Faculty of Medicine and Health Sciences

Thesis submitted for the degree of

Doctor in Medical Sciences

at the University of Antwerp to be defended by

Neil Hamilton Wood

Submitted in fulfilment of the requirements for the degree of **Doctor of Health Sciences (Medical Virology)**, in the School of Medicine, at the Sefako Makgatho Health Sciences University, South Africa

An investigation of the prevalence of Oral and Oropharyngeal Human Papillomavirus in selected South African population groups and tissue specimens

Faculty of Medicine and Health Sciences
Antwerp
2021

Faculty of Medicine
Sefako Makgatho Health Sciences University
2021

Supervisors:

Sefako Makgatho Health Sciences University:

Dr. RL Lebelo PhD	Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa. HIV and Hepatitis Research Unit, National Health Laboratory service, South Africa
Prof. Dr. SC Boy PhD	Department of Oral Pathology, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

Universiteit Antwerpen - University of Antwerp:

Prof. Dr. J Bogers PhD	Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), University of Antwerp, Belgium. Laboratory of Cell Biology & Histology, Antwerp, Belgium
Prof. Dr. O Vanderveken PhD	Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium; Department of ENT, Head and Neck Surgery, Antwerp University Hospital, Edegem, Belgium

Table of Content: **Page**

Additional Information:

Acknowledgements

My sincere gratitude and thanks to my supervisors and mentors for their guidance, constant inputs and abundance of patience exhibited towards me during this time:

To Dr Ramokone Lebelo, thank you for your thorough input and guidance. I have learnt a great deal from you and will be eternally grateful. I hope to have many future collaborations with you.

To Prof Dr Johannes Bogers, your example serves as an inspiration to me and your guidance and support were invaluable. Your positive reinforcement and continuous pushing towards the end-goal during difficult times are appreciated. Thank you also for your kind friendship.

To Prof Dr Olivier Vanderveken, my sincere gratitude for the patience and guidance shown to me. Thank you also for the wonderful exposure during my visit to the Universitair Ziekenhius Antwerpen. Your positive input and guidance are deeply appreciated.

To Prof Dr Sonja Boy, my sincere and deepest gratitude for your academic guidance and inputs. Your work-ethic serves as a pristine example to me. Thank you also for being there when times were dark and uncertain. Your faith in me has carried me many miles during extreme working conditions.

A special word of thanks to Dr Ina Benoy who worked tirelessly to keep me steering in the right direction, for her superb expertise both in the laboratory and in research, and for much needed guidance and support. Your wisdom and guidance are deeply appreciated and thank you for teaching me. Thank you also for taking care of me during my first stay in Antwerp and showing concern for my wellbeing.

I am deeply grateful to my beautiful family for their consideration and patience. The long periods of time spent away from them were difficult, but they carried this with grace and acceptance giving me loads of support. My family also had to tolerate long working hours and hours in front on spreadsheets and statistics. Thanks to my wife Megna and my daughters Neha and Leia.

A special word of thanks also to Prof Pagolang Motloba, Dr Cindy Simoens, Prof Dr Olalekan Ayo-Yusuf, Ms Nina Redzic, Mr Koketso Makua, Dr Petra Gaylard, Prof Maposhane Nchabeleng, Prof Gloria Selabe, Prof Mapaseka Seheri, Mr Jan Vervoort, Ms Kristin Deby, staff in the Department of Virology at SMU, and MeCRU staff at SMU.

My sincere gratitude to the following for financial, technical, academic and administrative support:

- VLIR-UOS collaboration
- WAKA HPV Africa network
- Sefako Makgatho Health Sciences University
- Universiteit Antwerpen

Dedicated to my late father, who passed on whilst this project was ongoing

Declaration:

I declare that I have produced and submitted the work "An investigation of the prevalence of Oral and Oropharyngeal Human Papillomavirus in selected South African population groups and tissue specimens." for purposes of defence for the joint PhD degree of Doctor of Medical Sciences at the University of Antwerp, Belgium, and Doctor in Health Sciences at Sefako Makgatho Health Sciences University, South Africa.

I declare that the thesis hereby submitted to Sefako Makgatho Health Sciences University and to the University of Antwerp for the joint doctoral degree has not previously been submitted by me for a degree at this or any other university; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

Ethical Clearance number: Sefako Makgatho Health Sciences University Research Ethics Committee **SMUREC/P/86/2016 PG**

_____ _____

Signature of candidate: Neil Hamilton Wood Date

ABBREVIATIONS AND ACRONYMS

CD	-	Cluster of Differentiation
CI	-	Confidence Interval
CoCoPop	-	Condition, Context and Population
DNA	-	Deoxyribonucleic Acid
FFPE	-	Formalin-Fixed Paraffin-Embedded
HIV	-	Human Immunodeficiency Virus
HNSCC	-	Head and Neck Squamous Cell Carcinoma
HPV	-	Human Papillomavirus
HR	-	High Risk
HRP	-	Horseradish Peroxidase
IQR	-	Interquartile Range
ISH	-	In Situ Hybridization
JBI	-	Joanna Briggs Institute
LR	-	Low Risk
NGFR	-	Nerve Growth Factor Receptor
OHC	-	Oral Health Centre
OP	-	Oropharynx
OPSCC	-	Oropharyngeal Squamous Cell Carcinoma
OR	-	Odds Ratio
OS	-	Oral Sex
PCR	-	Polymerase Chain Reaction
PRISMA	-	Preferred Reporting Items for Systematic Reviews and Meta-analysis
qPCR	-	Quantitative Polymerase Chain Reaction
SCC	-	Squamous Cell Carcinoma
STD	-	Sexually Transmitted Disease
SWB	-	Stringent Wash Buffer
TBST	-	Tris-buffered saline (TBS) and Polysorbate 20 (Tween 20)
UD	-	Undetermined
UK	-	United Kingdom
US	-	United States
USA	-	United States of America

SUMMARY

Introduction:

The Human papillomavirus (HPV) is the responsible aetiologic agent for a variety of benign and malignant epithelial lesions. A specific subset of the HPV family, high-risk HPV's, is firmly established as oncogenic in humans, capable of inducing cellular transformation with resultant squamous cell carcinomas in different anatomic locations. The World Health Organization recognizes squamous papillomas, verruca vulgaris, condyloma acuminatum and focal epithelial hyperplasia as the four main clinical entities of benign HPV-associated oral and oropharyngeal lesions (Piña et al., 2019).

Several meta-analyses published over a number of years indicate that the data contribution on oral and oropharyngeal HPV infection and sequelae from sub-Saharan Africa is sparse. Where papers were available, methodologic and population selection incompatibilities made their inclusion into systematic review difficult, or unsuitable. Given the period over which these data were published, it is clear there are significant lags in data collection and presentation and in methodologic evolution and standardization. Despite these challenges, this thesis has additionally produced a systematic review and meta-analysis of all South African data available from 1995 to 2020 on oral and oropharyngeal HPV studies, to highlight the significant data shortfall for this country in this regard, the first of which was published in 1995.

Study outline:

In order to present a full and comprehensive overview of the HPV-prevalence in South Africans in the Ga-Rankuwa, Pretoria area a multi-fronted study approach had to be designed. The first challenge was that there existed no data on the high-risk behaviors of oral sex practice and tobacco use for this population. Once this baseline was established, it was necessary not only to study HPV-prevalence in relation to these behaviors, but also to report on HPV prevalence from different population perspectives. Therefore, the study design included a general population, an HIV-seropositive population, a unique population of men who have sex with other men. This enabled the study to report on these population groups in a controlled manner taking specific risk factors and behaviors traditionally associated with HPV acquisition and transmission. Further to this, it was necessary to study HPV-prevalence in formalin-fixed, paraffin-embedded oral and oropharyngeal tissue specimens submitted to the Oral Pathology department over a ten-yar period. The intent was to investigate the prevalence of HPV in these

specimens, and also to demonstrate whether any HPV types were aetiologic drivers or possibly transient or passenger in nature.

Results:

Tobacco use and oral sex practice was studied in 847 participants from the Ga-Rankuwa, Pretoria region which showed that 19.7% were current smokers and 22.8% were actively practicing oral sex. About a third of smokers also practiced oral sex. This established an important baseline for these parameters.

Another study as part of this thesis investigated 149 participants from a general population and 72 from an HIV-management clinic. Regarding high-risk behaviours: smokers comprised 29.4% of this sample and 45.2% of these participants reported to have ever consumed alcohol. Alcohol consumption characteristics were investigated but nothing of significance was found in the context of this project. Open mouth kissing during teenage years was confirmed by 64.7% of participants, 40.3% have given oral sex with their mouth, and 44.8% confirmed to have received oral sex from their partner's mouth. Majority (64.7%) of the participants had open mouth kissed during their teenage years with a significant difference between the two groups where more dental clinic participants practiced open mouth kissing at a very early age (10-14 years). Of all the participants, 65.2% (144) confirmed they knew what oral sex (OS) was, and 40.3% (89) reported to have given OS with their mouths.

When studying the MSM population, data showed that 6% had high-risk HPV present in the mouth/oropharynx. This falls within the lower end of the global HPV prevalence spectrum for these anatomic sites in MSM. This cohort averaged 29 years of age, more than half were unemployed (53.3%), and 66,8% were HIV seropositive. The most common sexual practice was anal sex (69.4%) followed by oral sex (28.6%), and by rimming (9.6%).

Tissue specimens comprising of 67 benign HPV-associated lesions, 21 Squamous cell carcinomas that were used as test cases. Microscopically normal-appearing tonsillar tissue specimens (71) removed through elective tonsillectomy procedures served as a tissue-control. HPV-positive cervix carcinoma tissue blocks served as positive control whilst distilled water replaced HPV-probe cocktails on known HPV-positive specimens for use as negative control. All specimens were collected over a ten-year time period and were analysed for HPV through qPCR. An 11% overall HPV prevalence for all specimen groups (155) was found. A total of

20 positive results were obtained for 17 tissue specimens. Nine squamous cell carcinoma specimens were $p16^{INK4a}$ positive, but all of these were negative for qPCR and ISH. The single SCC that was HPV-33 and HPV-12 positive was negative for both $p16^{INK4a}$ and HPV using *in situ* hybridization (ISH). HPV-6 was the most frequently detected type.

Discussion and Conclusion:

The systematic review and meta-analysis confirmed that prevalence reporting for head and neck HPV in South Africa is lagging with only eleven reports in existence over a 25-year period. The overall pooled prevalence of 11% demonstrated in the systematic review is slightly higher than global averages. Populations comprising higher risk groups exhibit higher HPV prevalence rates which is in part influenced by the existence of comorbidities in these groups. Methodology and study design consistencies and standardization will improve prevalence reporting in this geographic region.

In the population group attending a university-based Oral Health Centre, tobacco use and the practice of oral sex were not significantly associated risk behaviors and could be considered independent risks for oral and oropharyngeal HPV infection and quite possible for the development of squamous cell carcinomas in the mouth and oropharynx. No significant relationships were found between the presence of HPV and demographic data or sexual, oral sexual, tobacco use, or alcohol use; and no associations were seen with number of genital sexual and of oral-sex partners.

An overall low HPV prevalence of 3.6% was found for the general population attending dental clinic and an HIV-management clinic. Separately, the dental clinic population's HPV prevalence was 4% and the HIV clinic population was 2.8%. It is made clear however, that oral/oropharyngeal mucosal infection by HPV is not implied by the detection of any HPV-DNA in these participants.

An interesting and unintended finding in the general population and HIV-seropositive cohorts was that only four out of the 221 participants had palatine tonsils present, and all four of these patients were from the HIV-seropositive cohort, and none of them were HPV positive. It could be that the lack of HPV-driven oropharyngeal squamous cell carcinomas is the result of early tonsillectomies in this population sample.

The HPV prevalence of 6% reported in the MSM population group is in alignment with global reports. The prevalence of oral/oropharyngeal HPV in this MSM cohort was influenced by sexual practices. Significantly, MSM participants who practiced rimming appeared to be at higher risk of HIV acquisition.

Data obtained from $p16^{INK4a}$, ISH and qPCR tests from the FFPE tissue specimens demonstrated the lack of concurrence. This reinforces the hypothesis that a passenger-infection theory is more likely in this specimen group. In addition, no HPV-driven oral/oropharyngeal SCC could be detected. Future methodology must include selected biomarker detection alongside PCR investigation for confirmation of HPV-driven lesion development.

The data from this project supports the proposal that HPV infection of the mouth represents a passenger infection and may propagate from this anatomic site, but it is not responsible for oral or oropharyngeal SCC in the South African population group involved. Given the findings presented here, it must be questioned whether vaccines are a requisite preventive measure for head and neck squamous cell carcinoma in this population group seeing that female vaccination is in process and ongoing.

SAMENVATTING

Introductie:

Het humaan papillomavirus (HPV) is het verantwoordelijk voor een verscheidenheid aan goedaardige en kwaadaardige epitheliale laesies. Van een specifieke subset van de HPV-familie, zogenaamde hoog-risico-HPV's, is inmiddels duidelijk aangetoond dat ze oncogeen zijn bij mensen, en dus in staat zijn tot het induceren van cellulaire transformatie resulterend in plaveiselcelcarcinomen op verschillende anatomische locaties. De Wereldgezondheidsorganisatie erkent plaveiselcelpapillomen, verruca vulgaris, condyloma acuminatum en focale epitheliale hyperplasie als de vier belangrijkste klinische entiteiten van goedaardige HPV-geassocieerde orale en orofaryngeale laesies.

Verschillende meta-analyses die de laatste jaren gepubliceerd werden, geven aan dat de gegevensbijdrage over orale en orofaryngeale HPV-infectie en de gevolgen ervan in sub-Sahara Afrika schaars is. Waar wel artikels beschikbaar waren, maakten methodologische onvolkomenheden en gebrekkige populatieselectie hun opname in de systematische review moeilijk of ongeschikt. Gezien de periode waarin deze gegevens werden gepubliceerd, is het duidelijk dat er aanzienlijke vertragingen zijn in het verzamelen en presenteren van gegevens en in methodologische evolutie en standaardisatie. Ondanks deze uitdagingen heeft dit proefschrif toch een systematische review en meta-analyse opgeleverd van Zuid-Afrikaanse gegevens die op dat moment beschikbaar waren over orale en orofaryngeale HPV-onderzoeken, om het aanzienlijke tekort aan gegevens in de literatuur voor dit land in dit opzicht toch te proberen op te vangen.

Overzicht van de studie:

Om een volledig en alomvattend overzicht te geven van de HPV-prevalentie bij Zuid-Afrikanen in Ga-Rankuwa, Pretoria, moest een zogenaamde “multi-fronted study”-benadering ontworpen worden. De eerste uitdaging was dat er voor deze populatie geen gegevens bestonden over het risicovolle gedrag van orale seks en tabaksgebruik. Toen deze baseline gegevens eenmaal konden worden vastgesteld, was het niet alleen nodig om de HPV-prevalentie in relatie tot deze gedragingen te bestuderen, maar ook om de HPV-prevalentie te rapporteren vanuit verschillende populatieperspectieven. Daarom omvatte de onderzoeksopzet een algemene populatie, een hiv-seropositieve populatie en een unieke populatie mannen die seks hebben met andere mannen (MSM). Hierdoor kon de studie op een gecontroleerde manier over deze bevolkingsgroepen rapporteren, waarbij gebruik werd gemaakt van specifieke

risicofactoren en gedragingen die traditioneel geassocieerd worden met HPV-acquisitie en -transmissie. Daarnaast was het nodig om de HPV-prevalentie te bestuderen in formaline-gefixeerde, in paraffine ingebedde orale en orofaryngeale weefselspecimens die over een periode van tien jaar werden ingediend bij de afdeling Orale Pathologie. Dit was bedoeld om de prevalentie van HPV in deze specimens te onderzoeken, en ook om aan te tonen of de gedetecteerde HPV stammen de etiologische drivers, of eerder drivers van voorbijgaande aard, of passagiers, waren.

Resultaten:
Tabaksgebruik en orale seks werden bestudeerd bij 847 deelnemers uit de regio Ga-Rankuwa, Pretoria, waaruit bleek dat 19,7% huidige rokers waren en 22,8% actief orale seks beoefende. Ongeveer een derde van de rokers beoefende ook orale seks. Dit zorgde voor een belangrijke baseline voor deze parameters.

Een andere studie als onderdeel van dit proefschrift onderzocht 149 deelnemers uit een algemene populatie en 72 uit een HIV-behandelingskliniek. Rokers maakten 29,4% uit van deze steekproef en 45,2% van deze deelnemers meldde ooit alcohol te hebben gebruikt. Kussen met open mond tijdens tienerjaren werd bevestigd door 64,7% van de deelnemers, 40,3% gaf orale seks met de mond en 44,8% bevestigde orale seks (OS) te hebben ontvangen via de mond van hun partner. De meerderheid (64,7%) van de deelnemers had tijdens hun tienerjaren met open mond gekust, met een significant verschil tussen de twee groepen waar meer deelnemers aan de tandheelkundige kliniek op zeer jonge leeftijd (10-14 jaar) zoenen met open mond beoefenden. Van alle deelnemers bevestigde 65,2% (144) dat ze wisten wat OS was, en 40,3% (89) gaf aan OS met de mond te hebben gegeven.

Bij het bestuderen van de MSM-populatie toonden gegevens aan dat 6% hoog-risico HPV in de mond / orofarynx had. Dit valt eerder binnen de ondergrens van het wereldwijde HPV-prevalentiespectrum voor deze anatomische locaties bij MSM. Deze cohorte was gemiddeld 29 jaar oud, meer dan de helft was werkloos (53,3%) en 66,8% was HIV seropositief. De meest voorkomende seksuele praktijk was anale seks (69,4%), gevolgd door orale seks (28,6%) en rimmen (9,6%).

Weefselspecimens bestaande uit 67 goedaardige HPV-geassocieerde laesies, 21 plaveiselcelcarcinomen en 71 tonsillaire stalen die dienden als controle over een periode van

tien jaar, werden geanalyseerd op HPV met inbegrip van qPCR. Er werd een algemene HPV-prevalentie van 11% gevonden voor alle specimengroepen (155). In totaal werden 20 positieve resultaten verkregen voor 17 weefsels. Negen plaveiselcelcarcinoomspecimens waren p16INK4a-positief, maar deze waren allemaal negatief voor qPCR en in-situ-hybridisatie (ISH). Het einige plaveiselcelcarcinoom datHPV-33 en HPV-12 positief was, bleek negatief voor zowel $p16^{INK4a}$ als ISH. HPV-6 was het meest gedetecteerde type.

Uitkomsten:

De systematische review en meta-analyse bevestigden dat de rapportering van prevalentiecijfers voor hoofd-hals-HPV in Zuid-Afrika achterblijft, met slechts een paar publicaties over een periode van 25 jaar. De totale gepoolde prevalentie van 11% aangetoond in de systematische review is iets hoger dan de wereldwijde gemiddelden. Populaties met groepen met een hoger risico vertonen hogere HPV-prevalentiecijfers, wat gedeeltelijk wordt beïnvloed door het bestaan van comorbiditeit in deze groepen. Het verhogen van consistentie en standaardisatie van de methodologie en studieopzet zullen de rapportering van de prevalenties in deze geografische regio potentieel verbeteren.

In deze bevolkingsgroep waren tabaksgebruik en het beoefenen van orale seks niet significant geassocieerd met risicogedrag en konden ze worden beschouwd als onafhankelijke risico's voor orale en orofaryngeale HPV-infectie en dus potentieel niet in verband te brengen met de ontwikkeling van plaveiselcelcarcinomen in de mond en orofarynx. Er werden geen significante relaties gevonden tussen de aanwezigheid van HPV en demografische gegevens of seksueel, oraal seksueel, tabaksgebruik of alcoholgebruik; en er werden geen associaties gezien met het aantal seksuele en orale sekspartners.

Een algemene lage HPV-prevalentie van 3,6% werd gevonden voor de algemene bevolking van een tandheelkundige kliniek en een HIV-behandelingskliniek. Los daarvan was de HPV-prevalentie van de populatie in de tandheelkundige kliniek 4% en de populatie van de HIV-kliniek 2,8%. Het wordt echter duidelijk gemaakt dat orale / orofaryngeale mucosale infectie door HPV niet wordt geïmpliceerd door de detectie van enig HPV-DNA bij deze deelnemers.

Een interessante en niet-intentionele bevinding in de algemene populatie en hiv-seropositieve cohorten was dat slechts vier van de 221 deelnemers keelamandelen hadden, en alle vier waren deze afkomstig uit het hiv-positieve cohort, en geen enkele was HPV-positief. Het kan zijn dat

het ontbreken van HPV-aangedreven orofaryngeale plaveiselcelcarcinomen het resultaat is van vroege tonsillectomieën in deze populatie.

De HPV-prevalentie van 6% gerapporteerd in de MSM-bevolkingsgroep komt overeen met internationale rapportering. De prevalentie van orale / orofaryngeale HPV in dit MSM-cohort werd beïnvloed door seksuele praktijken. Het is veelbetekenend dat MSM-deelnemers die rimmen beoefenden een hoger risico leken te lopen op het krijgen van HIV.

Gegevens verkregen uit het bestuderen van de FFPE ("formalin fixed and paraffin embedded ")-weefselspecimens toonden het gebrek aan overeenstemming tussen $p16^{INK4a}$ en ISH-positiviteit met qPCR aan. Dit versterkt de hypothese dat een passagiers-infectietheorie waarschijnlijker is in deze bevolkingsgroep. Bovendien kon geen HPV-aangedreven orale / orofaryngeale plaveiselcelcarcinoom worden gedetecteerd. Toekomstige methodologie moet naast PCR-onderzoek ook geselecteerde biomarkerdetectie omvatten voor bevestiging van HPV-gestuurde laesieontwikkeling.

De gegevens van dit project ondersteunen het voorstel dat HPV-infectie van de mond een infectie van een passagier is en zich kan voortplanten vanaf deze anatomische plaats, maar het is niet verantwoordelijk voor orale of orofaryngeale SCC in de betrokken Zuid-Afrikaanse bevolkingsgroep. Gezien de hier gepresenteerde bevindingen, moet de vraag worden gesteld of vaccins een noodzakelijke preventieve maatregel zijn voor plaveiselcelcarcinoom van het hoofd-halsgebied in deze bevolkingsgroep, aangezien vaccinatie bij vrouwen aan de gang is.

CHAPTER 1:

General Introduction

CHAPTER 1:

General introduction

Human Papillomavirus (HPV) mucosal infection is well described and studied withing the spectrum of cervical disease. The literature that focuses on oropharyngeal HPV prevalence and infection has steadily grown recently due to the association of a subset of HPV-driven squamous cell carcinoma (SCC), but to a much lesser extent for that studying the oral cavity. There is no reliable data or consensus on the true prevalence of HPV infection of the oral and oropharyngeal mucosae in South Africa. Data originating from South Africa on oral and oropharyngeal HPV prevalence and infection is very sparse, and this project has shown that the minimal data available from studies on South African population groups and specimens has many limitations. Despite this lack of data from South Africa, oral SCC incidence in South Africa is reported as 2.7/100 000 and SCC of the pharynx as 2.4/100 000 (Vogt et al., 2013). This makes it imperative to establish which portion of these are driven by HPV.

