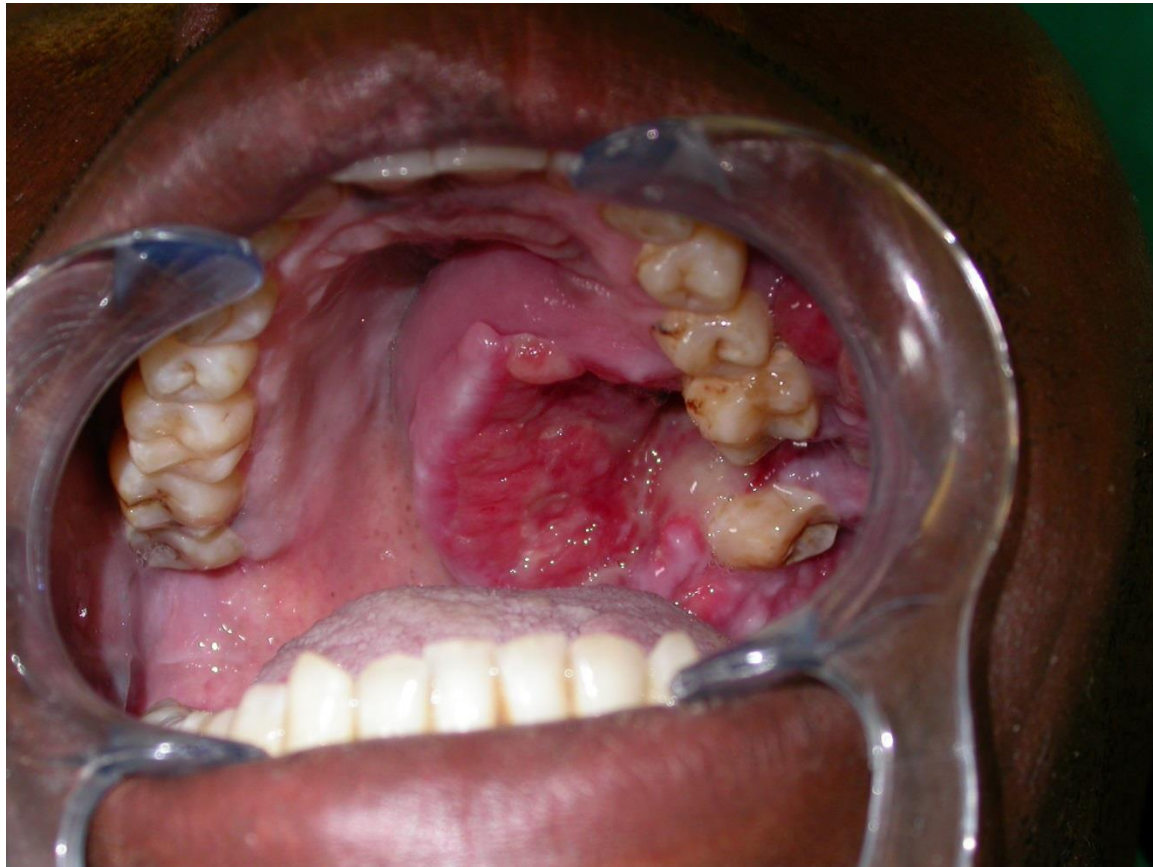
Background

Oral Squamous Cell Carcinoma



Background

Oral Squamous Cell Carcinoma



Background

- ▶ Manifestations of oral HPV infection:
 - Transitory (Subclinical) up to 50% of cases
 - Spontaneous regression in up to 30% of cases
 - Persistent infection
- ▶ Important to know whether infection is latent or whether virus is transcribed – mRNA

Dos Reis et al., 2009



Literature

- ▶ 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

South African studies concerned with oral/oropharyngeal HPV infection



Literature review

- ▶ A large child and adolescent study (N=1235) by Smith et al., (2007) reported:
 - 2.5% < 1 year
 - 0.8% 1–4 years
 - 1.2% 5–11 years
 - 1.5% 12–15 years
 - 3.3% 16–18 years with oral HPV 6,11,16 and 18 at 0.9%
- ▶ Specific HPV–type prevalence worldwide?
 - Not well reported for limitations mentioned

Literature review

- ▶ A Brazilian study of oral HPV infection in 100 children (3–13) 50 HIV + and 50 HIV –
 - Only HPV – 6, 11, 16 and 18 investigated
 - 6 HIV+ and 3 HIV– children had oral HPV infection
 - Mostly HPV 11, 16 and 18.
- ▶ Jeftha et al., (2003) reported oral HPV prevalence of 3.6% in HIV + children vs 12.3 HIV–

Pinheiro et al., 2011 – J Clin Pathol

Jeftha et al., 2003 – conference proceedings AADR



Literature review

- ▶ South African studies:
 - Focus on targeted cohorts
 - Mostly limited oral/oropharyngeal HPV identification to 2–4 types
 - Smaller sample sizes

- None included children
- None included a cohort on HAART

Literature review

- ▶ Comparisons of disease prevalence
- ▶ Prevalence rates for “oral” or for “oropharyngeal” regions depend on the anatomic sites investigated if a wash approach was not applied.

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
 - Results were contrasting to a meta-analysis by Miller and Johnstone (2001)
 - 7 were RT-PCR positive for HPV 16, none for HPV 18
 - All negative on ISH for both HPV 16 and HPV 18
- ▶ 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%
 - Stark contrast to Boy et al., (2007)

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)

Conclusion

- ▶ Significance of output:
 - We are able to study and design vaccination and/or targeted treatment strategies and improve prognostication abilities when we know and understand which HPV types infect our population groups.
 - This knowledge can be further applied to investigate the proposed chemical/HPV combined pathway of carcinogenesis.

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