Human papillomavirus is, according to the Centers for Disease Control and Prevention (CDC), the most commonly sexually transmitted disease (CDC, 2020). This prompted the general need to report on the high-risk behaviours relating to sexual practices within the different population groups studied. As the classic aetiology of oral and oropharyngeal squamous cell carcinoma (SCC), tobacco and alcohol use were studied alongside high-risk sexual practices and HPV prevalence, to establish a baseline for this South African population group. In order to be able to interpret any clinical data collected in this project, the establishment of this baseline concerning high-risk sexual and social behaviour was essential. This fundamental behavioural data proved to be important as the outcomes presented in this thesis differ to that presented in the international literature, especially from the developed countries. This is an important and novel contribution that will guide understanding in the influence of this difference in behavioural data in the acquisition and spread of oncogenic HPV types in South African populations.

Data on oral and oropharyngeal cancer from Africa is limited and usually presented as small series. Given the time period in which these data were published, it is clear there are significant lags in data collection and presentation and in methodologic evolution and standardization. Prevalence of oral and oropharyngeal mucosal HPV infection has not been

described for the general South African population. In addition, HPV-types infecting oral lesions have not been described for the general South African population.

Taking into consideration the larger HIV prevalence in South Africa than elsewhere, the importance of providing data on oral and oropharyngeal HPV infection becomes urgent. Current data suggests a much higher oral and oropharyngeal HPV prevalence in HIV-seropositive patients; and HPV infection rates of these sites are proportional to the level of immune suppression (Vogt et al., 2013). Oropharyngeal HPV infection rates are up to 3 times higher in HIV-seropositive patients than in patients with oral SCC (Adamopoulou et al., 2008). It is therefore clear that this project required a multifaceted approach to answer the overarching research question and study problem: "What is the prevalence of oral and oropharyngeal HPV infection in the South African population in the region North West of Pretoria and areas as serviced by the university hospitals?"

This study problem is managed in terms of the following research questions:

- What is the characteristics of oral sex practice, tobacco use, and alcohol consumption in this population group?
- What is the prevalence of oral and oropharyngeal HPV infection in an HIV-seropositive South African cohort attending a regional HIV-management clinic?
- What is the prevalence of oral and oropharyngeal HPV infection in a general South African cohort attending a regional dental hospital?
- What is the prevalence of oral and oropharyngeal HPV infection in population sample of men who have sex with men?
- Which HPV genotypes are the most prevalent in HPV-associated benign and malignant oral and oropharyngeal lesions?
- Which HPV genotypes are the most prevalent in the oral and oropharyngeal mucosae of the HIV-seropositive participants and how does this compare to that found in the general participants from the dental hospital?

In order to assess the prevalence rates for oral and oropharyngeal HPV infection in this South African geographic region, this study approached the research question by proposing to investigate HPV presence and/or prevalence in the following:

- Rinse and gargle specimens of dental clinic attendees.

- Rinse and gargle specimens of HIV-seropositive patients attending the HIV-management clinic.
- Men who have sex with men (MSM) residing in the same geographic area.
- Malignant and benign HPV-associated oral and oropharyngeal tissue specimens, with non-HPV-associated tonsillar control tissue specimens, all from the same geographic location.
- Further to the above, a systematic review was conducted, and a meta-analysis was synthesised to reflect the oral and oropharyngeal HPV-prevalence rate in South Africa.

In addition to the prevalence investigation in the mouth and oropharynx, demographic data, high-risk behaviour were recorded in the clinical component of this project (rinse and gargle specimens of the dental clinic attendees, HIV-management clinic attendees and the MSM participant group). The study aimed to find any meaningful or significant relationships between any behaviours and/or demographic data, and the presence of HPV in the mouth or oropharynx of the participants or tissue specimen. The study also aimed:

- To determine the prevalence of, and to genotype HPV in the oral and oropharyngeal mucosae of the HIV-seropositive participants, in the dental hospital attendees, and of MSM participants of all ages.
- To determine whether any relationship exists between the prevalence of any of the HPV genotypes in oral and oropharyngeal mucosae of HIV-seropositive participants and of the general population attending a regional dental hospital and age, gender, HIV-serostatus, use of highly-active antiretroviral therapy, smoking, alcohol use and self-reported sexual practices.
- 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 formalin-fixed paraffin-embedded (FFPE) tissue specimens.

The following chapters systematically deal with these questions and aims, and provide significant and, at times novel, insights into this topic.

CHAPTER 2:

Tobacco use and Oral Sex Practice among Dental Clinic Attendees

PUBLISHED:

Wood NH, Ayo-Yusuf OA, Gugushe TS, Bogers J-P (2019) Tobacco use and oral sex practice among dental clinic attendees. *PLoS ONE* 14(3): e0213729. https://doi.org/10.1371/journal. pone.0213729

CHAPTER 2

Tobacco use and Oral Sex Practice among Dental Clinic Attendees

Neil H Wood[1*], Olalekan A Ayo-Yusuf [2], Tshepo S Gugushe[3], John-Paul Bogers[4,5]

[1]Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, South Africa.
[2]Africa Centre for Tobacco Industry Monitoring & Policy Research, Sefako Makgatho Health Sciences University, South Africa.
[3]School of Oral Health Sciences, Sefako Makgatho Health Sciences University, South Africa.
[4]Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), University of Antwerp, Belgium
[5]Laboratory of Cell Biology & Histology, Antwerp, Belgium.

*Corresponding Author:
E-mail: neil.wood@smu.ac.za; drnhwood@gmail.com

PUBLISHED: Wood NH, Ayo-Yusuf OA, Gugushe TS, Bogers J-P (2019) Tobacco use and oral sex practice among dental clinic attendees. *PLoS ONE* 14(3): e0213729. https://doi.org/10.1371/journal. pone.0213729

Abstract

Tobacco use and oral sex (OS) are important risk factors for oral and oropharyngeal Human papillomavirus (HPV) infection. Little is known about the prevalence of OS practice in South Africa. This study aimed to determine the prevalence of OS practice and tobacco use in a South African patient population. This cross-sectional study used a structured questionnaire to collect socio-demographic characteristics, tobacco use, betel nut use and OS practice data from consenting adults (≥18 years; n=850). Oral sex practices were recorded for patients 18-45 years-old (n=514). Data analysis included chi-square and multiple logistic regression analyses. Of the study population, 55.2% (n=468) were female, 88% (n=748) self-identified as black Africans and 45.1% (n=383) were unemployed. Furthermore, 19.7% (n=167), 6.4% (n=54) and 2.1% (n=18) were current smokers, snuff users and betel nut users, respectively. Out of the 514 who answered the questionnaire in relation to OS, 22.8% (n=115) reported to practice it. Oral sex practice in the age group 18-45 years was most common among the self-identified white participants (41.9%); and among tobacco users than among non-tobacco users (30.9% vs. 20.5%; p=0.022). A multivariable-adjusted regression model showed that white South Africans were more likely to use tobacco than black Africans (OR=5.25; 95% CI=2.21-12.47). The practice of OS was more likely among those 18-35 years-old (OR=1.67; 95% CI=1.01-2.74) but had no significant association with tobacco use (OR=1.06; 95% CI=0.62-1.83). The observed age and ethnic differences in both risk behaviours suggest a need for targeted population intervention in order to reduce the risk for oral HPV infection.

Key words: oral sex, tobacco use, smoking

Introduction

The aetiopathogenesis of oropharyngeal squamous cell carcinoma (SCC) has been linked to high-risk human papillomavirus (HPV) infection [1-3]. While the incidence of SCC of the head and neck is diminishing, that of HPV-related oropharyngeal SCC is increasing [4]. This implies that different aetiologic mechanisms may be at play [5] and support the postulate that HPV-associated SCC is a distinct and separate clinical entity from tobacco and alcohol-associated SCC [6,7]. Earlier oral/oropharyngeal HPV studies were limited by the lack of a standardized meaning for the "oral" *vs* "oropharyngeal" anatomical compartments. This lead to ambiguity in some reports and care must be taken when interpreting results representative of these two distinct anatomic sites [8,9]. The oropharyngeal site is defined by Paquette and colleagues [9] as "...posterior one-third of the tongue, palatine and pharyngeal tonsils, bounded inferiorly by the epiglottis and superiorly by the soft palate.".

Oral and oropharyngeal SCC is the 6th most common cancer and also the 6th largest cause of cancer related deaths worldwide [10]. Patients diagnosed with oral SCC have a mean 5-year survival rate of about 50%. The most important risk factors of oral SCC are tobacco smoking, excessive alcohol intake, chewing betel quid and areca nut and a diet low in fresh fruits and vegetables [10].

Tobacco use has a long association with the development of head and neck malignancy and the use of alcohol and tobacco are well-known risk factors for the development of head and neck SCC [3,11,12]. Some association between smoking and prevalence of oral HPV infection exists, but more importantly, tobacco use has been associated with a reduced capacity for the clearance of oncogenic HPV-infection [13,14]. Although the biologic link responsible for increased prevalence of oral HPV in current smokers has not yet been fully defined, the rationale lies in the local oral/oropharyngeal mucosal pro-inflammatory milieu and the immune suppression induced by tobacco use, creating a favourable niche for HPV infection and persistence [15].

Infection by HPV is the most common sexually transmitted disease (STD) [16]. Although oral and oropharyngeal HPV infections are believed to be acquired by orogenital contact with an infected sexual partner, by mouth-to-mouth contact or by autoinoculation from another infected site [17], some studies report the majority of cases with oral HPV infection are not

the result of sexual transmission [18,19]. Nevertheless, it is important to understand the demographic characteristics of oral sex (OS) practice in order to further research on its influence in oral health, especially in resource-poor settings such as this study's population.

HPV-infection and SCC of the mouth and oropharynx have been associated with patients becoming sexually active at a younger age, having numerous sexual partners, and with practicing orogenital sex [20-22]. While there is a strong association between HPV and oropharyngeal SCC with about 50% of all cases of HPV- cytopositive oropharyngeal SCC being caused by high-risk HPV genotypes, in the case of oral SCC there is limited evidence causally linking HPV infection of the mouth to oral SCC [23-25].

Within the limited scope of evidence, the apparently lower frequency of HPV infection in oral and oropharyngeal SCC of South African cohorts [8,26] could be because the practice of OS may be less common among South Africans than among Western and Asian populations; and may differ between different racial groups [27,28]. Reports on the ethnic distribution of OS practice are also very limited in the international literature, and when available, it presents different prevalence rates for OS practice according to the geographic region of the study [4,29,30]. While a number of studies have investigated the characteristics of tobacco use and to a lesser extent the practice oral sex [14], most have been done separately despite the fact that both risk behaviours may be related and co-exist. The practice of OS is a known high-risk sexual behaviour that facilitates oncogenic HPV transmission [31].

The purpose of this study was to investigate the prevalence of tobacco use and the practice of OS among the patients attending the Sefako Makgatho Health Sciences University Oral Health Centre located in a peri-urban area of South Africa.

Material and methods

This cross-sectional study involved consenting adults (≥18 years; n=847) who attended for consultation at a university-based Oral Health Centre (OHC). Using a structured self-administered questionnaire, socio-demographic characteristics that included age, gender, self-identified race/ethnicity (Black African; Coloured (Mixed ancestry); White; Indian/Asian), tobacco smoking and/or snuff use (some days or everyday), betel nut use and OS practices were recorded. Oral sex practices were recorded only for patients 18-45 years-old (n=514).

Oral sex practice was determined by asking participants whether they were currently engaged in oral sex practice, having their mouth in contact with a partner's genitalia.

Data analysis included chi-square and multi-variable adjusted logistic regression analyses. Two separate regression models were reported for OS and tobacco use. In both instances the independent effect of one as a predictor-variable of the other as an outcome-variable was controlled for age, gender, ethnicity and employment status. All tests were two-tailed and p values of 0.05 or less considered as significant. Ethical clearance for this project was obtained from the Sefako Makgatho Health Sciences University Research Ethics Committee (MREC/D/187/2010:IR).

Results

The study sample comprised 847 patients who visited the university-based Oral Health Centre. Study participants self-identified their race and of the 847 patients, 88.3% (748 patients) self-identified as black African, 6% (55 patients) were white, 2 patients (0.3%) were Indian, only 1 patient (0.1%) was of mixed race and 41 did not identify their race. Of the study population (n=468), 55.2% were female and 26 participants did not indicate their gender on the questionnaire. Eighty-eight percent (n=748) were Black and 45.1% (n=383) were unemployed (Table 1). Owing to the small participation number, those participants who self-identified as 'unknown race', of Indian and of mixed race were excluded from further analysis.

Table 1: Socio-demographic characteristics of the study sample.

Characteristics		**% (n)**
Gender	Male	41.6 (n=353)
	Female	55.2 (n=468)
	Unknown	3.1(n=26)
Race		
	Black African	88.3 (n=748)
	Whites	6.5 (n=55)
	Indian/Asian	0.2 (n=2)

	Mixed race	0.1 (n=1)
	unknown	4.8 (n=41)
Age group		
	18-35 years	44.2 (n=368)
	36-45 years	22.5 (n=187)
	>45 years	33.3 (n=277)
Employment status		
	Employed	30.2 (n=257)
	Retired/Student	7.6 (n=64)
	Unemployed	45.1 (n=383)
	Unknown	17.1 (n=143)
Tobacco use		
	Current smoker	19.7 (n=167)
	Current snuff user	6.4 (n=54)
	Betel nut user	2.1 (n=18)
Practice oro-genital sex*	Current practice	22.8 (n=115)

*Only among those 18-45 years old

Four hundred and seventy-five of the 748 black patients and 36 of the 55 white patients answered the question relating to their sexual behaviour; 21.6% (99) of the black patients and 41.9% (13) of the white patients practiced OS (Table 2). Data for other race groups were excluded from analysis due to insufficient sample size (n=3).

Table 2: Association between socio-demographic characteristics, oro-genital sex and tobacco smoking.

Characteristics		Practice oro-genital sex* % (N=514)	p-value	Tobacco use % (N=847)	p-value
Gender	Male	32.1 (69)		38.6 (135)	
	Female	16.2 (46)		12.0 (56)	
			<0.001		<0.001
Race					

	Black African	21.6 (99)		21.7 (162)	
	Whites	41.9 (13)		53.7 (29)	
	'Other' (Excluded)	0.5 (3)		0.0 (0)	
			0.031		<0.001
Age group					
	>45 years	n/a		25.8 (71)	
	36-45 years	17.4 (28)		23.0 (43)	
	18-35 years	25.2 (89)		21.5 (79)	
			0.05		0.432
Employment status					
	Unemployed	24.8 (50)		23.8 (91)	
	Retired/Student/unknown	19.6 (31)		18.7 (39)	
	Employed	23.4 (36)		27.0 (69)	
			0.503		0.108
Tobacco user					
	No	20.5 (83)		-	
	Yes	30.9 (34)		-	
			0.022		
Practice oro-genital sex*	No	-		19.1 (76)	
	Yes	-		29.1 (34)	
					0.022

*Only among those 18-45 years that responded to this question

Of the participants, 19.7% (n=167), 6.4% (n=54) and 2.1% (n=18) were current smokers, snuff users and betel nut users, respectively (Table 1). Out of the 514 who answered the questionnaire in relation to OS, 22.4% (n=115) reported to practice OS (Table 2). Oral sex practice in the age group 18-45 years was significantly more common among white South Africans (41.9%) than among black South Africans; and among tobacco users than among non-tobacco users (30.9% vs. 20.5%; p=0.022) (Table 2). A multivariable-adjusted regression model showed that compared to black South Africans, white South Africans were more likely to use tobacco (OR=5.25; 95% CI=2.21-12.47) and practice OS (OR=2.38; 95%

CI=1.06-5.35). However, after controlling for confounding factors, the practice of OS was not significantly associated with tobacco use (OR=1.06; 95% CI=0.62-1.83) (Table 3).

Table 3: Multivariable-adjusted regression model of factors associated with tobacco use and practice of oro-genital sex among those 45 years old and younger.

Characteristics		**Practice oro-genital sex* % (N=514) OR (95% CI)**	**p-value**	**Tobacco use % (N=847) OR (95% CI)**	**p-value**
Gender	Male	1		1	
	Female	0.42 (0.27-0.67)	<0.001	0.15 (0.09-0.25)	<0.001
Race					
	Black African	1		1	
	Whites	2.38 (1.06-5.35)	0.035	5.25 (2.21-12.47)	<0.001
Age group					
	36-45 years	1		1	
	18-35 years	1.67 (1.01-2.74)	0.045	1.25 (0.74-2.13)	0.401
Employment status					
	Unemployed	1		1	
	Retired/Student/ unknown	1.15 (0.69-1.94)	0.593	0.50 (0.27-0.92)	0.026
	Employed	0.73 (0.41-1.31)	0.291	0.70 (0.40-1.23)	0.220

Tobacco user					
	No	1		-	
	Yes	1.08 (0.63-1.85)	0.776	-	
Practice oro-genital sex*	No	-		1	
	Yes	-		1.06 (0.62-1.83)	0.825

*Only among those 18-45 years that responded to this question

Discussion

This study showed that about 1 in 5 clinic attendees in this dental training institution practice OS. Furthermore, almost a third of those who practice OS were also current tobacco users. Consistent with the literature, tobacco use were more common among men (38.6%) than among women (12%) and this ratio (3.2:1) is closer than that of the national prevalence (4:1) [32-33]. Our study indeed showed that tobacco use in this predominantly black African population of dental clinic attendees (21.7%) was slightly higher than the reported national prevalence of 17.7% for this population group [33,34].

Despite South African data showing that oropharyngeal cancer in white South African population occurs at a much older age than other ethnic groups [35], no reports on ethnic distribution of OS practice are available for the South African population. However, broader population-based reports of OS practice demonstrate a wide variation between population groups.

Our finding of 32% prevalence of OS practice among males is comparable to 40% prevalence reported among high-risk male South African factory workers recently published [26]. However, the study by Vogt and colleagues [36] reports 84 % of men and 82% of women in heterosexual couples practiced oral sex which was consistent with data from Canada (71%) [28] and the US (80%) [31]. Conversely, another South African study of heterosexual couples, but in a different geographic location, reported that only 8.7 % of women and 6.2% of men reported to practice oral sex which is similar to that reported in China [37,38].

The differences in these reports could be due to different study designs, data collection methods, and analyses. The target population group also plays a role in the reporting of oral sex practice [28]. Conceivably, the practice of OS may be culturally inclined. The number of oral sex partners, the frequency of oral sexual events, and even the duration of each oral sexual event may all play a role in the extent to which OS practice is self-reported. However, these variables were not explored in detail due to the cultural and societal sensitivities surrounding this topic in this population group.

This study highlighted a significantly higher likelihood to practice OS among youth than older adults. This is consistent with the literature [28]. Furthermore, considering that OS is a significant source of exposure to HPV, OS may partly explain why HPV-associated oropharyngeal SCC is more common in younger people [10]. The practice of OS by younger adults has been characterised as a normative social practice that is less intimate and others do this in an effort to avoid pregnancy [39] and as a "benefit-provisioning mate retention behaviour" [40]. A study of 410 younger heterosexual adult women reported that OS was performed as a way to express love and care to their male partner [40]. The higher risk for OS among youths support targeted interventions such as the promotion of condom and dental dam in the prevention of oral HPV infection [41]

There were significant racial differences in the practice of OS and tobacco use with white South Africans most likely to report both risk behaviours for oral and oropharyngeal cancer. On the one hand, OS increases the risk of HPV-exposure and on the other hand, smoking reduces the clearance of HPV, which means that white South Africans who are more likely to both smoke and practice OS may be at a higher risk to develop oral and oropharyngeal infection. It is nevertheless pertinent to note that in this study, smoking was not significantly associated with OS practice, therefore neither of these risk behaviours can be used as a risk behaviour marker for the other.

The practice of OS was twice more common among white than black South Africans in this study. This relatively low frequency of OS, in particular among black South Africans, may explain why despite the fact that in South Africa the prevalence of genital HPV infection is as high as 22.1% among women [42] with one study demonstrating a prevalence of 68% [43], the prevalence of oral HPV infection (3.5-8.4%) [38,44] is relatively low. In fact, only about

20% of HIV-seropositive black women with genital HPV infection have concurrent oral HPV infection, and in only half of this 20% can the genital HPV genotypes be detected in the mouth [8]. Self-inoculation via the genital-oral route has been suggested as a source of oral HPV infection in the South African setting [38].

Study limitations

Some caution in the interpretation of our study findings in relation to the study's limitations would include the fact that the OS and tobacco behaviour were self-reported. It may indeed be that respondents provided sociably desirable responses and that this may be an under-representation of OS practice and of tobacco use. The findings of this study are limited to dental clinic attendees therefore may not be generalized to the general South African population.

Due to cultural and societal sensitivities associated with the practice of OS in this population group, the nature of the OS practice, including frequency of practice, was not further investigated. We believe that forcing this sensitive topic on this population would have greatly reduced participation and this project sensitised many participants and non-participants in this population to a topic considered taboo.

Despite these limitations, this study provides useful information for prioritizing public health interventions and for further research, which may include more in depth demographic and epidemiological profile of those who practice OS and the presentations of signs and symptoms of related infection.

Conclusion

The study findings suggest that tobacco use and the practice of oral sex are not significantly associated risk behaviours and thus could be considered independent risks for oral and oropharyngeal infection. Furthermore, age and ethnic differences in both risk behaviours suggest need for targeted population intervention in order to prevent and reduce the incidence of oral and oropharyngeal infection. Community engagement and further investigation are required concerning perceptions of oral sex practice and tobacco use.

Disclosure of potential conflicts of interest:

The authors declare that they have no conflicts of interest

Ethical Approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This project was specifically approved by the Sefako Makgatho Health Sciences University Research Ethics Committee, clearance number MREC/D/187/2010:IR.

Informed consent:

Consent was informed and obtained in writing from all individual participants included in this study.

References:

1. Petti S. Lifestyle risk factors for oral cancer. Oral Oncol. 2009;45: 340-350.

2. Shah JP, Gil Z. Current concepts in management of oral cancer surgery. Oral Oncol. 2009;45: 394-401.

3. Upile T, Jerjes W, Al-Khawalde M, Radhi H, Sudhoff H. Oral sex, cancer and death: sexually transmitted cancers. Head Neck Oncol. 2012;4: 31.

4. Farsi NJ, El-Zein M, Gaied H, Lee YCA, Hashibe M, Nicolau B, et al. Sexual behaviours and head and neck cancer: A systematic review and meta-analysis. Cancer Epidemiol. 2015;39: 1036-1046.

5. Shibosky CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 22-44 years. Cancer. 2005;103: 1843-1849.

6. Gillison M, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, et al. Distinct risk factor profiles for Human papillomavirus type 16-positive and Human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008;100:407-420.

7. Gillison ML, Castelsagué X, Chaturvedi A, Goodman MT, Snijders P, Tommasino M, et al. Eurogin roadmap: Comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. Int J Cancer. 2014;134:497-507.

8. Richter KL, Janse van Rensburg E, van Heerden WFP, Boy SC. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. J Oral Pathol Med. 2008;37: 555-559.

9. Paquette C, Evans MF, Meer SS, Rajendran V, Adamson CS, Cooper K. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in Black South Africans. Head and Neck Pathol. 2013;7: 361-372.

10. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45: 309-316.

11. International Agency for Research on Cancer (IARC). Tobacco smoking. IARC Monogr Eval Carcinog Risk Chem Hum. 38, Lyon: IARC, 1986.

12. Johnson N. Tobacco use and oral cancer: a global perspective. J Dent Edu. 2001;65: 328-339.

13. Giuliano AR, Sedjo RL, Roe DJ, Harri R, Baldwi S, Papenfuss MR, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes & Control. 2002;13: 839-846.

14. Fakhry C, Gillison ML, D'Souza G. Tobacco use and oral HPV-16 infection. JAMA. 2014;312: 1465-1467.

15. Kreimer AR, Pierce Campbell CM, Lin H, Fulp W, Papenfuss MR, Abrahamsen M, et al. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. Lancet. 2013;382: 877-887.

16. Mbulawa ZZ, Johnson LF, Marais DJ, Coetzee D, Williamson A. The impact of human immunodeficiency virus on human papillomavirus transmission in heterosexually active couples. J Infection. 2013;67: 51-58.

17. D'Souza A, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviours associated with prevalent oral human papillomavirus infection. J Infect Dis. 2009;199: 1263-1269.

18. Rintala M, Grenman S, Puranen M, Syrjänen S. Natural history of oral papillomavirus infections in spouses: A prospective Finnish HPV family study. J Clin Virol. 2006;35: 89-94.

19. Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Oral mucosa as a reservoir of human papillomavirus: Point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. Eur Urol. 2012;62: 1063-1070.

20. Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, et al. Oral human papillomavirus infection in adults is associated with sexual behaviour and HIV serostatus. J Infect Dis. 2004;189: 686-698.

21. Stanley M. Prevention strategies against the human papillomavirus: the effectiveness of vaccination. Gynecol Oncol, 2007;107: S19-S23.

22. Syrjanen, S. Human papillomaviruses in the head and neck carcinomas. N Engl J Med. 2007;365**:** 1993-1995.

23. Hobbs CG, Stern JA, Baily M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin Otolaryngol. 2006;31: 461-462.

24. Van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. Oral Oncol. 2010;46: 423-425.

25. Smith EM, Rubenstein LM, Haugen TH, Pawlita M, Turek LP. Complex etiology underlies risk and survival in head and neck cancer Human Papillomavirus, tobacco, and alcohol: a case for multifactor disease. J Oncol. 2015; ID571862, Doi:10.1155/2012/571862.

26. Davidson CL, Richter KL, van der Linde M, Coetsee J, Boy SC. Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: a pilot study. S Afr Med J. 2014;104: 358-361.

27. Johnson EH, Jackson LA, Hinkle Y, Gilbert D, Hoopwood T, Lollis CM, et al. What is the significance of black-white differences in risky sexual behaviour? J Natl Med Assoc. 1994;86: 745-749.

28. Vannier SA, O'Sullivan LF. Who gives and who gets: why, when, and with whom young people engage in oral sex. J Youth Adolescence. 2012;41: 572-582.

29. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. J Clin Oncol. 2015;33: 3235-3242.

30. Jelihovschi I, Bidescu AC, Tucaliuc SE, Iancu LS. Detection of human papillomavirus in head and neck squamous cell carcinomas: a literature review. Revista Medico-chirurgicala a Societatii de Medici si Naturalisti din lasi. 2015;119: 502-509.

31. D'Souza G, McNeel TS, Fakhry C. Understanding personal risk of oropharyngeal cancer: risk-groups for oncogenic oral HPV infection and oropharyngeal cancer. Ann Oncol. 2017;28: 3065-3069.

32. Peer N, Bradshaw D, Laubscher R, Steyn N, Steyn K. Urban-rural and gender differences in tobacco and alcohol use, diet and physical activity among young black South Africans between 1998 and 2003. Glob Health Action. 2013;6: 19216.

33. Reddy P, Zuma K, Shisana O, Jonas K, Sewpaul R. Prevalence of tobacco use among adults in South Africa: Results from the first South African National Health and Nutrition Examination Survey. SAMJ. 2015;105: 648-655.

34. Peer N, Bradshaw D, Laubscher R, Steyn K. Trends in adult tobacco use from two South African demographic and health surveys conducted in 1998 and 2003. S Afr Med J 2009;99: 744-749.

35. Ayo-Yusuf OA, Lalloo R, Johnson NW. Trends and ethnic disparities in oral and oro-pharyngeal cancers in South Africa 1992-2001. SADJ. 2013;68: 168-173.

36. Vogt SL, Gravitt PE, Martinson NA, Hoffman J, D'Souza G. Concordant oral-genital HPV infection in South Africa couples: evidence for transmission. Front Oncol. 2013;3: 1-7.

37. Ma Q, Pan X, Cai G, Yan J, Xu Y, Ono-Kihara M, et al. The characteristics of heterosexual STD clinic attendees who practice oral sex in Zhejiang province, China. Plos One. 2013;8, e67092.

38. Mbulawa ZZ, Johnson LF, Marais DJ, Coetzee D, Williamson AL. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. J Infection. 2014;68: 185-189.

39. Vannier SA, Byers ES. A qualitative study of university students‘ perceptions of oral sex, intercourse, and intimacy. Arch Sex Behav. 2013;42: 1573-1581.

40. Sela Y, Shackelford TK, Pham MN, Eule, HA. Do women perform fellatio as a mate retention behaviour? Pers Individ Dif. 2015;73: 61-66.

41. Gupta A, Perkins RB, Ortega G, Feldman S, Villa A. Barrier use during oro-genital sex and oral Human Papillomavirus prevalence: Analysis of NHANES 2009-2014. Oral Dis. 2018;00:1-8. https://doi.org/10.1111/odi.12997

42. De Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Munoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007;7: 453-459.

43. Mbulawa ZZ, Coetzee D, Marais DJ, Kamupira M, Zwane E, Allan B, et al. Genital human papillomavirus prevalence and human papillomavirus concordance in heterosexual couples are positively associated with human immunodeficiency virus coinfection. J Infect Dis. 2009;199: 1514-1524.

44. Marais DJ, Passmore JA, Denny L, Sampson C, Allan BR, Williamson AL. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. J Med Virol. 2008;80: 953e9.

CHAPTER 3

Oral and Oropharyngeal HPV prevalence in South Africa. A systematic review and meta-analysis

Under Review at time of Thesis submission:

Oral Surgery Oral Medicine Oral Pathology Oral Radiology

CHAPTER 3

Oral and Oropharyngeal HPV prevalence in South Africa. A systematic review and meta-analysis

Neil Hamilton Wood[1] Neil.wood@smu.ac.za; neilwood28@gmail.com

Pagollang David Motloba[2] Pagollang.Motloba@smu.ac.za

Louisa Nokukhanya Makwakwa[3] Nokukhanya.Makwakwa@smu.ac.za

Johannes Bogers[4] jpbogers@ambior.org; john-paul.bogers@uantwerpen.be

[1]Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

[2]Department of Community Dentistry, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

[3]Clinical Manager, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

[4]Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Belgium.

Corresponding author:

Neil H Wood – neil.wood@smu.ac.za, BOX D26, Medunsa, 0204, South Africa

Word Count: 2874

Author Contribution Statement: NHW and JB conceptualized the work. NHW PDM and NLM made substantial contributions to the design of the work. NHW, NLM and PDM contributed towards the acquisition, analysis and interpretation of data. NHW and PDM wrote the first draft. NHW, JB and PDM provided substantial intellectual content and input. NHW, PDM, NLM and JB revised the work critically for important intellectual content. NHW, PDM, NLM and JB gave final approval of the version submitted for publication. NHW, PDM, NLM and JB agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. NHW is responsible for the overall work and stands as guarantor.

Abstract:

Objectives: There is a shortage of prevalence data for HPV infection in the head and neck in Southern African populations. In addition to cervical cancer, this sexually transmitted oncogenic virus is responsible for a subset of head and neck cancer and is transmitted via oral sexual routes, and through other forms of intimate contact between anatomical sites lined by mucosa. This systematic review and meta-analyses aimed to synthesize data for the prevalence of head and neck HPV infection in South Africa.

Methods: Original research papers from South Africa reporting on the prevalence of HPV in the head and neck was systematically reviewed using PubMed, Ovid Medline, Embase and the Cochrane Library. A meta-analysis on the prevalence data was conducted for 16 papers that met the inclusion criteria.

Results: This systematic review and meta-analysis reports a pooled prevalence for head and neck HPV infection of 11%. The study shows both a shortage of, and a data lag for, HPV prevalence studies in the head and neck of Southern African populations. Technological improvement over time, differences in data collection methodology, differences in laboratory analysis processes and differences in the selection of study populations including various population risk levels all influence the prevalence measurement outcome.

Conclusion: Prevalence reporting for head and neck HPV in South Africa is lagging with only a few reports in existence over a 25-year period. The overall pooled prevalence of 11% is slightly higher than global averages. Populations comprising higher risk groups exhibit higher HPV prevalence rates which is in part influenced by the existence of comorbidities in these groups. Methodologic and study design consistencies and standardization will improve prevalence reporting in this geographic region.

Key Words: HPV prevalence, oropharynx, oral, HPV, human papillomavirus

Introduction

The Human papillomavirus (HPV) is classified as oncogenic to humans (group 1 infectious agent) and the oropharynx is an anatomic site associated with high-risk (HR)-HPV-induced epithelial transformation.[1] This is the most common sexually transmitted pathogen that includes oro-genital and oral-oral transmission routes.[2] The virus infects the basal keratinocytes of a micro-lacerated mucosal surface.[3]

Clinically productive HPV infection of the skin or mucosae result in benign or malignant lesions. The benign HPV-induced lesions are typically single exophytic proliferations often described as having a cobblestone-like surface or finger-like projections. These lesions are usually of a normal mucosal coloring unless traumatized or secondarily infected.[3] There are also reports that describe HPV association with oral leukoplakic lesions that exhibit dysplastic change, with oral lichen planus and with erythroplakia, although these may represent incidental findings. Associations with malignancies have been reported for cases of verrucous carcinomas.[4-6] However, a subset of head-and-neck carcinomas driven by HPV infection as seen in younger, sexually active individuals is now well recognized.[7-10]

It appears that some HPV types exhibit stronger gender bias than others,[11-13] although this association may be artefactual or biased, as many HPV-prevalence studies focus purely on the cervix. In a study from the US, men had three times higher asymptomatic HPV infection than women.[14] The reasons for this gender disparity remain unclear.[9]

High-risk HPV-types may be found in benign HPV-associated oral lesions and low-risk (LR)-HPV types may be identified in malignant HPV-associated lesions like oropharyngeal SCC (OPSCC).[15] Some view the presence of HPV in the mouth as being an oral HPV carrier, or as a passenger infection[16-18]; and these individuals are probable transmitters of the virus[19] through oral sex practices and through open mouth kissing.[2]

The prevalence of HPV in the mouth and oropharynx has been studied globally, but there is an apparent lack of substantial data from sub-Saharan Africa, but specifically, South Africa. A 2012 systematic review and meta-analysis that reported findings on malignancies resultant from infectious agents also found a significant lack of prevalence studies from developed areas.[20] Only 3 South African studies were included in a 2018 systematic review,[9] and the

Human papillomavirus and related diseases report cited only two South African studies, both dealing with oral/oropharyngeal squamous cell carcinoma.[21]

Given the epidemiologic shift in HR-HPV infection of the head and neck and its closer association with OPSCC,[9] it becomes even more urgent to estimate the burden of HPV infection of South Africans in terms of incidence and persistence. The systematic synthesis of these epidemiologic data would improve our understanding of the diseases and of its natural course in this geographic setting.

Methodology

Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) was adopted in conducting this meta-analysis. The CoCoPop (Condition, Context and Population) framework was adopted to generate the following keywords - **Condition** - Oral HPV, oropharyngeal HPV; **Context** - South Africa, English language; **Population** - Human subjects. Two authors (NHW and LNM) performed searches of PubMed, EBSCOhost, MEDLINE and Embase. For additional articles, grey literature, conference proceedings and reference lists from previously published research were reviewed. To augment the search, MeSH and Emtree subject headings were used individually and in combination.

Study selection

Studies were included if they satisfied the following criteria: (i) human subjects, (ii) described the incidence, prevalence of oral or oropharyngeal HPV (ii) South African population. The following studies were excluded (i) systematic reviews, (ii) meta-analyses, (iii) case control studies, (iv) case studies, (v) studies of nonhuman subjects.

Quality Assessment

The study quality was assessed using the Critical Appraisal Checklist for Studies Reporting Prevalence Data published by the JBI. The tool scores the studies on a total score of 9 points, with higher scores indicative of better design and quality. Two researchers (NHW and LNM), blindly assessed the studies and assigned a rating of "poor" (≤3), "fair" (4–7), or "good" (≥8) to each study. Only studies achieving a rating of good by both reviewers were included in the

analysis. Any disagreement was resolved through discussion between the researchers, or by third reviewer (DPM).

Data Extraction

A form specially developed for this study was used by two independent authors to extract information. The variables of interest included: authors and date of publication; characteristics of the study population (gender, age, sexual orientation); site of sample collection (oral cavity, oropharynx), method of sample collection (oral brush, rinse and gargle, and tissues blocks), prevalence of HPV (crude prevalence and population size).

Data analysis

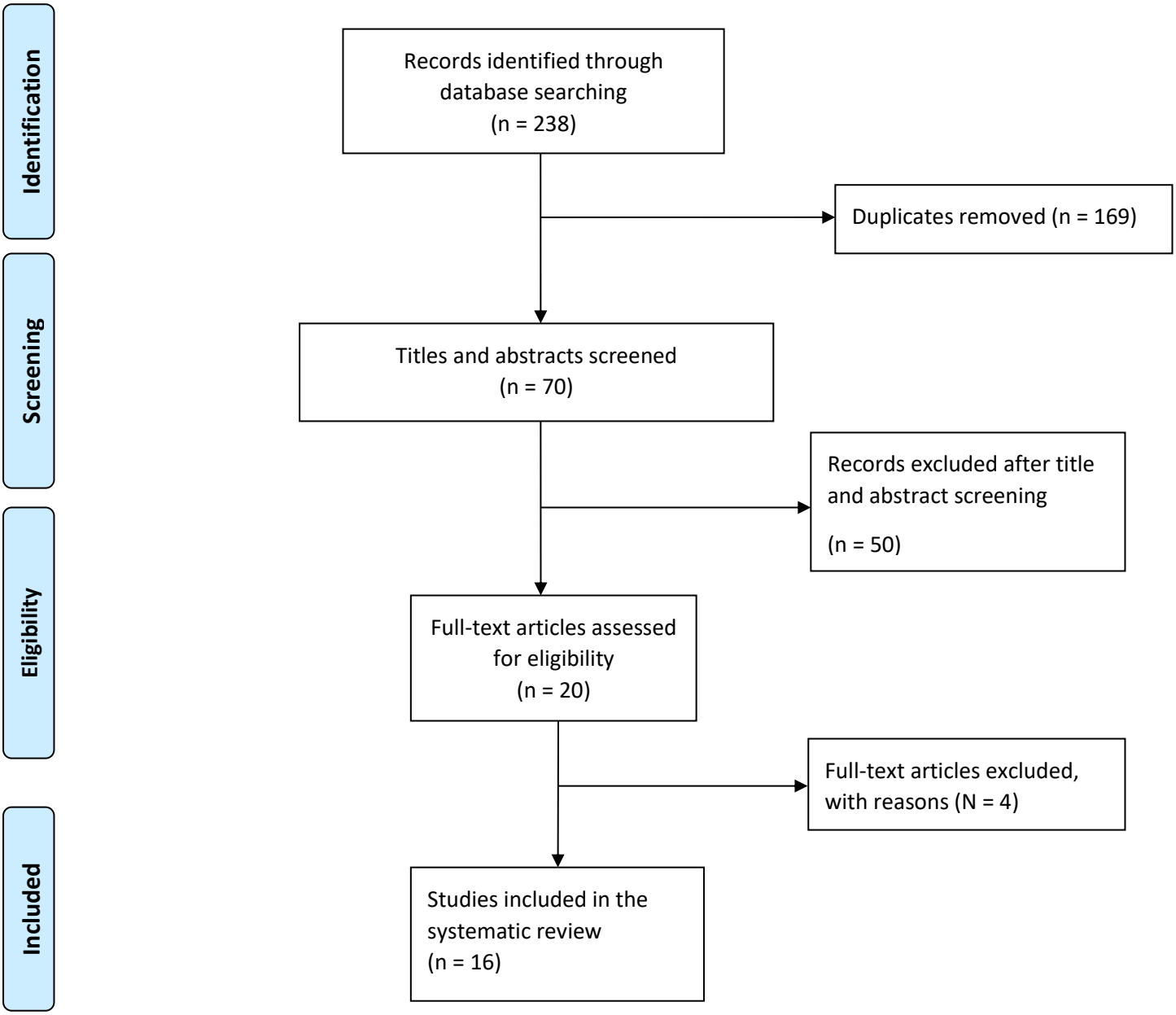
The measure of effect size (Prevalence of HPV) was computed using the Metaprop command for the meta-analysis of proportions in Stata®. Metaprop is appropriate for proportions, which range from 0 or 100% and guarantees that CIs remain within the 0 to 1 range. This stability is achieved by using the binomial distribution to model within-study variability. In this study, the effect size was calculated together with the corresponding 95% CI using the Wald method executed with the cimethod (score) command. Forest plot was generated to show the individual and pooled effects size, 95% CI, the author's name, publication year and study weights (both for primary studies and this systematic review/meta-analysis), based on subgroups. The random effects model was used to compute the overall estimate of prevalence and the 95% confidence interval. The Cochran's I^2 index was calculated to measure heterogeneity among studies, with $p < 0.05$ indicative of heterogeneity. The I^2 values of <25%, between (25% and 50%), and of >50% reflected, mild, moderate and high heterogeneity respectively.

Subgroup analysis was performed to assess sources of heterogeneity among the studies. Variables included in the subgroup analysis included publication date, site and method of sample collection. Additional sensitivity analysis was done using the "leave-one-out" approach to evaluate the robustness of the pooled results. By removing one study at a time, the weighted or disproportional influence of a single study on the overall prevalence was evaluated. Publication bias was checked by visual inspection of funnel plots of prevalence and precision and statistical tests.

Results:

A total of 239 records were obtained from a comprehensive literature search. After the screening of 70 abstracts and titles, 20 full texts were reviewed, of which 16 satisfied the inclusion and exclusion criteria and were included in the analysis (Figure 1). The 16 studies were published between 1995 and 2020 with the total sample size of 2478 and 230 confirmed cases of HPV (Table 1). Larger heterogeneity was anticipated for the small South African data pool.

Figure 1: PRISMA flow diagram detailing literature-search details



Study	Date	Site	Population / Sample type	Risk	N	% Prevalence (n)	Specimen
Mbulawa et al.[22]	2014	Oral	Heterosexual couples	Low	442	8,4 (37)	Oral brush
Boy et al.[16]	2006	Oral and OP	Tissue	UD	59	11,9 (7)	FFPE tissue blocks
Chikandiwa et al.[23]	2018	Oral and OP	HIV+ Men	High	181	1,7 (3)	Rinse and Gargle
Muller et al.[24]	2016	OP	MSM	High	200	11,5 (23)	OP brush
Davidson et al.[25]	2014	Oral and OP	Men	Low	125	5,6 (7)	Rinse and Gargle
Richter et al.[26]	2008	Oral	HIV+ Women	High	30	20 (6)	Oral brush
Vogt et al.[27]	2013	Oral and OP	Heterosexual couples	Low	68	14,7 (10)	Oral brush
Paquette et al.[18]	2013	OP	Tissue	UD	51	94,1 (48)	FFPE tissue blocks
Van Rensburg et al.[28]	1995	Oral	Tissue	UD	66	1,5 (1)	FFPE tissue blocks
Van Rensburg et al.[29]	1996	Oral	Tissue	UD	146	3,1 (2)	FFPE tissue blocks
Mistry et al.[30]	abstract	Oral and OP	MSM	High	199	6 (12)	Rinse and Gargle
Marais et al.[31]	2008	Oral	Women with cervical disease	High	105	26,7 (28)	Oral Brush
Marais et al.[32]	2006	Oral	Dental clinic attendees	Low	116	3,4 (4)	Oral Brush
Sekee et al.[33]	2018	OP	Tissue	UD	20	5 (1)	FFPE tissue blocks
Bulane et al.[34]	2020	OP	Tissue	UD	449	7,3 (33)	FFPE tissue blocks
Wood et al.[2]	2020	OP	Dental clinic attendees & HIV+ patients	UD &High	221	3,6 (8)	Rinse and Gargle

Table 1: Demographics of populations and methods of included studies.

OP = Oropharynx; FFPE = Formalin-fixed paraffin-embedded; UD = undetermined

Heterogeneity assessment

Heterogeneity of the studies was assessed using Cochran's Q and I^2 statistics. The random-effects model was adopted to pool the study-specific prevalence rates and adjust for variability, attributable to large heterogeneity ($P<0.000$ and $I^2=95.07$). To better understand the methodological and clinical variation we undertook subgroup according to source and type of specimen type, publication date and risk profile.

Subgroup and sensitivity analysis

Prevalence statistics for the pooled HPV studies (n=16), as well subgroup and sensitivity analysis estimates are summarized in table 2. The estimated overall prevalence of the HPV was 0.11 (95% CI = 0.06 - 0.17). The sensitivity analysis was furthered by removing two studies, [18, 31] which reported significantly higher prevalence and small sample sizes (table 2). Subgroup analyses revealed that HPV was more prevalent in specimens that were sourced from the oropharynx (0.237; 95% CI = 0.059 - 0.67) than in those from the oral cavity (0.072; 95% CI = 0.03 - 0.163), and in the case where specimen were sourced from both sites (0.069; 95% CI = 0.037 - 0.125). Similarly, studies that were published before 2010, reported higher prevalence than the ones published in the last 10 years, (0.237; 95% CI = 0.059 – 0.67) and (0.237; 95% CI = 0.059 – 0.67) respectively. Equally, the prevalence differed according to the level of risk and type of specimen used in the detection of HPV. These proportions did not differ significantly among the subgroups except for specimen type (p=0.044) (table 2).

Table 2: Subgroup analysis of oral HPV by site, specimen and date

Subgroup	(n) studies	Prevalence (%)	95% Confidence Interval	Heterogeneity I^2 (%); p-value
Overall	16	11.0	6.0 – 17.0	95.07; 0.000
Site				
Oral	6	8.0	2.0 – 16.0	91.03; 0.00
Oropharynx	4	27.0	3.0– 62.0	98.49; 0.00
Oral & Oropharynx	6	6.0	3.0 – 10.0	74.50; 0.00
Specimen type				
Rinse & Gargle	4	4.0	2.0 – 7.0	49.21; 0.12
Oral Brush	6	13.0	7.0 – 19.0	85.31; 0.00
FFPE tissue blocks	6	16.0	1.0 – 40.0	97.87; 0.000
Date of publication				
Before 2010	6	8.0	2.0 – 19.0	91.21; 0.000
2010 and beyond	10	12.0	5.0 – 22.0	96.35; 0.000
Sensitivity analysis				
n-1[18]	15	7.0	4.0 – 10.0	85.57; 0.000
n-2[18,31]	14	7.5	5.1 – 11.6	72.13; 0.000

The exclusion of the outlier study by Paquette and colleagues,[18] resulted in significant decrease in prevalence, (0.07; 95% CI = 0.04 – 0.1) (Table 2). The additional exclusion of population risk levels e.g. the inclusion of studies on men who have sex with men, and to differences in geographic location between the included studies.

Discussion

The prevalence of HPV infection of the head and neck is well documented globally. However, these data are not representative of the South African community. Among the factors supporting this statement is the relative lack of analyses from general populations in South Africa, with the bulk of the studies reporting on HPV detection in the head and neck coming from focussed groups, all with varying confounding factors that contribute towards HPV acquisition (Table 1). Several meta-analyses have been done over a number of years with very few to no papers being included from the South African setting. The 2010

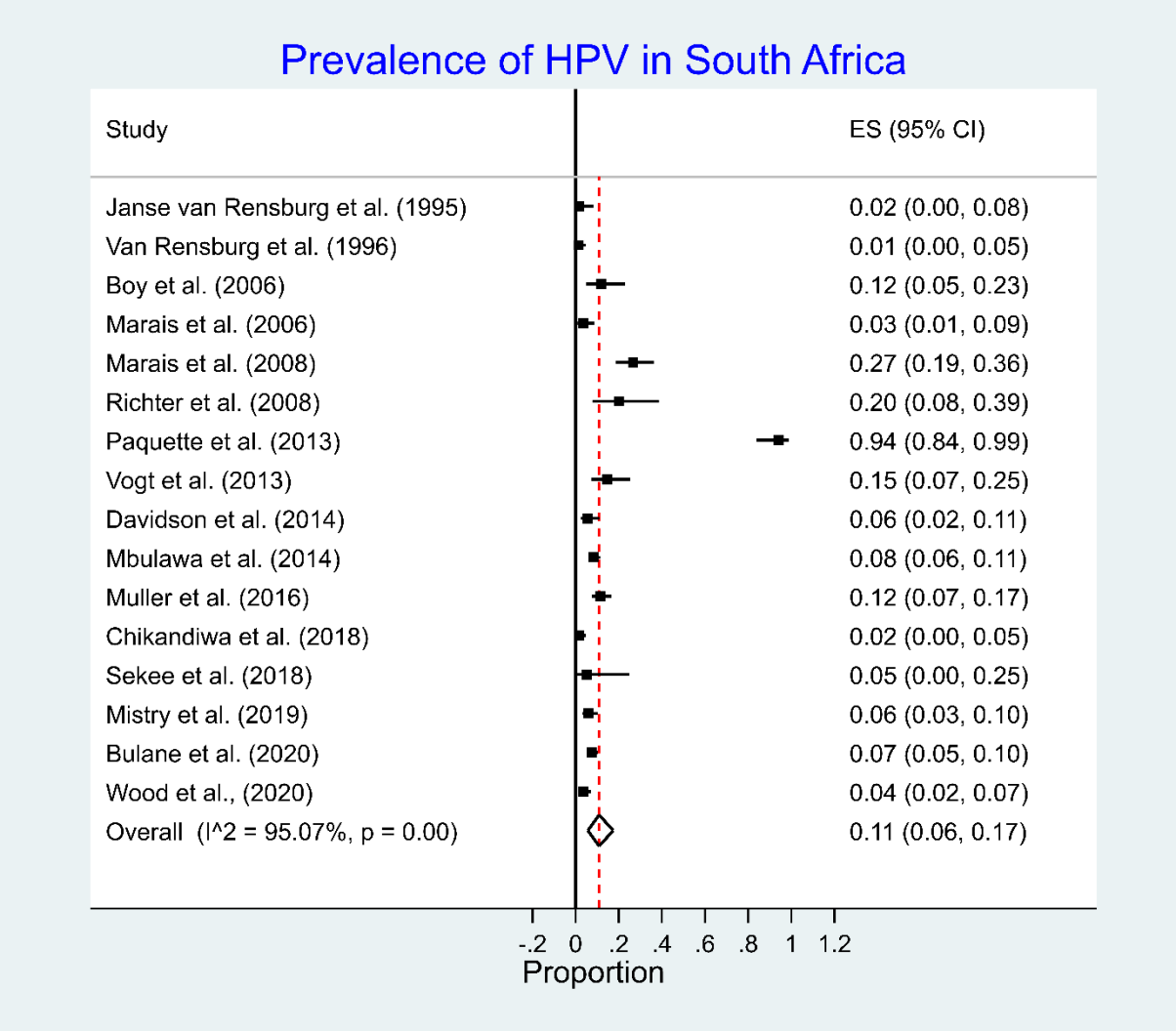
systematic review by Kreimer and colleagues included only one paper from South Africa.[35] The *Human Papillomavirus and Related Diseases Report* cited 2 South African studies, but these were not included in the 9 studies found suitable to present a picture of the global HPV burden in the head and neck.[21]

The detection of HPV-DNA does not imply infection by HPV but may represent a passenger infection or carrier status. Some individuals may however harbour asymptomatic, but definite HPV infection of which a smaller portion, usually HR-HPV persists, and subsequently increases the risk for malignant transformation.[3, 18] Persistence data for HR-HPV infection of the head in neck in South Africans are lacking.

Specimen collection, sampling, analyses methods

Patient demographics, geographic location, population risk profiles, sampling methods, anatomical sites sampled, tissue specimen type and quality, storage and transport, and detection methods all influence the reported prevalence rates of HPV-infection.[3, 9, 13, 26, 36] The salivary detection of HPV is influenced by immune efficiency, smoking and the sensitivity and specificity of the PCR-technique applied.[19, 37] Detection frequency of different HPV types is also influenced by the specific intra-oral site infected, and the presence or absence of oral diseases such as periodontitis or oral lichen planus that expose basal keratinocytes.[3, 38] Oral wash specimens do not allow for oral site discrimination but come into contact with portions of the oropharynx and can therefore not be considered as an exclusively oral specimen.
study by Marais *et al*.,[31] had a negligible influence of the HPV prevalence rate, (0.075; 95% CI = 0.051 – 0.116). Overall, these two studies resulted in a 33.5% increase in the overall estimate of HPV (7.5% to 11.0%), which provides critical explanation on the source of the observed heterogeneity in this particular study (Figure 2).

Figure 2: Forest plot – estimated prevalence of oral and oropharyngeal HPV detection in South Africa



Publication Bias

The risk of publication bias was studied by funnel plot analysis, Egger's test and Begg's correlation. The statistical tests point to absence of bias; Egger's test (p=0.415), Begg's (p=0.293) respectively. We conclude that there is no evidence of publication bias in this meta-analysis. Therefore, the large heterogeneity cannot be attributed to publication bias, but rather to clinical and methodological variations among the included studies, to differences between

This meta-analysis validates the impact of various study designs and conduct on the estimation of the prevalence of HPV. Therefore, the observed heterogeneity can be reasonably attributed to differences in clinical and methodological differences, and to some extent population risk

level. The heterogeneity of the published papers makes a meaningful systematic review highly challenging, yet necessary.[13]

Data on HPV-associated malignancies of the head and neck:

HPV-16 carcinogenicity is established for oral/oropharyngeal and laryngeal squamous cell carcinoma (SCC), and is considered a requisite aetiologic factor for a molecular and clinically distinct subset of head and neck SCC.[7, 20, 21, 35] Proportionally, oral/oropharyngeal cancers that originate from HR-HPV infection may be small, but HPV-16 is responsible for the vast majority of these SCC (40-60%).[35, 39] Data from the U.S. clearly shows that HPV-driven head and neck SCC has overtaken cervical SCC.[9]

Data on oral and oropharyngeal cancer from Africa is limited when compared to contributions from other geographic regions, and is usually presented as small series.[27, 40] A systematic review published in 2013 on prevalence of HPV-infection in oropharyngeal and non-oropharyngeal head and neck malignancies reported no data from Africa and state that the research on HPV-infection in these lesions are needed from Africa.[13] Similarly, the meta-analyses by Dayyani and colleagues[41] and by de Martel and co-authors[20] did not include any data from Southern Africa. The *Human papillomavirus and related diseases report*[21] cited only two papers[16, 29] from South Africa with regard to HPV-associated head and neck SCC further highlighting the lack of good data from South Africa.

Evaluation of case-control studies in which tumour tissue was analysed for HPV E6 and E7 oncoprotein expression showed that the HR-HPV was prevalent in 13% of lesion tissue in studies originating outside of North America, Europe, Australia, and Japan.[20] The South African studies reporting HPV detection in oral/oropharyngeal SCC tissue specimens reflect a range of 1.4% to 94.1% (Table 1). Due to this high level of heterogeneity, subgroup analysis could not be undertaken to estimate the pooled prevalence of HPV related to the SCC. The prevalence of oral and oropharyngeal mucosal HPV infection remains undescribed for the general South African population, despite the existence of smaller sampled cohorts.

Systematic review

The association between HPV and oral and or pharyngeal carcinoma necessitates the estimation of HPV presence in a variety of population subgroups. Large heterogeneity renders the computation of the summary estimate challenging. The clinical and methodological

heterogeneity between the studies included in this systematic review varied greatly. First, the lag bias which is characterised by delayed publication of HPV papers in South Africa extends over 15-year period. This phenomenon could account for differences in diagnostic and identification of HPV as underpinned by advanced in technology and developed protocols. The prevalence of HPV increased from an average of 8.0% in the 1990s to 12.0% a decade later. Six studies compared to ten (10) were published in these periods, suggesting the presence of publication bias. Similar findings in the systematic review of 66 papers by Tam and colleagues[9], indicated the increase in HPV prevalence from 3.0% to 7.9% in the 1990's to the 2010's. Secondly, variations in methodologies, population risk profiles and geographic location differences, choice of anatomical sites and specimens, and differences in laboratory investigations may reflect the passage of time, thus further accounting for greater heterogeneity. Despite the design shortcomings, this meta-analysis represents the recent attempt to provide a summarised estimate of the burden of the prevalence of oral and oropharyngeal HPV in South Africa.

The prevalence of HPV detection ranges between 6.2% and 17.7% compared to our estimate of 11.0%.[42, 43] However, detection increases when studying groups with associated risk factors for HPV transmission/infection. An HPV prevalence of 10% and more has been reported in studies among high-risk men who have sex with other men and also in women diagnosed with cervical diseases. In HIV-seropositive patients, head and neck HPV-detection can be up to 30%, with chances for oral detection of HPV being up to three times higher in HIV-seropositive patients.[44, 45] Benign and potentially malignant oral lesions are associated with HPV prevalence estimates of around 18.6% and 24.5% respectively.[46] Trzcinska and colleagues reported 29% of head and neck squamous papillomas as positive for HPV.[47] The higher approximation of HPV in our study could therefore be attributed to the inclusion of populations at high risk, the deployment of more sensitive diagnostic techniques in the latter decade and increasing the spectrum of any HPV subtype identification. As reported in table 1, the effects of specimen collection and processing, and of risk factors had a major impact on the high HPV estimates and observable heterogeneity.

Conclusion

The pooled estimate of 11% of oral and oropharyngeal HPV infection from the limited number of South African studies provides a reasonable approximation of HPV prevalence despite the

limitations shown in this study. The increasing prevalence of head and neck HPV among the high-risk groups is attributable to coexistence with comorbidities. The improvement in technology over time has increased the positive detection rate, which could explain the spike in the reported prevalence. This meta-analysis demonstrated the effect of knowledge lag on the estimation of HPV prevalence. To counteract this phenomenon, studies must be standardised and must use the most robust protocols for the detection of HPV. This will permit comparability and computation of reliable estimates of prevalence for the South African population.

Prospective incidence and persistence studies are critical for other vulnerable or high-risk population groups to determine whether preventive vaccination is required, or exclusive vaccination of the female population would confer sufficient protection in these particular groups.

REFERENCES:

1 IARC. Monographs on the evaluation of carcinogenic risks to humans, volume 100. A review of carcinogen-Part B: biological agents. Lyon: International Agency for Research on Cancer, 2011.

2 Wood NH, Makua KS, Lebelo RL, *et al*. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-positive cohort from Pretoria, South Africa. *Adv in Virol* 2020;ID 2395219. https//doi.org/10.1155/2020/2395219

3 Syrjänen S. Oral manifestations of human papillomavirus infections. *Eur J Oral Sci* 2018;126(Suppl 1):49–66.

4 Syrjänen KJ. Human papillomavirus (HPV) infections and their associations with squamous cell neoplasia. *Arch Geschwulstforsch* 1987;57:417–444.

5 Syrjänen S, Lodi G, von Bültzingslöwen I, *et al*. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Dis* 2011;17(Suppl 1):58–72.

6 Grce M, Mravak-Stipetic M. Human papillomavirus-associated diseases. *Clin Dermatol* 2014;32:253–258.

7 Chaturvedi AK, Anderson WF, Lortet-Tieulent J, *et al*. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol* 2013;34:4550-4554.

8 Leemans CR, Snijders PJ, Brakenhoff RH. The molecular landscape of head and neck cancer. *Nature Rev* 2018;18:269-282.

9 Tam S, Fu S, Xu L, *et al*. The epidemiology of oral human papillomavirus infection in healthy populations: A systematic review and meta-analysis. *Oral Oncol* 2018;82:91-99.

10 Yete S, D'Souza W, Saranath D. High-risk Human papillomavirus in oral cancer: Clinical implications. *Oncology* 2018;94:133-141.

11 Komlos KF, Kocjan BJ, Kosorok P, *et al*. Tumor-specific and gender-specific pre-vaccination distribution of human papillomavirus types 6 and 11 in anogenital warts and laryngeal papillomas: A study on 574 tissue specimens. *J Med Virol* 2012;84:1233-1241.

12 Danielewski JA, Garland SM, McCloskey J, *et al*. Human papillomavirus type 6 and 11 genetic variants found in 71 oral and anogenital epithelial samples from Australia. *PLOS ONE* 2013;8:e63892. https://doi.org/10.1371/journal.pone.0063892

13 Mehanna H, Beech T, Nicholson T, *et al*. Prevalence of Human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer – systematic review and meta-analysis of trends by time and region. *Head & Neck* 2013;35:747-755.

14 Sanders AE, Slade GD, Patton LL. National prevalence of oral HPV infection and related risk factors in the U.S. adult population. *Oral Dis* 2012;18:430–441.

15 Prabhat MP, Lakshmi CR, Madhavi NS, *et al*. Multifocal epithelial hyperplasia of oral cavity expressing HPV 16 gene: a rare entity. *Case Reports in Dent* 2013; ID 871906. https://doi.org/10.1155/2013/871306

16 Boy S, Janse van Rensburg E, Engelbrecht S, *et al*. HPV detection in primary intra-oral squamous cell carcinomas – commensal, aetiological agent or contamination? *J Oral Pathol Med* 2006;35:86–90.

17 Fatahzadeh M, Schlecht NF, Chen Z, *et al*. Oral human papillomavirus detection in older adults who have human immunodeficiency virus infection. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013;115:505-514.

18 Paquette C, Evans MF, Meer SS, *et al*. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in black South Africans. *Head and Neck Pathol* 2013;**7**:361-372.

19 Gaester K, Fonseca LA, Luiz O, *et al*. Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors. *Sci Rep* 2014;4:6592.

20 De Martel C, Ferlay J, Francheschi S, *et al*. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012;13:607–15.

21 Bruni L, Albero G, Serrano B, *et al*. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. *Summary Report*, 17 June 2019. https://www.hpvcentre.net/statistics/reports/XWX.pdf, (accessed 3 August 2020).

22 Mbulawa ZZA, Johnson LF, Marais DJ, *et al*. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. *Journal of Infection*. 2014;68:185-189.

23 Chikandiwa A, Pisa PT, Chersich MF, *et al*. Oropharyngeal HPV infection: prevalence and sampling methods among HIV-infected men in South Africa. *Int J STD & AIDS* 2018;29:776-780.

24 Muller EE, Rebe K, Chirwa TF, *et al*. The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. *BMC Infect Dis* 2016;16:440.

25 Davidson CL, Richter KL, van der Linde M, *et al*. Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: A pilot study. *SAMJ* 2014;104:358–361.

26 Richter KL, Janse van Rensburg E, van Heerden WFP, *et al*. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. *J Oral Path Med* 2008;37:555-559.

27 Vogt SL, Gravitt PE, Martinson NA, *et al*. Concordant oral-genital HPV infection in South Africa couples: evidence for transmission. *Frontier Oncol: Cancer Epidem Prev* 2013;3:1-7.

28 Van Rensburg EJ, van Heerden WF, Venter EH, *et al*. Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population. *S Afr Med J* 1995;85:894-896.

29 Van Rensburg EJ, Engelbrecht S, Van Heerden WF, *et al*. Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. *Anticancer Res* 1996;16:969-973.

30 Mistry HB. Dissertation: Prevalence of oral and oro-pharyngeal Human papillomavirus infection in a selected cohort of men from a rural/semi-rural area in Gauteng, South Africa. Sefako Makgatho Health Sciences University, Pretoria, South Africa, 2011. https://www.smu.ac.za/library/

31 Marais DJ, Passmore DS, Denny L, *et al*. Cervical and oral Human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *J Med Virol* 2008;80:953–959.

32 Marais DJ, Sampson C, Jeftha A, *et al*. More men than women make mucosal IgA antibodies to Human papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population. *BMC Infect Dis* 2006;6:95.

33 Sekee TR, Burt FJ, Goedhals D, *et al*. Human papillomavirus in head and neck squamous cell carcinomas in a South African cohort. *Papillomavirus Res* 2018;6:58-62.

34 Bulane A, Goedhals D, Seedat RY, *et al*. Human papillomavirus DNA in head and neck squamous cell carcinomas in the Free State, South Africa. *J Med Virol* 2020;92:227-233.

35 Kreimer AR, Bhatia RK, Messeguer AL, *et al*. Oral Human papillomavirus in healthy individuals: A systematic review of the literature. *Sex Transm Dis* 2010;37:386-391.

36 Gillison ML, Broutian T, Pickard RK *et al*. Prevalence of oral HPV infection in the United States, 2009-2010. *JAMA* 2012;307:693-703.

37 Adamopoulou M, Viaraktaris E, Nkenke E, *et al*. Prevelance of human papillomavirus in saliva and cervix of sexually active women. *Gynecol Oncol* 2013;129:395-400.

38 Pinheiro RS, de Franca TR, Rocha B, *et al*. Human papillomavirus coinfection in the oral cavity of HIV-infected children. *J Clin Pathol* 2011;64:1083-1087.

39 D'Souza G, Kreimer AR, Viscidi R, *et al*. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;356:1944 –1956.

40 Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2008;45:309-316.

41 Dayyani F, Etzel CJ, Liu M, *et al*. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol* 2010;2:15.

42 Mravak-Stipetic M, Sabol I, Kranjcic J, *et al*. Human papillomavirus in the lesions of the oral mucosa according to topography. *PLoS ONE*. 2013;8:e69736.

43 Matos, LL, Miranda GA, Cernea CR. Prevalence of oral and oropharyngeal human papillomavirus infection in Brazilian population studies: a systematic review. *Brazil J Otorhinolaryngol* 2015;81:554-567.

44 D'souza G, Carey TE, William WN Jr, *et al*. Epidemiology of head and neck squamous cell cancer among HIV-infected patients. *J Acquir Immune Defic Syndr* 2014;65:603-610.

45 Beachler DC, D'Souza G. Oral HPV infection and head and neck cancers in HIV-infected individuals. *Curr Opin Oncol* 2013;25;503-510.

46 Jayaprakash V, Reid M, Hatton E, *et al*. Human papillomavirus types 16 and 18 in epithelial dysplasia of oral cavity and oropharynx: a meta-analysis, 1985-2010. *Oral Oncol* 2011;47:1048-1054.

47 Trzcinska A, Zhang W, Gitman M, *et al*. Papillomas as detected by real-time PCR and Sanger sequencing. *Head and Neck Pathol* 2020;14:428-434.

CHAPTER 4:

Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV positive cohort from Pretoria, South Africa.

PUBLISHED:

Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken O, Bogers J. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-Positive cohort from Pretoria, South Africa. *Advances in Virology* 2020; 2395219.

https://doi.org/10.1155/2020/2395219.

CHAPTER 4:

Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV positive cohort from Pretoria, South Africa.

Neil H Wood[1] Neil.wood@smu.ac.za; neilwood28@gmail.com
Koketso S Makua[2] koketsomakua@gmail.com
Ramokone L Lebelo[3] Ramokone.Lebelo@smu.ac.za; Lisbeth.Lebelo@gmail.com
Nina Redzic[4] nina.redzic@hotmail.com
Ina Benoy[4] ibenoy@ambior.org
Olivier M Vanderveken[6] olivier.vanderveken@uza.be; olivier@ovanderveken.be
Johannes Bogers[4] jpbogers@ambior.org; john-paul.bogers@uantwerpen.be

1 Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa.
2 Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa.
3 HIV and Hepatitis Research Unit, National Health Laboratory service, Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa.
4 Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Belgium.
5 Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium;
6 Department of ENT, Head and Neck Surgery, Antwerp University Hospital, Edegem, Belgium

PUBLISHED: Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken O, Bogers J. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-Positive cohort from Pretoria, South Africa. *Advances in Virology* 2020; 2395219.
https://doi.org/10.1155/2020/2395219.

Abstract

Introduction: Studies on HPV prevalence in the head and neck region of South Africans are sparse. Of the available reports in the literature, none report on the association between HPV-DNA presence in the mouth and oropharynx in relation to high-risk behaviours such as oral sex practice or tobacco and alcohol use.

Materials and methods: Following ethical clearance and informed consent, patients attending a regional HIV-management clinic, and patients attending a dental hospital were recruited to this study. The participants completed an interview-based questionnaire obtaining demographic information, data on HIV serostatus, and behavioural data including sexual practices, tobacco and alcohol use, and a rinse-and-gargle specimen was taken. Specimens were analysed for HPV DNA on 3 separate PCR/qPCR platforms. Statistical analyses were performed for associations between study group and categorical variables, HPV status, and data from the questionnaires.

Results: Of 221 participants, 149 were from a general population and 72 from the HIV-management clinic. Smokers comprised 29.4% of the sample and 45.2% of participants reported to have ever used alcohol. Open mouth kissing during teenage years was confirmed by 64.7% of participants, 40.3% have given oral sex with their mouth, and 44.8% confirmed to have received oral sex from their partner's mouth. Seven participants (3.2%) had detectable α-HPV DNA, and 1 (0.4%) had detectable β-HPV DNA in their rinse-and-gargle specimens. Two participants were from the HIV-management clinic, and 6 from the general dental population (overall 3.6%).

Conclusion: Five high-risk HPV, 2 low-risk HPV, and one β-HPV types were detected. The low prevalence of 3.6% compares well to similar studies in different cohorts studied in South Africa, and falls within the global oral/oropharyngeal prevalence spectrum. Only 4 participants, all from the HIV-management clinic, had palatine tonsils. No significant relationships were found between HPV presence and demographic data or sexual, oral sexual, tobacco use, or alcohol use; and no associations were seen with numbers of sexual and oral-sex partners.

Key Words:

Human Papillomavirus, prevalence, oral, tonsil, oropharyngeal, oral sex, tobacco, smoking

Introduction

The human papillomavirus (HPV) is epitheliotropic and requires access to the basal cells of epithelium to initiate a complex sequence of events that additionally relies on specific host reactions and interactions to successfully infect the basal keratinocyte [1]. Mucosal infection by high-risk (HR)-HPV subtypes have an established association with an increased risk to develop cervical, anal, penile and oropharyngeal carcinoma [2,3].

Taking into consideration the high HIV prevalence in South Africa and the bidirectional risks in acquisition of either infection, HIV or HPV, in cases where the one precedes the other, the importance of providing prevalence data on oral and oropharyngeal HPV infection becomes urgent [2,4]. The "Human papillomavirus and related diseases" report [5] shows both a deficiency, and a comparative reporting lag, in data from Africa when compared to other geographic regions. This is reflected in various systematic reviews done on this topic [5-7].

The practice of oral sex (OS), open mouth kissing, having multiple sexual partners, and a compromised immune system increase the risk of acquiring oral/oropharyngeal HPV infection in general population groups [8-12]. The virus spreads through direct contact with infected genital mucous membranes or with bodily fluids. In the South African context, the practice of oral sex, open mouth kissing, smoking, and alcohol consumption have not been adequately studied in relation to HPV infection.

Smoking has been shown to influence HPV clearance from the mouth, and in cases of HPV-positive SCC, prognosis is worsened with concomitant smoking [13,14].

This study describes the prevalence of HPV-DNA detected in the mouths and oropharynges of a dental clinic population and of an HIV-seropositive clinic population. High-risk sexual behaviours and habits that also include tobacco and alcohol use are reported.

Materials and methods

Inclusion of patients

Patients who attended the University oral health centre and the HIV management clinic were recruited as a convenience sample. The dental patients attended the institution for treatment of oral health related issues that were unrelated to HPV infection, and the patients attending the HIV clinic came for follow-up and treatment management visits which were also unrelated to the study.

Data collection

An interview-based questionnaire was completed prior to oral/oropharyngeal specimen collection. The questionnaire was developed from a previous project [15] and existing literature. Data collected included age, gender, smoking habits, alcohol use, oral sex (OS) contact/practice, and HIV sero-status among others. In the case of the HIV-seropositive cohort, the most recent CD4+ T cell count and HIV viral load were recorded from the patient file. The questionnaires were sequentially numbered to ensure anonymity, and de-linked from patient names, hospital file-numbers, or other possible identifiers.

Specimen collection and transportation

Participants rinsed for 15 seconds and then gargled for 15 seconds with 10 ml of saline, and then spit the contents into a Thinprep® vial containing Preservcyt® solution. The specimens were stored at 4°C until they were transported to the laboratory. The material in the vial is fixed by Preservcyt® and can be preserved for 6 months at 15-30°C. For the purposes of this report, oral wash implies sampling of the oral and oropharyngeal mucosae. The vials were numbered with the corresponding questionnaire.

HPV testing

All oral washes were tested for the presence of HPV with 3 different assays: Abbott HR-HPV assay and the RIATOL qPCR HPV genotyping assay, both focussing on the presence of α-papilloma viruses and the AML β-human papillomavirus typing assay.

The Abbott *m*2000™ RealTime system was used for the qualitative HPV detection with the HR-HPV assay. This real time multiplex PCR detection kit reports only limited HPV DNA typing results: presence of HPV 16, HPV 18 and other HR-HPV (pooled signal derived from

the following HR-HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The assay is optimized using probe specificity design and an internal Human Beta Globin control which is a sample validity control for cell adequacy, sample extraction and amplification efficiency. The assay is clinically optimised for cervical cancer screening. This clinically-based assay cut-off is also used in this study for the detection of HR-HPV in oral washes.

The Riatol qPCR HPV genotyping assay is an ISO certified, fully automated, clinically validated laboratory-developed test [16]. This qPCR HPV DNA test not only detects 14 HR-HPV types (HPV16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 56 E7, 58 E6, 59 E7, 68 E7), but also reports selected potential high-risk HPV types (HPV 53 E6, 66 E6 and 67 L1) and two low-risk (LR)-HPV types (HPV6 E6, 11 E6). Cellularity control was performed on every sample, by amplification of the beta-globin gene. Based on the beta-globin standard curve, DNA concentration (ng/µl) was determined in every sample. Samples with a DNA concentration below 0,12 ng/µl are considered as invalid and reported as inconclusive. Type specific HPV positivity is defined as having a positive amplification signal for that specific HPV type, independently of the beta-globin signal.

Specimens were also tested for a spectrum of β-papillomaviruses with the AML lab developed assay. The assay involves a TaqMan real-time PCR containing type-specific primers and consensus probes capable of detecting multiple β-HPV types. In total 5 multiplex consensus reaction are performed: three with double targets detecting HPV types 1/63, 3/10, 7/41 and two with triple targets detecting HPV types 2/27/57 and 4/60/65.

Statistics

Sample size estimation was based on the estimation of the proportion of HPV-positive patients in each study group, taking the general global and locally reported prevalence rates into consideration. Based on an overestimated HPV prevalence of 20%, 5% precision and the 95% confidence level, a sample size of 246 would be required for each group [17].

The X^2 test was used to assess the relationship between study group and categorical variables. Fisher's exact test was used for 2x2 tables or where the requirements for the X^2 test could not be met. The strength of the associations was measured by Cramer's V and by the phi-coefficient respectively.

The relationship between study group as well as between age and lifetime sex partners was assessed by the t-test (or ANOVA for more than two categories). Data analysis was carried out using SAS® (version 9.4 for Windows®). The 5% significance level was used.

Ethical approval

This study received ethical clearance from the Sefako Makgatho Health Sciences University Research and Ethics Committee (SMUREC/P/86/2016). Individuals received an information sheet and provided informed consent prior to participation. Participants had the opportunity to ask questions and not one person was disadvantaged or prejudiced in any way in the event of opting not to participate in the study.

Results

A total of 221 participants were enrolled, 149 from the general population attending the dental clinic, and 72 attending the regional HIV-management clinic. The actual sample sizes of 72 (HIV clinic) and 149 (dental clinic) used in this study correspond to a precision of 9.2% and 6.5% respectively (rather than 5.0%), which could be a limitation of the study. For a sample size of 221 this study has the resolution to determine differences between these two datasets. The power value informed the degree of confidence to which our sample size is sufficient in order to see differences between these datasets. A power value of 90% is deemed to be an acceptable level of confidence. Based on these analyses, for a sample size of 221 with a comparison difference of 0.018, the power value is 97.8%. The sample size is therefore sufficient to determine any differences between this dataset and global data. Patients attending these tertiary healthcare institutions are from feeder regions generally considered as being in lower socio-economic areas.

Participants' ages ranged from 20 to 74 years with a mean age of 43.8 years. Majority of the participants were black and more than half were female (Table 1).

Table 1: Demographic data for the study population

		Overall (n=221)		**Group**				
				Dental Clinic (n=149)		**HIV Clinic (n=72)**		**p-value for between-group test**
Variable	**Category**	**n**	**%**	**n**	**%**	**n**	**%**	
Gender	Male	101	46.5	67	45.0	34	47.2	>0.99
	Female	116	53.5	78	52.3	38	52.8	
	Unknown	4						
Ethnicity	Black	210	96.8	138	95.2	72	100.0	0.41
	Caucasian	4	1.8	4	2.8	0	0.0	
	Indian	2	0.9	2	1.4	0	0.0	
	Mixed heritage	1	0.5	1	0.7	0	0.0	
	Unknown	4						

The age demographic represented by means, standard deviation and range

Age	n	n=219	n=147	n=72	0.10
	Mean	43.8	42.7	46.0	
	Std Deviation	14.0	15.0	11.5	
	Range	20-74	20-74	23-72	

Sixteen-point three percent (n=36) of all participants (29.4%), were current smokers with no significant difference between the clinic groups in the number of cigarettes smoked per day. Fifty-four-point eight percent of participants confirmed to have consumed alcohol. Of those who confirmed to have consumed alcohol (n=121), 36.4% consumed alcohol in the week prior to the study and 33.1% reported consuming alcohol during the four months before the study (Table 2). There was no significant difference between the HIV-management clinic and dental clinic participants on the amount of alcohol consumed. Thirteen participants used snuff tobacco (5.9%) (data not shown).

Table 2: Tobacco and alcohol use in both participant groups

Variable	**Category**	**Overall n=221 (%)**		**Group Dental Clinic n=149 (%)**		**HIV Clinic n=72 (%)**		**p-value for between-group test**
Ever used tobacco	No	156	70.6	104	69.8	52	72.2	0.75
	Yes	65	29.4	45	30.2	20	27.8	
Current smoker	No	185	83.7	121	81.2	64	88.9	0.18
	Yes	36	16.3	28	18.8	8	11.1	
Number of cigarettes per day - grouped (if current smoker; n=36)	1-3	11	31	8	29.6	3	37.5	0.60
	4-8	14	40	10	37.0	4	50.0	
	9 or more	10	29	9	33.3	1	15.5	
	Unknown	1						
Ever used alcohol	No	100	45.2	69	46.3	31	43.1	0.67

	Yes	121	54.8	80	53.7	41	56.9	
Last alcoholic drink - grouped (if use alcohol; n=121)	1-7 days	44	36.4	28	35.0	16	39.0	0.049 (V=0.22)
	1 week - 3 months	37	30.6	30	37.5	7	17.1	
	4 months or more	40	33.1	22	27.5	18	43.9	
Number of alcoholic drinks per week - grouped (if use alcohol; n=121)	1	33	29.7	18	25.7	15	36.6	0.46
	2-3	38	34.2	26	37.1	12	29.3	
	4 or more	40	36.0	26	37.1	14	34.1	
	Unknown	10						

Majority (64.7%) of the participants had open mouth kissed during their teenage years (15-19 years) with a significant difference between the two groups where more dental clinic participants practiced open mouth kissing at a very early age (10-14 years) (Table 3). There was also a significant difference in the number of people who practiced open mouth kissing in the past 2 years between the two groups, with data showing that some HIV positive people did not perform kissing in the past 2 years. Of all the participants, 65.2% (n=144) confirmed they knew what OS was, and 40.3% (n=89) reported to have given OS with their mouths and 44.8% (n=99) reporting to have received OS. Only one of these men tested positive for oral/oropharyngeal HPV and was from the dental clinic group, who indicated he did not give, nor receive OS from his single OS partner during the past year. There was a significant association between age and number of lifetime OS partners within the Dental clinic group (n=145; p=0.006)(Table 3). Interestingly the mean age of those with no OS partners was older than that for those with OS partners.

Table 3: Distribution of kissing and sexual behaviour in both participant groups

Variable	**Category**	**Overall n=221 (%)**		**Group Dental Clinic n=149 (%)**		**HIV Clinic n=72 (%)**		**p-value for between-group test**
	10-14 years	20	9.7	17	12.6	3	4,2	

Age at first open mouth kiss	15-19 years	134	64.7	81	60.0	53	73.6	0.039 (phi=0.22)
	20-24 years	39	18.8	24	17.8	15	20.8	
	25-30 years	6	2.9	6	4.4	0	0.0	
	30 years or older	8	3.9	7	5.2	1	1.4	
	Unknown	14						
Number of people kissed with open mouth in last 2 years	0	21	10.4	0	0.0	21	29.2	<0.0001 (V=0.46)
	1	85	42.3	61	47.3	24	33.3	
	2-3	52	25.9	36	27.9	16	22.2	
	4 or more	43	21.4	32	24.8	14	15.3	
	Unknown	20						
Age of first sexual encounter	10-14 years	19	9.1	12	8.8	7	9.7	0.81
	15-19 years	126	60.3	81	59.1	45	62.5	
	20 years or older	16	30.6	44	32.1	20	27.8	
	Unknown	12						
Number of OS partners (lifetime)	0	112	51.6	76	52.4	36	50.0	0.64
	1-2	61	28.1	38	25.5	23	31.9	
	3 or more	44	20.3	31	20.8	13	18.1	
	Unknown	12						
Number of partners given OS to (lifetime) - grouped (if lifetime OS partners >=1; n=105)	1	41	43.2	31	46.3	10	35.7	0.59
	2-3	34	35.8	22	32.8	12	42.9	
	4 or more	20	21.1	14	20.9	6	21.4	
	Unknown	10						

The HIV-management clinic represents a population of exclusively HIV-positive patients, whereas 4.0% of the dental clinic attendees self-reported to be HIV positive. The HPV (α-papillomavirus) prevalence for the dental clinic population (n=149) analysed by the Riatol assay only was 1.4% (95% CI: 0.2-4.9%) (n=2) compared to the 2.8% (95% CI: 0.3-9.8%) (n=2) for the HIV clinic (n=72) with no significant difference between these groups. All specimens except 2 had a positive Beta Globin control. Only 3 participants from the dental clinic, and none from the HIV clinic had HPV-DNA detectable by the Abbott platform. The

combined proportion of oral/oropharyngeal α-HPV-positivity for this study (n=221) was 3.2% (n=7) (Table 4). This compares favourably to international reports. CD4+ T cell counts are available, but no significant associations or relationships were seen with statistical analyses. These patients were overall well controlled.

Palatine tonsils were absent in 100% of the dental clinic participants group, and in 94.4% of the HIV-clinic participants group. None of the α-HPV-positive participants (n=7) had tonsils. Not a single participant presented with HPV-associated lesions during clinical examination.

Table 4: HPV results of oral wash specimens for dental clinic and HIV clinic

Overall for dental clinic and HIV clinic populations			
Test platform*	**Category**	**N=221**	**%**
Abbott HR HPV	Negative	202	91.4
result	Positive (HPV 16, 2x HPV-other)	3	1.4
	Unknown (not run)	16	7.2
Riatol qPCR HPV	Negative	213	96,3
genotyping result	low risk HPV positive (HPV 53, 67)	2	0.9
	high risk HPV positive (HPV 16, 35)	2	0.9
	DNA insufficient run	2	0.9
	Non evaluable (technical error)	2	0.9
AML β-HPV	Negative	216	97.7
genotyping result	Positive (HPVgoup 4/40/65)	1	0.5
	DNA insufficient	2	0.9
	Non evaluable (technical error)	2	0.9

**All specimens were tested on the three different platforms*

Table 5 shows the 8 participants that were positive for HPV, 2 from the HIV-clinic and 5 from the dental clinic, had detectable α-HPV DNA (Table 5). One participant, from the dental clinic, had detectable β-HPV DNA. The discrepancy between these two HPV tests is generally seen in HPV assays where the overall concordance is rather low, especially in low-viral-load samples (as here). No significant associations were found between HPV presence and any demographic factor or high-risk behaviour, nor with number of sex or of OS partners (supplementary file).

Table 5: Summary of the HPV-positive participants from both populations (N=221)

Age	Gender	Type identified	Test platform	Population	Smoking current	OS given**	Alcohol use*
31	F	HPV-16	Abbott	Dental clinic		Yes	
66	F	HR-HPV	Abbott	Dental clinic		Yes	
35	M	HR-HPV	Abbott	Dental clinic			
28	M	HR-HPV 35	Riatol	Dental clinic	Yes	Yes	Yes
49	M	HR-HPV 16	Riatol	HIV clinic		Yes	
36	M	LR-HPV 53	Riatol	Dental clinic	Yes		Yes
46	M	LR-HPV 67	Riatol	HIV clinic			
66†	M	*β-HPV 4/60/65*	Riatol	Dental clinic			Yes

**during the 30 days preceding specimen collection* ***OS given with participant's mouth*

†*only participant where β-papillomavirus was detected as either HPV-4, or HPV-60, or HPV-65*

Discussion

There are limited data describing the spectrum of HPV genotypes infecting the oral and oropharyngeal mucosae of the general South African population, and there are similarly no reliable data describing the types of HPV present in oral/oropharyngeal HPV-associated benign and malignant lesions of South African populations. Previous studies from South Africa on oral/oropharyngeal HPV infection were further limited by smaller specific cohorts [18-22]. The authenticity of the lower overall prevalence rate (3.2%) found in this study is further supported by other independent studies from South Africa [18,22,23].

In general, HPV prevalence studies are centred around the α-papillomaviruses because of the strong oncogenic association and transduction potential of E6/E7 oncoproteins. However, it is not strange to find HPV from other genera in the mouth and oropharynx and some even associated with benign lesions and more recently, potentially malignant lesions [24-26]. However, Agalliu and co-workers [27] suggested that HPV other than α-papillomaviruses may have a part to play in the aetiology of head and neck SCC, being the first to report a positive association between infection with β- and/or γ-HPV subtypes. Only one participant

from this study had demonstrable β-HPV DNA, and we can only speculate that this was a passenger-infection (background infection) as described in the literature [19,21].

Although this study did not interrogate multiple HPV-types in the individual specimens, it did look at HPV-16, HPV-18 and other HR-HPV types. The finding of multiple types infecting a single individual is not infrequently reported. It is entirely possible for one patient to have two HPV types infecting the same mucosal field, and also possible for one patient to have one type affecting more than one mucosal field, e.g. oro-genital as evident by numerous concordance studies for different anatomical locations lined by mucosa. No incidental finding of multiple HPV- type infections were made, and the 8 HPV positive specimens in this study all had single HPV subtypes detected as determined by the three independent assays.

Oral and oropharyngeal HPV prevalence studies report prevalence rates of up to 10.0% for patients considered otherwise healthy. Many of these do not present data on HPV genotypes. In the USA, prevalence of oral HPV infection in a group of 14-69 years old participants was 6.9% and higher among men. The study showed a HR- HPV prevalence of 3.7% and a low-risk HPV prevalence of 3.1% [28]. Another US study demonstrated oral HPV-infection prevalence of 2.4 % in 18-30-year olds, and confirmed the oral-to-oral and oral-to-genital transmission routes of HPV [29]. These fall within the range of a 2010 meta-analysis of oral HPV prevalence globally which showed a prevalence of under 5.0% across different demographic strata [30]. The combined prevalence of 3.2% found in this study is within this spectrum.

A Swedish study of oral HPV-infection in youths between the ages of 15-23 years demonstrated higher HPV prevalence rates for males and females at 9.3% and 9.5% respectively. However, females with concomitant cervical HPV-infection had significantly higher oral HPV prevalence rates (17.1%) when compared to those without cervical HPV infection [31]. South African studies on HPV prevalence in the mouth and oropharynx are limited to 14. Of these, only 2 were conducted from oral rinse and gargle samples [22,23], 6 used oral brushes to collect samples [18,20,32-35], and 6 were performed on formalin-fixed paraffin-embedded SCC samples [19,21,36-39]. This highlight the importance for data on HPV infection in the head and neck region of South Africans.

An investigation of oral/oropharyngeal HPV presence in 128 South African male factory workers revealed an oral/oropharyngeal HPV prevalence of 5.6% [23]. Chikandiwa and colleagues [22] recently demonstrated an oral/oropharyngeal HPV prevalence of 1.8% in a male population from Cape Town in South Africa. These compare favourably with the prevalence of 3.2% found in this study. Evidence for oral-genital HPV transmission from a South African study reveals oral/oropharyngeal HPV prevalence of 15.0% for the 34 adults examined, but unfortunately did not analyse HPV subtypes detected [33]. A novel South African pilot study was in agreement with the Swedish data: when the oral and cervical mucosae were examined for HPV presence in 30 HIV-seropositive women, Richter and co-workers found that oral mucosal HPV presence was higher (20.0%) in those HIV-positive females with concomitant cervical HPV-infection [20]. The authors used a brush-biopsy technique to collect the samples. In contrast, Meyer et al., [40] found no significant difference in the prevalence rates of oral HPV-infection when comparing women with cervical HPV-infection (5.7%) to those without (5.1%).

The group led by Mbulawa [34] proposed that in the African setting, oral/oropharyngeal HPV is acquired from sexual partners, but in woman it may also be due to autoinoculation. The authors reported oral HPV infection in 8.4% of study participants. Further to this, it is conceivable that when using saliva as a lubricant, autoinoculation and cross infection with HPV may occur [41].

High risk sexual practices such as unprotected sexual intercourse, having multiple sexual partners and the practice of oral sex/anal sex need to be investigated concomitantly with oral/oropharyngeal HPV prevalence, because these are classic risk factors for HPV transmission. However, data on oral-sexual practice from South Africa are very limited [17], and no information relating this behaviour to different types of HPV infecting the oral and oropharyngeal mucosae in South Africans exists [23]. The proportion of participants who reported to have ever practiced oral sex were 46.3% for the dental clinic attendees, and 50.0% for the patients attending the HIV management clinic (47.5% combined). This is much higher than a previous report published from the same area which revealed that 22.4% of participants practiced oral sex [15]. In this current study, no link between the practice of oral sex and HPV presence could be demonstrated following analysis of the data, in part because of the extraordinary low prevalence of HPV detected.

Global data suggests a much higher oral/oropharyngeal HPV prevalence for HIV-seropositive patients; and HPV infection rates of these mucosal sites are proportional to the level of immune suppression [33]. This study does not align with these global data in that only 2/72 (2.7%) HIV-positive participants had detectable HPV, and none had any clinically evident HPV lesions. This was no different when compared to the general dental participants in which 6/149 (4%) had detectable HPV (Table 5). Oropharyngeal HPV infection rates are up to 3 times higher in HIV-seropositive patients than in patients with oral SCC [42]. Conversely, it is also reported that the risk for HIV infection is increased in instances of a preceding HPV infection [4]. The natural course and progression of HPV infection also appears to be different to that in HIV-negative individuals due to the HIV-induced immune alteration [43].

It is still unknown whether early or later HPV infection plays any significant role in the mouth/oropharynx; nor has latency of HPV in humans been established conclusively [44]. However, several reports of immune reconstitution inflammatory syndrome following highly active antiretroviral treatment have been described with clinical manifestations of HPV infection that include rampant proliferation of oral and peri-oral papules and wart-like projections [43,45,46]. In the HIV-seropositive cohort of this study, no HPV-associated oral lesions were identified, and an oral/oropharyngeal HPV prevalence of 2.8% (2/72) in this particular cohort was found.

Tobacco and alcohol use are considered the traditional risk factors in the development of oral/oropharyngeal squamous cell carcinoma. However, a slow decline in tobacco use globally has also been accompanied by a rise in oropharyngeal cancer incidence [47]. A distinct subset of oropharyngeal HPV-induced carcinomas is already recognized and are increasing in prevalence with HPV-16 seemingly responsible for 40-60% of these SCC's [48-50]. The odds of developing oropharyngeal SCC rises more than 13 times when HPV 16 is found in keratinocytes shed from the oral/oropharyngeal mucosa [49]. Oral SCC incidence in South Africa is reported as 2.7/100 000 and SCC of the pharynx as 2.4/100 000 [33].

In a report on trends regarding tobacco use in South Africa, the prevalence of smoking among black South Africans was 22.7% and 36.6% among Caucasians; and the trend reflected a decrease in smoking [21,51]. Another South African study from a different geographic area reported 19.7% of participants to be smokers, and 6.4% used snuff tobacco [15]. This study

reports 29.4% of participants who indicated to have ever used tobacco, and 16.3% to be current smokers. Thirteen participants (5.9%) used snuff. Aside from delaying HPV clearance, it is also known that smokers with concomitant HR-HPV infection in oropharyngeal SCC have a significantly higher risk of disease recurrence than non-smoking counterparts [13].

HPV infection has a stronger association with tonsillar SCC than SCC of the mouth [20]. The dispersed reticular network of epithelial cells found in the tonsillar crypts expresses a primitive differentiation. These CD44-positive and nerve growth factor receptor (NGFR)-positive cells have been shown to have a higher proliferative potential than regular mucosal counterparts. These proliferating cells also express CK5 which is a basal cell marker. The micro-structural arrangement of these cryptal epithelial reticulations leads to the cells being exposed to the external environment, making it easier for HPV-access. No micro-laceration or wounding is required. The proof of principle for this was published by Kang and colleagues [52]. However, the influence of cryptal surface progenitor cells has not been well-studied with regards to maintenance of cryptal epithelial cells. Similarly, very little information exists on the influence of HPV on the different epithelial cell population found in the tonsil [52].

An interesting incidental finding from this study's population groups showed that 98.2% of participants had their palatine tonsils removed. None of the oral/oropharyngeal HPV prevalence studies we interrogated included data on the presence or absence of palatine tonsils. It is therefore conceivable that the absence of tonsils could play a preventive role in HPV-associated SCC of the head and neck. This needs further investigation.

It could be that the oral cavity serves the passenger infection model as a reservoir to inoculate the tonsils and other more distant mucosal sites [19,21]. The "oral HPV reservoir theory" was also postulated in the context of HIV seropositive adults by Fatahzadeh and colleagues [53]. In order to further advance the knowledge frontier on the natural course and impact of oral/oropharyngeal HPV infection, the importance of early detection for prevention, persistence studies cannot be overlooked, and must be standardized methodologically. Females receiving the HPV vaccine are believed to receive protection for HR-HPV types currently associated with head and neck cancer. This must be done in parallel to cost-vs-benefit analyses and determination of optimal vaccine administration time in these cohorts

[30]. It remains to be seen if this herd-immunity will result in coverage of other high-risk populations such as men who have sex with men, and HIV-positive cohorts practicing high risk behaviours.

Conclusion

The overall prevalence of oral/oropharyngeal HPV DNA in dental clinic attendees and in attendees of an HIV-management clinic is low in this report. This data compares favourably to other South African papers in different cohort studies and also falls within the expected global prevalence range. One participant presented with β-papillomavirus DNA present in the rinse and gargle specimen.

Oral/oropharyngeal mucosal infection by HPV is not implied by the detection of any HPV-DNA in these participants. The possibility that these represent passenger infections that serve to inoculate other anatomical sites lined by mucosa must be considered. Single-site co-infection with multiple HPV types may be detected only as a singular HPV positivity using conventional PCR platforms and should be individually identified through sequencing [21]. A significant finding was that a large portion of the population did not have palatine tonsils which raises the question whether tonsillectomy is prophylactic in the South African population, however, with such limited data on HPV-positive oral and oropharyngeal SCC, this is a question that remains to be answered. Similarly, the value of vaccination in order to prevent a very distinct subset of head and neck cancer must be studied.

Data Availability

Dataset is uploaded at the time of submission of this manuscript as a supplementary file, without restrictions named dataset.xlsx

Conflicts of Interest

The authors declare that they have no conflicts of interest emanating from the publication of this paper.

Funding Statement

This work was funded by the Sefako Makgatho Health Sciences University Research Development Grant. This work received additional funding from the Flemish government (IUC-VLIR-OUS) and the funding agency had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

References

1. Letian T, Tianyu Z. Cellular receptor binding and entry of human papillomavirus. Virology Journal 2010; 7:2.

2. Syrjänen S. Oral manifestations of human papillomavirus infections. Eur J Oral Sci 2018; 126(Suppl. 1):49–66.

3. Yete S, D'Souza W, Saranath D. High-risk Human papillomavirus in oral cancer: Clinical implications. Oncology 2018; 94:133-141.

4. Houlihan CF, Larke NL, Watson-Jones D, Smith-McCune KK. Shibosky S, Gravitt PE, Smith JS, Kuhn L, Wang C, Hayes R. Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis. AIDS 2010; 26:2211-2222.

5. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human papillomavirus and related diseases in the world. Summary Report, 17 June 2019. https://www.hpvcentre.net/statistics/reports/XWX.pdf, accessed 27 November 2019.

6. Kreimer AR, Clifford GM, Boyle P, et al. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 15:467-475.

7. De Martel C, Ferlay J, Francheschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13:607–15.

8. D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009; 199:1263–1269.

9. Feller L, Khammissa RA, Wood NH, Lemmer J. Epithelial maturation and molecular biology of oral HPV. Infect Agents Cancer. 2009; 4:16. doi: 10.1186/1750-9378-4-16.

10. Adamopoulou M, Viaraktaris E, Nkenke E, Avgoustidis D, Karakitsos P, Sioulas V, Nisyrios T, Yapijakis C. Prevelance of human papillomavirus in saliva and cervix of sexually active women. Gynecol Oncol. 2013; 129:395-400.

11. Betiol J, Villa LL, Sichero L. Impact of HPV infection on the development of head and neck cancer. Braz J Med Biol Res. 2013; 46(3):217-26.

12. Cubie HA. Diseases associated with human papilloma virus infection. Virology 2013; 445:21-34. http://dx.doi.org/10.1016/j.virol.2013.06.007

13. Maxwell JH, Kumar B, Feng FY, Worden FP, Lee JS, Eisbruch A, Wolf GT, Prince ME, Moyer JS, Teknos TN, Chepeha DB, McHugh JB, Urba SG, Stoerker J, Walline HM, Kurnit DM, Cordell KG, Davis SJ, Ward PD, Bradford CR, Carey TE. Tobacco Use in Human Papillomavirus–Positive Advanced Oropharynx Cancer Patients Related to Increased Risk of Distant Metastases and Tumor Recurrence. Clin Cancer Res 2010; 16:1226–1235.

14. Gaester, K. Fonseca LAM, Luiz O, Assone T, Fontes AS, Costa F, Duarte AJ, Casseb J. Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors. Sci Rep 2014; 4:6592. DOI:10.1038/srep06592

15. Wood NH, Ayo-Yusuf OA, Gugushe TS, Bogers J. Tobacco use and oral sex practice among dental clinic attendees. PLoS ONE 2019; 14(3):e0213729. https://doi.org/10.1371/journal. pone.0213729

16. Benoy I, Xu L, Vanden Broeck D, Poljack M, Valenčak AO, Arbyn M, Bogers J. Using the VALGENT-3 Framework to Assess the Clinical and Analytical Performance of the RIATOL qPCR HPV Genotyping Assay. J Clin Virol 2019; 120:57-62.

17. Daniel WW (2013). Biostatistics: A Foundation for Analysis in the Health Sciences. 10th edition. New York: John Wiley & Sons, Chapter 6, p191.

18. Marais DJ, Sampson C, Jeftha A, Dhaya D, Passmore JS, Denny L, Rybicki EP, Van der Walt E, Stephen LXG, Williamson A. More men than women make mucosal IgA antibodies

to Human papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population. BMC Infect Dis 2006; 6:95.

19. Boy S, Janse van Rensburg E, Engelbrecht S, Dreyer L, van Heerden M, van Heerden E. HPV detection in primary intra-oral squamous cell carcinomas – commensal, aetiological agent or contamination? J Oral Pathol Med 2006; 35:86–90.

20. Richter KL, Janse van Rensburg E, van Heerden WFP, Boy SC. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. J Oral Path Med 2008; 37:555-559.

21. Paquette C, Evans MF, Meer SS, Rajendran V, Adamson CS-C, Cooper K. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in black South Africans. Head and Neck Pathol. 2013; 7:361 – 372.

22. Chikandiwa A, Pisa PT, Chersich MF, Muller EE, Mayaud P, Delany-Moretlwe S. Oropharyngeal HPV infection: prevalence and sampling methods among HIV-infected men in South Africa. Int J STD & AIDS 2018; 29(8):776-780.

23. Davidson CL, Richter KL, van der Linde M, Coetsee J, Boy SC. Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: A pilot study. SAMJ. 2014; 104:358–361.

24. Bottalico D, Chen Z, Dunne A, Ostoloza J, McKinney S, Sun C, Schlecht NF, Fatahzadeh M, Herrero R, Schiffman M, Burk RD. The oral cavity contains abundant known and novel human papillomaviruses from the betapapillomavirus and gammapapillomavirus genera. J Infect Dis 2011; 204:787–92.

25. Paolini F, Rizzo C, Sperduti I, Pichi B, Mafera B, Rahimi SS, Vigili MG, Venuti A. Both mucosal and cutaneous papillomaviruses are in the oral cavity but only alpha genus seems to be associated with cancer. J Clin Virol 2013; 56:72-76.

26. Martin E, Dang J, Bzhalava D, Stern J, Edelstein ZR, Koutsky LA, Kiviat NB, Feng Q. Characterization of three novel human papillomavirus types isolated from oral rinse samples of healthy individuals. J Clin Virol 2014; 59:30-37.

27. Agalliu I, Gapstur S, Chen Z, Wang T, Anderson RL, Teras L, Kreimer AR, Hayes RB, Freedman ND, Burk RD. Associations of Oral α-, β-, and γ-Human papillomavirus types with risk of incident head and neck cancer. JAMA Oncol 2016; 2:599-606.

28. Gillison ML, Broutian T, Pickard RK, Tong Z, Xiao W, Kahle L, Graubard BI, Chaturvedi AK. Prevalence of Oral HPV Infection in the United States, 2009-2010. JAMA. 2012; 307:693-703.

29. 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. Sex Transm Dis 2012; 39:559-566.

30. Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giulliano AR. Oral Human papillomavirus in healthy individuals: A systematic review of the literature. Sex Transm Dis 2010; 37:386-391.

31. Du J, Nordfors C, Näsman A, Sobkowiak M, Romanitan M, Dalianis T, Ramqvist T. Human Papillomavirus (HPV) 16 E6 Variants in Tonsillar Cancer in Comparison to Those in Cervical Cancer in Stockholm, Sweden. PLoS ONE 2012; 7:e36239.

32. Marais DJ, Passmore DS, Denny L, Sampson C, Allan BR, Williamson A. Cervical and Oral Human Papillomavirus Types in HIV-1 Positive and Negative Women With Cervical Disease in South Africa. J Med Virol 2008; 80:953–959.

33. Vogt SL, Gravitt PE, Martinson NA, Hoffmann J, D'Souza G. Concordant oral-genital HPV infection in South Africa couples: evidence for transmission. Frontier Oncol: Cancer Epidem Prev 2013; 3(303):1-7.

34. Mbulawa ZZA, Johnson LF, Marais DJ, Coetzee D, Williamson A-L. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. Journal of Infection. 2014; 68:185-189.

35. Muller EE, Rebe K, Chirwa TF, Struthers H, McIntyre J, Lewis DA. The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. BMC Infect Dis 2016; 16:440.

36. Van Rensburg EJ, van Heerden WF, Venter EH, Raubenheimer EJ . Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population. S Afr Med J 1995; 85: 894– 896.

37. Van Rensburg EJ, Engelbrecht S, Van Heerden WF, Raubennheimer EJ, Schoub BD . Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. Anticancer Res 1996; 16: 969– 973.

38. Sekee TR, Burt FJ, Goedhals D, Goedhals J, Munsamy Y, Seedat RY. Human papillomavirus in head and neck squamous cell carcinomas in a South African cohort. Papillomavirus Res 2018; 6:58-62.

39. Bulane A, Goedhals D, Seedat RY, Goedhals J, Burt F. Human papillomavirus DNA in head and neck squamous cell carcinomas in the Free State, South Africa. J Med Virol 2020; 92:227-233.

40. Meyer MF, Huebbers CU, Siefer OG, Vent J, Engbert I, Eslick GD, Valter M, Klussmann JP, Preuss SF. Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection. Oral Oncol 2014; 50:27-31.

41. Butler LM, Osmond DH, Jones AG, Martin JN. Use of saliva as a lubricant in anal sexual practices among homosexual men. J Acquir Immune Defic Syndr. 2009; 50:162–167.

42. Adamopoulou M, Vairaktaris E, Panis V, Nkenke E, Neukam FW, Yapijakis C. HPV detection rate in saliva may depend on the immune system efficiency. In Vivo 2008; 22:599-608.

43. Cameron JE, Hagensee ME. Oral HPV Complications in HIV-Infected Patients. Current HIV/AIDS Reports 2008; 5:126–131.

44. Egawa N, Egawa K, Griffin H, Doorbar J. Human Papillomaviruses; Epithelial Tropisms, and the Development of Neoplasia. Viruses 2015; 7:3863-3890.

45. Feller L, Khammissa RA, Wood NH, Malema V, Meyerov R, Lemmer J. Focal epithelial hyperplasia (Heck disease) related to highly active antiretroviral therapy in an HIV-seropositive child - a report of a case, and a review of the literature: case study. SADJ 2010; 65:172-175.

46. Moodley A, Wood NH. HIV-associated oral lesions as an indicator for HAART failure: a review. SADJ 2012; 67:348-352.

47. Vokes EE, Agrawal N, Seiwert TY. HPV-associated head and neck cancer. J Natl Cancer Inst 2015; 107:djv34.

48. Zumbach K, Hoffmann M, Kahn T, et al. Antibodies against oncoproteins E6 and E7 of human papillomavirus types 16 and 18 in patients with head-and-neck squamous-cell carcinoma. Int J Cancer 2000; 85:815–818.

49. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007; 356:1944–1956.

50. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: An emerging epidemic of human papillomavirus-associated cancers? Cancer 2007; 110:1429–1435.

51. Van Walbeek C. Recent trends in smoking prevalence in South Africa: some evidence from AMPS data. S Afr Med J. 2002; 92:468-72.

52. Kang SY, Kannan N, Zhang L, Martinez V, Rosin MP, Eaves CJ. Characterization of Epithelial Progenitors in Normal Human Palatine Tonsils and Their HPV16 E6/E7-Induced Perturbation. Stem Cell Reports 2015; 5:1210-1225.

53. Fatahzadeh M, Schlecht NF, Chen Z, Bottalico D, McKinney S, Ostoloza J, Dunne A, Burk RD. Oral human papillomavirus detection in older adults who have human immunodeficiency virus infection. 2013; 115:505-514.

CHAPTER 5

Oropharyngeal high-risk HPV prevalence, HIV status, and risk behaviours in a cohort of South African men who have sex with men

This chapter was completed as part of a master's thesis supervised by myself and Dr RL Lebelo and formed part of an overarching protocol. The master's student is indicated as the first author for the publication

Under review at time of thesis submission:
Interdisciplinary perspectives on Infectious Diseases

CHAPTER 5

Submitted to: Interdisciplinary Perspectives on Infectious Diseases

Oropharyngeal high-risk HPV prevalence, HIV status, and risk behaviours in a cohort of South African men who have sex with men

Harshita B Mistry[1] hashi717@gmail.com

Ramokone L Lebelo[2] Ramokone.Lebelo@smu.ac.za; Lisbeth.Lebelo@gmail.com

F Matshonyonge[2] drmatshox@gmail.com

Maphoshane Nchabeleng[3] Maphoshane.Nchabeleng@smu.ac.za

Matsontso Mathebula[4] Matsontso.Mathebula@smu.ac.za

Johannes Bogers[5] jpbogers@ambior.org; john-paul.bogers@uantwerpen.be

Neil H Wood[6] Neil.wood@smu.ac.za; neilwood28@gmail.com

1 Master student, Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

2 HIV and Hepatitis Research Unit, National Health Laboratory service, Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa.

3 Department of Microbiology, Sefako Makgatho Health Sciences University, Pretoria, South Africa

4 Mecru Clinical Research Unit, Sefako Makgatho Health Sciences University, Pretoria, South Africa

5 Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Belgium.

6 Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

Correspondence should be addressed to: Neil H Wood; neil.wood@smu.ac.za

Abstract

Data lag is evident when observing studies focussing on HPV prevalence in the head and neck of men who have sex with men (MSM) in Southern Africa. Sexual behaviours, other than anal intercourse, and associated factors are similarly underreported. One hundred and ninety-nine MSM were enrolled in this study. Participants completed a questionnaire followed by a clinical oral examination, and a rinse-and-gargle specimen in Thinprep® vials containing Preservcyt® solution was collected. Detection and typing for high-risk HPV were done by an automated system (Abbott® m2000sp). Six percent of MSM in this cohort had high-risk HPV present in the mouth/oropharynx. This cohort averages 29 years of age, more than half were unemployed (53.3%), and 66,8% were HIV seropositive. The most common sexual practice was anal sex (69.4%) followed by oral sex (28.6%), and by rimming (9.6%). A significant association between oral insertive sex and oral/oropharyngeal HPV status was demonstrated (p=0.0038; phi coefficient=0.20). An incidental but significant association between rimming and HIV status was found (p=0.0046; phi coefficient=0.19). The HPV prevalence of 6% reported in this study is in alignment with global reports. The prevalence of oral/oropharyngeal HPV in this MSM cohort was influenced by sexual practices. MSM participants who practiced rimming appear to be at higher risk of HIV acquisition.

Key Words:

MSM, HPV, HIV, Oral, Oropharyngeal, Oral sex, Rimming

Introduction

The Human papillomavirus (HPV), a non-enveloped DNA virus, is the most common sexually transmitted agent. The virus is responsible for a variety of benign lesions, potentially malignant lesions, and malignancies in the form of squamous cell carcinoma (SCC) of different anatomic sites lined by mucosa [1-3]. The association with SCC of the head and neck anatomic region, specifically the oropharynx, is well established [4].

Studies on head and neck HPV infection involving MSM populations are very limited. The 2019 *Information Centre on HPV and Cancer (ICO) Report* [5] demonstrates that there are no reliable data for HPV infection of the head and neck anatomical region in healthy South Africans. Only two studies from South Africa relating to head and neck SCC were included in the report. Only one study from Cape Town investigated oral/oropharyngeal HPV presence

in MSM, along with other anatomic sites [6]. This underpins the urgent need for baseline studies in the Southern African region. This plays a role in increased heterogeneity making the syntheses of meta-analyses for systematic reviews for this geographic area particularly challenging.

MSM are more prone to acquiring oral/oropharyngeal HPV infection due to the practice of high-risk sexual behaviours. This study investigated the presence of oral/oropharyngeal HPV in a group of 200 men-who-have-sex-with-men (MSM), and forms a important baseline contribution to the Southern-African database.

Materials and Methods

This cross-sectional descriptive study was performed in the Clinical Research Unit (MeCRU) at Sefako Makgatho Health Sciences University in Pretoria, South Africa. This unit falls within a 28 km radius of four townships (Ga-Rankuwa, Soshanguve, Mabopane and Mothutlung). The villages of Winderveldt and Mmakau, and the North-western suburbs of Pretoria form part of the catchment area served by the clinic.

Inclusion of participants

Men who self-declared to have sex with other men, who were 18 years or older, resided in targeted areas, and who consented to participate in this study were included. One point two million people fall within the rural and peri-urban areas served by MeCRU. Contact with the MSM occurred in environments where they felt comfortable to interact with staff/data collection teams.

Data and specimen collection

Participants received an information leaflet upon arrival at the research unit. Thereafter a group discussion took place and informed consent was obtained. Participants completed a self-administered questionnaire. Demographic data were collected followed by intra-oral examination and rinse specimen collection. The examination was performed in a fluorescent lighted room with a LED headlamp using standard infection control procedures. From an ethical perspective, patients were referred to the dental hospital for diagnosis and management of any abnormalities seen during their examination.

After the clinical oral examination, participants rinsed their mouth with saline for 15 seconds and gargled for another 15 seconds, then spit the contents into a Thinprep® vial containing the Preservcyt® solution. Rinse specimens were then delivered to the laboratory on the same day for processing and no obstacles were encountered. The specimens were processed according to standard operating procedures of the Department of Virology, Health Science Laboratory, Sefako Makagatho Health Sciences University.

HPV-testing

HPV detection and typing was done using the Abbott® *m*2000™ RealTime system which is a qualitative assay used to detect 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), and to genotype HPV 16 and HPV 18. This real-time, multiplex PCR detection kit was designed to provide clinically meaningful results through the multiplex PCR, and through the clinically-based assay cut off. The assay is clinically optimised for cervical cancer screening and also optimized for high reliability through the probe specificity design and Human-Beta Globin internal control. The clinically-based assay cut-off is utilized in this study for the detection of HR-HPV in oral washes.

Statistics

It was calculated that at least 123 MSM should be recruited for an expected frequency of 25% HPV in the population and taking into account globally and locally reported prevalence rates for oral/oropharyngeal HPV (5% precision, 95% confidence level). A sample size of 200 participants was finally decided on, but one participant was deemed a screen failure. The screen failure was defined as an enrolled participant that subsequently withdrew consent for sample collection.

The data were extracted from the questionnaire to protect the data integrity and anonymity and entered into a Microsoft® Excel® database. Laboratory results were recorded on a data collection sheet and included data on 14 high-risk (HR)-HPV positivity including the two HR-HPV types (16 and 18). Data from the intra-oral examinations were noted in each participant's file and entered into the spreadsheet.

Descriptive data were summarized as categorical values by frequency and percentage tabulation. Continuous variables were summarized by the mean, standard deviation where

applicable and median. The chi-squared test was used to assess the relationship between HPV status and race, HIV status, current sex practices, number of partners and Socio-economic status. The relationship between HPV status and age was assessed by the Wilcoxon rank sum test, since the data did not meet the assumption of the independent samples t-test. The strength of associations was measured by the Cohen's d for parametric tests and the r-value for the non-parametric tests and reported where applicable. Data analysis was carried out using SAS® (version 9.4 for Windows®) and the 5% significance level was used.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Consent was informed and obtained in writing from all individual participants included in this study. This project was specifically approved by the Sefako Makgatho Health Sciences University Research Ethics Committee, clearance number SMUREC/D/300/2016.

Results

Twelve participants (6%) were HR-oral HPV positive with only one of these being HR-HPV 16 as a single infection.

HIV serological tests revealed that 66.8% of the participants were infected with HIV and of these 10 (7.5%) were HPV positive compared to only 2 (3.1%) of those who are HIV negative. The majority of the participants were unemployed (53.3%) compared to 36.0% who were employed and there was a number of students enrolled in the study (10.7%). The mean age of participants was 29 years and the median age 27 years (IQR 24-31y; range 18-47y). The majority of participants where younger than 35 years of age and there was a decline in the age demographic just after 27 years of age. Most of the participants self-identified as black (98.5%) and the remainder as dual heritage (1.5%). Of those who identified themselves as black 6.2% were HPV positive while none of the dual heritage individuals had HPV infection (Table 1). One participant was a screen failure.

Table 1: Comparison of HPV status according to demographic data

		Overall		HPV status				p-value
				Negative		Positive		
Variable	**Category**	**N=199**	**%**	**N=186**	**row %**	**N=12**	**row %**	
Race*	**Black**	196	98,5	183	93,8	12	6,2	>0.99
	Dual heritage	3	1,5	3	100,0	0	0,0	
Social Economic	**Unemployed**	105	53,3	99	94,3	6	5,7	0,41
Status(SES)	**Employed**	71	36,0	65	91,5	6	8,5	
	Student	21	10,7	20	100,0	0	0,0	
	Unknown	2						
HIV status	**Positive**	133	66,8	123	92,5	10	7,5	0,34
	Negative	66	33,2	63	96,9	2	3,1	

** Self-declared by participants*

The mean age of the oral HPV-positive participants was 30,3; the standard deviation was 6,2. The median age for oral HPV positive participants was 29. The age range between the oral HPV positive and negative was the same; positive being between 20-42 years and negative being between 18-47 years (Table 2).

Table 2: Comparison of age and HPV status amongst MSM

Age (y)										**p-value**
HPV	**N Obs**	**N**	**Mean**	**Std Dev**	**Median**	**IQR**		**Min**	**Max**	
Negative	**186**	186	27,9	6,4	27	24	31	18	47	0,15
Positive	**12**	12	30,3	6,2	29	27	34	20	42	

The most commonly reported 'current sex practice' was anal sex (69.4%) followed by oral sex (28.6%), and by rimming (9.6%) (Table 3). Current practice was defined as any of the described forms of sexual contact having occurred in the 6 months leading up to participant enrolment. Fourteen percent of participants who practised oral sex were positive for HR-oral HPV, which was statistically significant (p=0.0057). About 67.5% of the participants reported having multiple partners in the 6 months preceding the study. Almost half (47.1%) of the participants reported to have had 3 or more sexual partners in the 6 months before enrolment. Only 4 out the 90 participants that had 3 or more partners during these 6 months had detectable oral/oropharyngeal HPV. Nine participants reported to have no partners in the past 6 months before enrolling into this study, and 2 of these were positive for oral/oropharyngeal HPV.

A large number of participants (80,9%) practiced oral receptive sex (participant's mouth), 60.8% performed oral insertive sex (partner's mouth), while 52,8% performed both. There was a significant association between oral insertive sex and oral/oropharyngeal HPV status (p=0.0038; phi coefficient=0.20). Of those who ever practiced oral insertive sex, 10.0% were HPV-positive (n=12), compared to 0.0% of those who never practiced oral insertive sex specifically (n=78) (Table 3). These data demonstrated a significant association between oral sex practice and HPV status (p=0.0057; phi coefficient=0.21). Of those who practiced oral sex in the last 6 months, 14.0% were HR-HPV positive (n=57), compared to only 2.8% of those who did not practice oral sex (n=142). For the MSM that performed oral receptive sex and oral insertive sex, oral HR-HPV prevalence was 7.5% and 10.0% respectively (Table 3).

Table 3: Association of sexual behaviour and HPV status

Variable	**Category**	**Overall**		**HPV status**				**p-value**
				Negative		**Positive**		
		N=199	**%**	**N=186**	**%**	**N=12**	**%**	
Current sex practice	**Anal sex**	138	69,4	*	*	*	*	
	Oral sex	57	28,6	49	86,0	8	14,0	0,0057
	Rimming	19	9,6	17	89,5	2	10,5	0,32
	Vaginal sex	1	0,5	*	*	*	*	
Number of partners in past 6 months	**0**	9	4,7	7	77,8	2	22,2	0,051
	1	53	27,7	52	98,1	1	1,9	
	2	39	20,4	35	89,7	4	10,3	
	3 or more	90	47,1	85	95,5	4	4,5	
	Unknown	8						
Sex practices ever done with a male partner	**Oral receptive**	161	80,9	149	92,5	12	7,5	0,13
	Oral insertive	121	60,8	108	90,0	12	10,0	0,0038
	Rimming bottom	92	46,2	86	93,5	6	6,5	0,80
	Rimming top	39	19,6	35	89,7	4	10,3	0,26
	Anal bottom	15	7,5	*	*	*	*	
	Anal top	5	2,5	*	*	*	*	

The results further indicate that more participants (46,2%) performed rimming bottom (having anus licked by partner) compared to rimming top (licking a partner's anus) (19,6%).

A significant association between rimming and HIV status (p=0.0046; phi coefficient=0.19) was observed. Of those who practiced rimming in the preceding 6 months, 94.7% were HIV-

positive, compared to 63.9% HIV positivity in those who did not practice rimming (n=180) during the same time (Table 4).

Table 4: Association with sexual behaviour and HIV status

				HIV status				**p-value**
				Positive		**Negative**		
		Number	**%**	**Number**	**%**	**Number**	**%**	
				133	**66,8**	**66**	**33,2**	
Current	**Oral sex**	57	28,6	37	64,9	20	35,1	0,74
Sex Practise	**Rimming**	19	9,6	18	94,7	1	5,3	0,0046
Number of	**0**	9	4,7	5	55,6	4	44,4	0,58
partners in	**1**	53	27,7	35	66,0	18	34,0	
past 6	**2**	39	20,4	23	59,0	16	41,0	
months	**3 or more**	90	47,1	63	70,0	27	30,0	

None of the study participants presented with any intra-oral HPV-associated or HPV-like lesions when examined. Other intra-oral abnormalities (non-HPV related) were observed in 26.1% (n=52) of the participants and recorded as incidental findings. Within this group of 52 participants, the most commonly recorded oral/dental abnormality was dental caries (51.9%).

Discussion

Studies on HPV in MSM populations are generally limited to vaccination studies that are focussed on anal and/or penile infections as measurements of vaccination outcomes. However, as with other sexually transmitted pathogens, the most probable sources for acquiring oral/oropharyngeal HPV infection in an MSM population are the practice of oral sex (specifically fellatio), of rimming, and of using saliva as a lubricant for anal sex [7-9], and these practices are rarely reported on. Data on the oral lesions or oral abnormalities found in the population described as MSM is sparse [2,10], and head and neck HPV prevalence data for this group are extremely limited in South Africa [6].

As a result of perceived changes in sexual behaviour in addition to the higher prevalence rates of anal and penile HPV infection seen in MSM [2,10], it is conceivable that the prevalence of oral/oropharyngeal HPV infection may be higher in this high-risk cohort than in the general population. The prevalence of oral/oropharyngeal HPV infection is estimated to be 4,5% in healthy individuals of all genders globally, but recent studies suggest that

oral/oropharyngeal HPV prevalence among HIV-infected MSM may range between 20-45% [11]. Data from oral specimens of 1688 healthy men in the US, Mexico and Brazil were analysed and only 4% had oral HR-HPV DNA positivity, with HPV 16 been the most commonly detected genotype.

A recent global meta-analysis investigating oral HPV infection in MSM found that MSM did not have significantly increased prevalence rates of oral HPV infection compared to the general population but highlighted a stand-out group of men who have sex with both men and women that had a significantly higher oral HPV infection rate. The meta-analysis demonstrated an average prevalence of 13,7% for oral presence of any HPV type, and 5,9% for high-risk HPV presence in the mouths of MSM [10]. The number of sexual partners over the 6 years preceding the study was not associated with any of the parameters examined in this study population which seems counterintuitive given results from genital-HPV-related investigations.

Reports on HPV detection in the mouth and oropharynx of general South African populations and on oral/oropharyngeal tissue specimens are limited and are reflected in Table 5. Furthermore, oral/oropharyngeal HPV infection prevalence data amongst South African MSM are limited to one HPV prevalence study showing 11,5% oral HPV detection in an MSM population from Cape Town [6].

Table 5: South African studies reporting on oral/oropharyngeal HPV presence

Authors	Year	Population sample	HPV types investigated
Wood et al. [16]	2020	149 Dental clinic attendees and 72 HIV-management clinic attendees	14 x HR, 3 x potential HR, 2 x LR; 12 x βHPV
Bulane et al. [17]	2020	449 OSCCa cases	16, 18, 31, 33, 45, 58
Sekee et al. [18]	2018	20 OSCCa cases	16, 18, 31, 33, 45, 58
Chikandiwa et al. [19]	2018	181 HIV seropositive men	37 HPV types
Muller et al. [6]	2016	200 MSM	24 x LR; 13 x HR
Davidson et al. [20]	2014	125 male factory workers	19xLR; 3pHR; 15xHR
Mbulawa et al. [21]	2014	221 heterosexual couples (442 participants)	37 HPV types
Vogt et al. [22]	2013	34 couples	15xHR; 22 other
Paquette et al. [23]	2013	55 OSCCa† cases	37 HPV types
Marais et al. [24]	2008	115 women with confirmed cervical disease	37 HPV types
Richter et al. [25]	2008	30 women, oral scraping	19xLR; 3pHR; 15xHR

Boy et al., [26]	2006	59 OSCCa cases	16 and 18
Marais et al. [27]	2006	116 Dental clinic attendees	11, 16, 18
Van Rensburg et al. [28]	1996	146 OSCCa cases	6, 11, 16, 18
Van Rensburg et al. [29]	1995	66 OSCCa cases	6, 11, 16, 18

*HR=High Risk; LR=Low Risk; pHR=probable High Risk

†OSCCa=Oral/Oropharyngeal Squamous Cell Carcinoma

The data from the study presented in this chapter shows a prevalence of 6,1% that falls within the lower end of the global prevalence range and is lower than the only other South African study focussed on MSM which was at 11,5% [6]. Although the prevalence in this study is lower than for the Cape Town study, the trend is the same. None of the participants had multiple HPV types present in the cohort presented here. Studies done outside of South Africa report a varying range of oral/oropharyngeal prevalence rates: In the UK King and colleagues [12] showed the prevalence of HR oral/oropharyngeal HPV in MSM was 5.9% comparable with the current report. Three other HPV oral MSM studies [11,13, 14], report an oral/oropharyngeal HR-HPV prevalence of 2,0%, 9,0% and 17,0% respectively. Further in the spectrum, an Australian study reported one of the highest overall oral/oropharyngeal HPV-positivity in 37 of 170 rinses (21.7%) [15] which further confirms this South African MSM study is within the global prevalence spectrum, but towards the lower end thereof.

Six percent of the 199 participants presented in this study had oral/oropharyngeal HR-HPV, and only one of these was positive for HPV-16. This falls within the lower end of the global HPV prevalence range and is consistent with other South African data. This lower HPV-positivity indicates the need for a much larger sample size and for a prospective approach which will be valuable to fully understand the dynamics of oral/oropharyngeal HPV infection in this local MSM population.

The UK MSM study found the median age of participants to be 30 [12] and Muller and colleagues [6] found the median age of MSM participants in Cape Town was 32 years. The data presented here found a much younger median age (27 years) of participants. This suggests the probability that mainly younger individuals openly identify as MSM.

King and co-workers [12] found 75% of their participants were white. Demographic data showed 31% black, 24,5% dual heritage and 41,9% white men identified as MSM. This

South African report's MSM cohort mostly self-identified as black, and mostly reside in the rural feeder areas serviced by this tertiary institution. The study by Muller and co-workers [6] was conducted in an urban area in Cape Town, which could explain the racial demographic variation. It is difficult to correlate global statistics to sub-Saharan Africa as the majority of MSM recruited to global studies are Caucasian, and the MSM in this study were predominantly black participants. The catchment area for this study's participants is semi-urban and rural areas compromising mainly townships in which most of the residents are of African heritage.

Education data revealed that 78,7% of MSM in this study completed high school and 47,1% completed tertiary studies. However, in the semi-rural setting of our study we found the majority of our participants to be unemployed, and a small number of students. The majority of the participants in this study were unemployed (53.3%) followed by 36.0% who were employed and a number of students (10.7%). In this report, HPV positivity is not significantly associated with age, race or with socio-economic status.

Infection by HPV is associated with an increased risk for HIV acquisition [30]. Reports suggest a higher HIV infection rate among MSM who are already infected with HPV [31,32]. It is unknown whether HPV infection predisposes to subsequent HIV infection or is a marker of increased HIV infection risk. It is also unclear whether prevalent HPV infection or the immune response associated with clearing that HPV infection, or both, plays a role in potentiating HIV acquisition. The first evidence that found an association between HPV infection and increased risk of HIV acquisition came from studying MSM cohorts.

A meta-analysis showing a higher oral/oropharyngeal HPV prevalence in HIV-positive than in HIV-negative MSM also revealed a pooled prevalence for HPV 16 of 3,0% in HIV-negative MSM and 4,7% in HIV-positive MSM; a pooled prevalence of all HR-HPV types of 9,1% in HIV-negative MSM and 16,5% in HIV-positive MSM; and a pooled prevalence for the presence of any HPV type of 17,1% in HIV-negative and of 28,9% in HIV-positive MSM. The analysis confirmed that oral/oropharyngeal HPV presence was higher in HIV-positive MSM [10].

Oral/oropharyngeal HPV prevalence among HIV-infected MSM may range between 20-45%, which is considerably higher than the prevalence of MSM that are HIV negative [6,11]. The

three cities study in South Africa revealed HIV prevalence rates in MSM are 22,3% in Cape Town, 26,8% in Johannesburg, and 48,2% in Durban [33]. In this study from Ga-Rankuwa, 66,8% of the recruited MSM participants were HIV seropositive.

Oral HPV has been reported with higher frequency in HIV-positive MSM greater than 40 years of age. A comparative study shows the majority of participants as Caucasian 96.5% with a median age of 39 years for HIV negative participants, and 44 years for HIV-positive participants [15]. Muller and colleagues did not compare HPV presence within different HIV status groups, however, they found that 52,3% of their South African MSM participants were HIV positive, with 17.1% of these being oral/oropharyngeal HPV-positive (all genotypes) compared to 7.1% HPV presence in their HIV-negative participants. In this much younger cohort (mean=30 years), this study found an overall oral/oropharyngeal HPV prevalence of 6% (n=12). The majority of this study's MSM were HIV-positive (66,8%) and this group showed an HPV prevalence of 7,5% (n=10)(Table 1). The finding that the majority of these MSM are HIV-positive (66,8%) is in line with other worldwide and local studies, albeit in the higher end of the spectrum.

An earlier Australian MSM study presented oral/oropharyngeal HPV prevalence at 19,0% amongst HIV-positive participants, compared to 7,0% in HIV-negative participants [14]. HPV 16 was present in 44,0% of HIV-positive MSM and in 0,8% of HIV-negative MSM, confirming that HPV oral/oropharyngeal infection was more common in MSM with HIV infection. There were 23 different genotypes detected in the HIV-positive MSM, and 15 genotypes detected in the HIV-negative MSM of the Australian cohort [15]. HIV-positive MSM show an increased prevalence of oral/oropharyngeal HPV infection in those who report to have occasional partners. This report found that no oral health-related factors modified risk to acquire oral HPV, as was the case in the Australian MSM group. The LR-HPV types were more common in HIV infected MSM (18%) than the HR-HPV (5%). Interestingly, HPV 16 was found most frequently in HIV-negative participants (5%). The study clearly showed a higher oral/oropharyngeal HPV prevalence in HIV-positive than in HIV-negative MSM [15], which is also the case in the data presented in this paper.

Muller and colleagues [6] interrogated the possible correlation between oral HPV incidence and HIV-positivity and suggested roles for HIV-related immune-suppression and risky sexual practises. An incidental finding from this study was that rimming was significantly associated

with HIV-positivity. This could suggest that in this MSM population group, rimming is a risk factor to acquire HIV, and it is recommended that patients who identify as MSM, who have HPV infection, and report the practice of rimming, should be screened for HIV infection.

Differences in the same individual with regard to the number of oral sex partners and genital sex partners could partly influence any perceived lower oral HPV infection prevalence. Oral/oropharyngeal and genital HPV infections appear to be influenced differently when assessing these parameters in the contexts of gender and age associations, but appear to have a higher prevalence in men than in women. The reasons for the reported increase in males are unclear despite investigations into co-factor exposure, gender differences in sexual behaviour, or even gender differences in natural disease progression [34].

The oral specimens and data of 1688 healthy men in the US, Mexico plus Brazil was analysed and HPV 16 was the most commonly detected genotype. Even though oral sex was common practice amongst Brazilian (94%), Mexican (84%) and of US (96%) men cohorts, oral sexual behaviours were not associated with increased chances of identifying oral HPV infection. Smoking was significantly associated with oral HPV infection in these cohorts, and of those who practised oral sex, 3.8% were oral HPV positive [35]. D'Souza et al., [36] performed a study amongst 2116 men and women in the USA and demonstrated that 85.4% of men and 83.2% of women had had oral sex. Men had more lifetime sexual partners and a higher oral HPV 16 prevalence. The HPV 16 oral prevalence with another concomitant oral HPV infection was also higher in men. Thirty seven point four percent of men who participated in the US study had more than 10 lifetime sexual partners [36]. In this study no association could be found between the number of sexual partners, and HPV prevalence or HIV serostatus.

Amongst UK MSM, it was reported that 64% had greater than 30 lifetime sex partners. More specifically, sex practises like oral receptive sex leads to an increased exposure of the oropharyngeal mucosa to HPV [12]. Mbulawa and co-workers [21] confirmed that oral HPV infection in a South African population is acquired from sexual partners where the number of sexual partners and sexual practise increase the risk for oral HPV infection. Although some studies, as is the case in this study, show no association between oral HPV infection and higher numbers of genital or oral sex partners, the bulk of the literature clearly demonstrates the relationship [36-38].

Data from Davidson et al., [20] shows that 40,8% of the South African male factory worker cohort (and only 3 of 7 HPV-positive participants) confirmed to practise oral sex. Two participants that were positive for HR-HPV types practised oral sex with more than one partner. Although oral sex was not practised by most participants, it was practised by those who were positive with HR-HPV types. However, a recent study on oral sexual practices of dental clinic attendees from the general population in same catchment area determined that 18-35 year olds tended to practice more oral sex, and 21,8% of the total cohort confirmed to practice oral sex [39]. Males reported a significantly higher prevalence of oral sex practice than the females, and at 32% compares favourably to that reported by Davidson et al., [20], and also of the current oral sex practice reported in this study of 28,6% (n=57). Although sexual orientation was not interrogated by Wood and co-workers [39], another study by the group revealed that 64,7% of individuals started open-mouth kissing during their teenage years [16]. In the same population, 40.3% of participants gave oral sex to their partner, and 44,8% received oral sex from their partner. It is therefore conceivable that between oral sex practice and kissing, the spread of HPV could be higher. More studies into the different types of sexual practices in relation to oral and oropharyngeal HPV infection among South Africans are needed.

Ninety-five point five percent of the MSM in Cape Town practised oral-receptive sex and 43,2% practised rimming [6]. This study found that a lower number of participants (80,9%) practised oral receptive sex when compared to the Cape Town study, while 60.8% performed oral insertive sex. This study revealed that oral/oropharyngeal HR-HPV presence was significantly increased in participants who performed oral insertive sex (10%), when compared to those who performed oral receptive sex (7.5%) (p=0.0038)(Table 3). In our study, 14% of those MSM who reported to practice oral sex in the preceding 6 months were HPV positive. This study demonstrates that oral insertive sex is a significant risk factor in the acquisition of oral/oropharyngeal HPV infection in this population group (p=0.0057). In contrast, Muller et al., [6] did not identify any independent risk factors associated with oropharyngeal HPV infection.

In this study, HPV infection has a statistically significant presence in participants who performed oral insertive sex as opposed to those who performed oral receptive sex (p=0.0038) (Table 3). The results further indicate that more participants (46,2%) performed

rimming bottom compared to rimming top (19,6%) which is very similar to the figures reported by Muller and colleagues [6]. In the Muller study, 95,5% of the MSM practised oral-receptive sex and 43,2% practised rimming. In our MSM population a large number of participants (80,9%) practised oral receptive sex while 60.8% performed oral insertive sex.

For the MSM participants who performed oral receptive sex and oral insertive sex, HR-oral HPV prevalence was 7.5% and 10.0% respectively. The most commonly reported 'current sex practice' was anal sex (69.4%). The second most common sex practice was oral sex at 28.6% followed by rimming (9.6%). Significantly, 14% of participants who practised oral sex were positive for oral HR-HPV (p=0.0057) compared to only 2.8% of those who did not practice oral sex.

Conclusion

The globally reported prevalence rate of oral/oropharyngeal HPV infection is higher in the high-risk MSM cohort than in the general population. It is known that HPV infection is frequent in these men, but data on their HPV-related oral/oropharyngeal diseases are sparse; and data from a younger black MSM group is very rare. The prevalence data points in this report cannot be extrapolated to the general South African MSM population, but provides an additional component to the baseline for research into oral/oropharyngeal HPV-infection of MSM.

Although lower for MSM cohorts, the 6% HR-HPV oral/oropharyngeal prevalence in this study is in alignment with global reports. The prevalence of oral/oropharyngeal HPV in this MSM cohort was influenced by sexual practices. Different detection methods of the HPV, the difference in specimen collection methods, processing, storage, method of DNA extraction, sensitivity and specificity amongst the various global and local studies makes correlation challenging.

The significant associations found between oral insertive sex and oral/oropharyngeal HPV status (p=0.0038; phi coefficient=0.20), and between rimming and HIV status (p=0.0046; phi coefficient=0.19) supports the ano-oral-genital/genital-oral-anal HPV transmission routes in this population group, showing how HR-HPV gains access to the mouth and oropharynx. Further studies can be designed to determine the natural history and burden of HPV-

associated diseases in the South African MSM community, and also for the effective HPV prevention strategies in these population groups. The current targeted interventions and HPV vaccination of boys to reduce the burden of HPV related diseases amongst MSM in South Africa requires further controlled prospective studies to provide the supporting evidence.

Data Availability

Dataset is uploaded at the time of submission of this manuscript as a supplementary file, without restrictions named dataset.xlsx

Conflicts of Interest

The authors declare that they have no conflicts of interest emanating from the publication of this paper.

Funding Statement

This work was funded by the Sefako Makgatho Health Sciences University Research Development Grant. This work received additional funding from the Flemish government (IUC-VLIR-OUS) and the funding agency had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

Acknowledgement

We acknowledge the MeCRU team at the Sefako Makgatho Health Sciences University for support and assistance.

References:

1. IARC. Human papillomaviruses. IARC Monogr Eval Carcinog Risks Hum 2007; 90:1-636.

2. Kreuter A, Wieland U. Human papillomavirus-associated diseases in HIV-infected men who have sex with men. Curr Opin Infect Dis 2009; 22:109-114.

3. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010; 401:70–79.

4. Tam S, Fu S, Xu L, Krause KJ, Lairson DR, Miao H, Sturgis EM, Dahlstrom KR. The epidemiology of oral human papillomavirus infection in healthy populations: a systematic review and meta-analysis. Oral Oncol 2018; 82:91-99.

5. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report, 17 June 2019. https://www.hpvcentre.net/statistics/reports/XWX.pdf, accessed 27 November 2019.

6. Muller EE, Rebe K, Chirwa TF, Struthers H, McIntyre J, Lewis DA. The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. BMC Infect Dis 2016; 16:440.

7. Hui B, Fairley CK, Chen M, Grulich A, Hocking J, Prestage G, Walker S, Law M, Regan D. Oral and anal sex are key to sustaining gonorrhoea at endemic levels in MSM populations: a mathematical model. Sexually transmitted infections. 2015; 91:365-9.

8. Vanhommering JW, Lambers FAE, Schinkel J, Geskus RB, Arends JE, Van de Laar TJW, Lauw FN, Brinkman K, Gras L, Rijnders BJA, Van der Meer JTM, Prins M. Risk factors for sexual transmission of hepatitis C virus among human immunodeficiency virus-infected men who have sex with men: A case-control study. OFID 2015; 2(3): doi: 10.1093/ofid/ofv115.

9. Chow EP, Cornelisse VJ, Read TR, Lee D, Walker S, Hocking JS, Chen MY, Bradshaw CS, Fairley CK. Saliva use as a lubricant for anal sex is a risk factor for rectal gonorrhoea among men who have sex with men, a new public health message: a cross-sectional survey. Sex Transm Infect 2016; pii: sextrans-2015-052502. doi: 10.1136/sextrans-2015-052502.

10. King EM, Oomeer S, Gilson R, Copas A, Beddows S, Soldan K, Jit M, Edmunds WJ, Sonnenberg P. Oral Human papillomavirus infection in men who have sex with men: a systematic review and Meta-Analysis. PLoS ONE 2016; 11(7): e0157976.

11. Mooij, S.H. The epidemiology of human papillomavirus in HIV-negative and HIV-infected men who have sex with men Thesis, University of Amsterdam (UvA), the Netherlands ISBN: 978-90-6464-855-7 http://dare.uva.nl/dissertaties.

12. King EM, Gilson R, Beddows S, Soldan K, Panwar K, Young C, Jit M, Edmunds WJ, Sonnenberg P. Oral human papillomavirus (HPV) infection in men who have sex with men: prevalence and lack of anogenital concordance: Table 1. Sexually Transmitted Infections 2015; 91:284–286. https://doi.org/10.1136/sextrans-2014-051955

13. Beachler DC, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, Wiley DJ, Minkoff H, Reddy S, Stammer EE, Gillison ML, D'Souza G. Risk Factors for Oral HPV Infection among a High Prevalence Population of HIV-Positive and At-Risk HIV-Negative Adults. Cancer Epidemiology Biomarkers & Prevention 2012; 21:122–133. https://doi.org/10.1158/1055-9965.EPI-11-0734

14. Read TRH, Hocking JS, Vodstrcil LA, Tabrizi SN, McCullough MJ, Grulich AE, Garland SM, Bradshaw CS, Chen MY, Fairley CK. Oral Human Papillomavirus in Men Having Sex with Men: Risk-Factors and Sampling. PLoS ONE 2012; 7:e49324. https://doi.org/10.1371/journal.pone.0049324

15. Rollo F, Latini A, Pichi B, Colafigli M, Benevolo M, Sinopoli I, Sperduti I, Laquintana V, Fabbri G, Frasca M. Prevalence and determinants of oral infection by Human Papillomavirus in HIV-infected and uninfected men who have sex with men. PloS ONE 2017; 12:e0184623.

16. Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken OM, Bogers J. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-positive cohort from Pretoria, South Africa. Adv in Virol 2020; https://doi.org/10.1155/2020/2395219

17. Bulane A, Goedhals D, Seedat RY, Goedhals J, Burt F. Human papillomavirus DNA in head and neck squamous cell carcinomas in the Free State, South Africa. J Med Virol 2020; 92:227-233.

18. Sekee TR, Burt FJ, Goedhals D, Goedhals J, Munsamy Y, Seedat RY. Human papillomavirus in head and neck squamous cell carcinomas in a South African cohort. Papillomavirus Res 2018; 6:58-62.

19. Chikandiwa A, Pisa PT, Chersich MF, Muller EE, MayaudP, Delany-Moretlwe S. Oropharyngeal HPV infection: prevalence and sampling methods among HIV-infected men in South Africa. Int J STD & AIDS 2018; 29(8):776-780.

20. Davidson CL, Richter KL, van der Linde M, Coetsee J, Boy SC. Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: A pilot study. SAMJ. 2014; 104(5):358 – 361.

21. Mbulawa ZZA, Johnson LF, Marais DJ, Coetzee D, Williamson A-L. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. Journal of Infection. 2014; 68:185-189.

22. Vogt SL, Gravitt PE, Martinson NA, Hoffmann J, D'Souza G. Concordant oral-genital HPV infection in South Africa couples: evidence for transmission. Frontier Oncol: Cancer Epidem Prev 2013; 3(303):1-7.

23. Paquette C, Evans MF, Meer SS, Rajendran V, Adamson CS-C, Cooper K. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in black South Africans. Head and Neck Pathol. 2013; 7:361 – 372.

24. Marais DJ, Passmore DS, Denny L, Sampson C, Allan BR, Williamson A. Cervical and Oral Human Papillomavirus Types in HIV-1 Positive and Negative Women With Cervical Disease in South Africa. J Med Virol 2008; 80:953–959.

25. Richter KL, Janse van Rensburg E, van Heerden WFP, Boy SC. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. J Oral Path Med 2008; 37:555-559.

26. Boy S, Janse van Rensburg E, Engelbrecht S, Dreyer L, van Heerden M, van Heerden E. HPV detection in primary intra-oral squamous cell carcinomas – commensal, aetiological agent or contamination? J Oral Pathol Med 2006; 35:86–90.

27. Marais DJ, Sampson C, Jeftha A, Dhaya D, Passmore JS, Denny L, Rybicki EP, Van der Walt E, Stephen LXG, Williamson A. More men than women make mucosal IgA antibodies to Human papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population. BMC Infect Dis 2006; 6:95.

28. Van Rensburg EJ, Engelbrecht S, Van Heerden WF, Raubennheimer EJ, Schoub BD . Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. Anticancer Res 1996; 16: 969– 973.

29. Van Rensburg EJ, van Heerden WF, Venter EH, Raubenheimer EJ . Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population. S Afr Med J 1995; 85: 894– 896.

30. Houlihan CF, Larke NL, Watson-Jones D, Smith-McCune KK. Shibosky S, Gravitt PE, Smith JS, Kuhn L, Wang C, Hayes R. Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis. AIDS 2010; 26:2211-2222.

31. Dietz CA, Nyberg CR. Genital, oral, and anal human papillomavirus infection in men who have sex with men. The Journal of the American Osteopathic Association 2011; 111:s19-s25.

32. Van Rijn VM, Mooij SH, Mollers M, Snijders PJF, Speksnijder AGCL, et al. Anal, Penile, and Oral High-Risk HPV Infections and HPV Seropositivity in HIV-Positive and HIV-Negative Men Who Have Sex with Men. PLoS ONE 2014; 9(3):e92208. doi:10.1371/journal.pone.0092208

33. Human Sciences Research Council. Three cities study: high levels of HIV infection found among men who have sex with men: The South African Marang Men's Project. 2014. http://www.hsrc.ac.za/en/research-data/view/7142. accessed: 26 Nov 2019

34. Gillison Maura L., Xavier Castellsagu_e, Anil Chaturvedi, Marc T. Goodman, Peter Snijders, Massimo Tommasino. Eurogin Roadmap: Comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. Int J Cancer 2014; 134:497–507.

35. Kreimer AR, Villa A, Nyitray AG, Abrahamsen N, Papenfuss M, Smith D, Hildesheim A, Villa LL, Lazcano-Ponce E, Giuliano AR. The epidemiology of Oral HPV infection among a multinational sample of healthy men. Cancer Epidemiol Biomarkers Prev 2011; 20:172–82.

36. D'Souza G, Cullen K, Bowie J, Thorpe R, Fakhry C. Differences in Oral Sexual Behaviors by Gender, Age, and Race Explain Observed Differences in Prevalence of Oral Human Papillomavirus Infection. PLoS ONE 2014; 9:e86023. https://doi.org/10.1371/journal.pone.0086023

37. Edelstein ZR, Schwartz SM, Hawes S, Hughes JP, Feng Q, Stern ME, O'Reilly S, Lee SK, Xi LF, Koutsky LA. Rates and determinants of oral human papillomavirus (HPV) infection in young men. Sex Transm Dis 2012; 39:860-867.

38. Cook RL, Thompson EL, Kelso NE, Friary J, Hosford J, Barkley P, Dodd VJ, Abrahamsen M, Ajinkya S, Obesso PD, Rashid MH, Guiliano AR. Sexual behaviours and other risk factors for oral human papillomavirus infections in young women. Sex Transm Dis 2014; 8:486-492.

39. Wood NH, Ayo-Yusuf OA, Gugushe TS, Bogers J. Tobacco use and oral sex practice among dental clinic attendees. PLoS ONE 2019; 14(3):e0213729. https://doi.org/10.1371/journal. pone.0213729

CHAPTER 6

Prevalence of Human Papillomavirus in oral and oropharyngeal tissue specimens from a university oral health centre in Pretoria, South Africa

Under revision at the time of thesis submission:
Oral Surgery Oral Medicine Oral Pathology Oral Radiology

CHAPTER 6

Submitted to: Oral Surgery Oral Medicine Oral Pathology Oral Radiology

Prevalence of Human Papillomavirus in oral and oropharyngeal FFPE tissue specimens from a university oral health centre in Pretoria, South Africa

Neil H Wood[1] neil.wood@smu.ac.za; drnhwood@gmail.com

Ramokone L Lebelo[2,3] Ramokone.Lebelo@smu.ac.za

Ina Benoy[4,5] ibenoy@ambior.org

Sonja C Boy[6] Sonja.iaop@gmail.com

Elna Cowley[6] Elna.Cowley@smu.ac.za

Olivier M Vanderveken[7,8] olivier.vanderveken@uza.be; olivier@ovanderveken.be

Johannes Bogers[4,5] jpbogers@ambior.org; john-paul.bogers@uantwerpen.be

1 Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa.

2 Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa.

3 HIV and Hepatitis Research Unit, National Health Laboratory service

4 Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), University of Antwerp, Belgium.

5 Laboratory of Cell Biology & Histology, Antwerp, Belgium

6 Department of Oral Pathology, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa

7 Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium;

8 Department of ENT, Head and Neck Surgery, Antwerp University Hospital, Edegem, Belgium

Correspondence to: Neil H Wood neil.wood@smu.ac.za

Abstract:

Background:
This study investigated the presence of HPV in benign and malignant HPV-associated oral and oropharyngeal formalin-fixed, paraffin-embedded tissue specimens. The prevalence of oral and oropharyngeal HPV is scantily studied in South African populations when compared to the USA and Europe, and only limited data is available with a lack of methodologic standardization.

Methodology:
159 tissue specimens comprising of 67 benign HPV-associated lesions, 21 Squamous cell carcinomas, and 71 tonsillar specimens that served as control spanning a ten-year time period were included in the study. Specimens were processed for qPCR analysis using the Riatol qPCR HPV test, and sections were prepared for in situ hybridization using the Dako® Wide Spectrum HPV Biotinylated DNA Probe. Malignancies were stained for $p16^{INK4a}$ with the Ventana Cintec p16 histology probe (Roche).

Results:
An 11% overall HPV prevalence for all specimen groups (n=155) was found. A total of 20 positive results were obtained for 17 tissue specimens of which 1 was a squamous cell carcinoma involving both the oral and oropharyngeal anatomic regions, 3 were tonsillar control specimens, and 13 were benign HPV-associated lesions. HPV-6 (5%) was the most detected type followed by HPV-11 (2%), -12 (1.2%), -58 (1.2%), and HPV-16, -18, -33, -56, and -67 had one detection each. Three tissue specimens had two HPV-types present for each. Nine squamous cell carcinoma specimens were $p16^{INK4a}$ positive, but all of these were negative for qPCR and ISH. The single SCC that was HPV-33 and HPV-12 positive was negative for both $p16^{INK4a}$ and ISH.

Conclusion:
Although the overall prevalence was 11% in this study, the lack of concurrence between $p16^{INK4a}$ and ISH positivity with qPCR results demonstrate that a passenger-infection theory is more likely in this population group. No HPV-driven lesions could be identified. Future methodology must include selected biomarker detection alongside PCR investigation for confirmation of HPV-driven lesion development.

Key Words: HPV prevalence, oropharynx, oral, squamous cell carcinoma

Introduction:

The human papillomavirus (HPV) has an established epitheliotropism. In order to achieve infection and cellular transduction, this enveloped, icosahedral DNA-virus requires access to basal epithelial keratinocytes to initiate a complex sequence of events. In the non-lymphoepithelial tissues of the oropharynx and mouth, requisite exposure of the basement membrane zone through epithelial wounding facilitates viral uptake into the basal cell for processing to establish infection. HPV can directly infect the keratinocytes of the reticulated epithelial network in the lymphoepithelial tonsillar crypt and the association between HPV infection and the epithelial cells of the tonsillar crypts is well recognized (Kang et al., 2015; Syrjänen, 2018).

Although the natural progression of oral mucosal HPV infection is still largely unclear, the majority of oral/oropharyngeal HPV infections are cleared early. Most HPV infections are brief and without clinical sequelae, however persistent mucosal infection by high-risk (HR)-HPV subtypes have an established association with an increased risk to develop oropharyngeal squamous cell carcinoma (SCC) (Cubie, 2013; Prue et al., 2017; Syrjänen, 2018).

Clinically productive HPV infection of the oral and oropharyngeal mucosae result in benign or malignant lesions, the latter being more uncommon. The benign HPV-induced lesions are typically singular exophytic proliferations, frequently described as having a cobblestone-like surface, or with finger-like projections. Commonly referred to as 'warts', the spectrum of papillomas are usually sessile with a broad base compared to its overall size. However, it is not uncommon to encounter semi-pedunculated or pedunculated lesions. Multiple or multifocal lesions may also occur, and this is one of the hallmark features of focal/multifocal epithelial hyperplasia in which multiple individual lesions may coalesce to form larger irregular ones (Feller et al., 2011).

Benign HPV-associated epithelial proliferations are usually of a normal skin or mucosal colour unless irritated physically, traumatized or secondarily infected. Focal epithelial hyperplasia (Heck's disease), verruca vulgaris, condyloma acuminatum and oral squamous papillomas represent the four main entities within the benign spectrum of HPV-induced oral/oropharyngeal lesions, referred to here as benign-HPV-associated lesions from an aetiologic perspective (Feller et al., 2011; Cubie, 2013; Egawa et al., 2015). The HPV types most commonly associated with these lesion-types are HPV-6 and -11, but this is not

exclusive as other LR-HPV and HR-HPV types have also been described in these lesions (Feller et al., 2011; Piña et al., 2019).

The relationship between HR-HPV and SCC of different anatomic sites is well-known, and HPV-16 is recognised by the International Agency for Research on Cancer (IARC) as a group 1 infectious agent (oncogenic virus) in the oropharynx (IARC, 2011; Egawa et al., 2015). Compared to the oropharynx, there is considerably less information regarding HPV infection of the oral cavity (Syrjanen 2018; Egawa et al., 2015).

This study investigated the presence of HR-HPV and low-risk (LR)-HPV types in HPV-associated and non-HPV-associated formalin-fixed paraffin-embedded (FFPE) benign and malignant tissue specimens from oral and oropharyngeal anatomic sites

Materials and methods:

Tissue specimens of oral and oropharyngeal lesions diagnosed histopathologically as oral SCC, oropharyngeal SCC, and benign oral and oropharyngeal HPV-associated lesions that included verruca vugaris, condyloma acuminatum, squamous papilloma and focal epithelial hyperplasia were identified from the archives of the Department of Oral Pathology at the Sefako Makgatho Health Sciences University and retrieved for sectioning and analysis. Tonsillar tissue removed by tonsillectomy, which was without any apparent HPV involvement, served as a control.

The data collected from the tissue submission forms in relation to the specific tissue specimens included (where available):
Age, Gender, Anatomic Location (oral / oropharyngeal), type of lesion (benign / malignant / tonsillar non-HPV), *in situ* hybridization (ISH) result, $P16^{INK4a}$ result (in cases of malignancies), Concentration of DNA in specimen, Concentration of DNA in specimen sufficient/not, HPV present (If yes: Type of HPV detected).

An Oral Pathologist screened freshly prepared histopathology slides to confirm the original diagnosis of the lesion. Further tissue sections from confirmed lesions were then prepared for DNA extraction, amplification, and genotyping. Specimens that were confirmed as malignancies were sectioned and stained for $p16^{INK4a}$ expression; and *in situ* hybridization (ISH) was performed on all specimens that tested positive of HPV on qPCR, and also on a randomly selected number of qPCR HPV-negative specimens in order to determine the

intranuclear presence or absence of HPV. Sections were 5 microns thick and depending on the size of the original specimen 5-10 sections were included to ensure adequate DNA availability. All instruments and equipment, including the microtome blade and mounting, were meticulously cleaned with xylene and rinsed with alcohol between sectioning of tissue blocks and controls to prevent contamination.

An ISO-certified, automated qPCR (Riatol qPCR HPV test) is a clinically validated laboratory developed assay (Benoy et al., 2019). Samples were processed in batches of 91 and stored in a liquid-based cytology medium (Thinprep, Hologic Inc., Manchester, UK) and when received were placed in the Sample Transfer System STS (Hologic Inc., Manchester, UK). Aliquots of 2ml were transferred in a deep-well plate and then placed in the Medium Throughput Automation MTA (Hologic Inc., Manchester, UK) using the Genfind® DNA extraction kit (Hologic Inc., Manchester, UK) exploiting standard boom extraction with magnetic beads. Subsequent DNA amplification was performed on the LightCycler 480 type I (Roche), using a series of real-time qPCR reactions. The presence of 14 different HR-HPV genotypes is confirmed through real-time PCR reactions (TaqMan) targeting type-specific viral gene sequences. HPV types detected by the Riatol qPCR HPV test includes 14 HR-HPV types (HPV 16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 56 E7, 58 E6, 59 E7, 66 E6, 68 E7), and selected potential HR or low-risk HPV types (HPV 6 E6, 11 E6, 53 E6, 67 L1). Ultra-low volumes (6 µl) are used in 8 multiplex reactions with cellularity control done for every sample (beta-globin gene amplification).

Results are reported as hrHPV negative, hrHPV positive or inconclusive. DNA concentration (ng/µl) is determined for every sample based on the beta-globin standard curve. Samples with a DNA concentration below 0,12 ng/µl are considered as invalid and reported as inconclusive. This cut-off is chosen based on previous analyses demonstrating that, consistency is not guaranteed below this cut-off. An inconclusive result included no or insufficient material/cells for analysis. A sample is considered negative for RIATOL qPCR HPV if none of the 14 HPV tests showed a positive signal and the DNA concentration is above 0,12 ng/µl. Type-specific HPV positivity is defined as having a positive amplification signal for that specific HPV type, independent of the beta-globin signal.

In situ hybridization was performed on 117 samples after formalin-fixed paraffin-embedded tissue specimens were de-paraffinized in xylene and hydrated in ethanol. Target retrieval was

performed using 150 ul Proteinase K (ready to use) per slide at room temp for 20 min. The Dako® GenPoint Tyramide Signal Amplification System for Biotinylated Probes (Dako K0620) was used according to manufacturer's instructions. The Dako® Wide Spectrum HPV Biotinylated DNA Probe (Dako® Y1404) which targets genomic DNA of HPV 6, 11, 16, 18, 31, 33, 35, 45, 51 and 52 was used for hybridization. Denaturing was performed at 92℃ for 5 min, hybridization at 37℃ for 18 hours and slides were then placed in TBST (Dako® Wash Buffer 10X S3006) at room temperature for 20 minutes. Stringent wash was used to wash slides in SWB at 48℃ for 30 min, followed by three 5 minute rinses with TBST. Primary Streptavidin-HRP (1:150) was applied using 150 ul per slide at room temperature for 15 min. Thereafter, slides were rinsed thrice with TBST for 5 min/rinse. A few drops of Biotinyl Tyramide were applied to each slide at room temp and left for 15 minutes and then rinsed three times with TBST for 5 min/rinse. Secondary-HRP was applied at room temp for 15 minutes and the slides again rinsed with TBST thrice for 5 min/rinse. Chromogen visualization was then applied at room temperature for couple seconds and washed in running tap water for 5 minutes. Mayer's Hematoxylin was used as a counter stain, applied for 2 min and rinsed with tap water. The slides were mounted with Faramount Aqueous Mounting Medium (Dako® S3025) and covered with glass coverslips.

All the oral/oropharyngeal malignant specimens were sectioned for $p16^{INK4a}$ staining. Following xylene de-paraffinization, $p16^{INK4a}$ staining was done using the Dako® Envision™ kit and the Flex mini kit (high pH) with the Ventana Cintec p16 histology probe (Roche), according to the manufacturer's instructions. Sections were mounted using Dako® Faramount and screened under a light microscope. Positivity for p16INK4a staining was recorded when more than 70% of tumour cells had stained nuclei and cytoplasm, and the tumour was in the anatomically correct region (i.e. oropharynx). For tumours that stained positive in this manner and were not in the oropharynx, ISH must be performed in order to confirm HPV positivity.

Descriptive analysis of the data was carried out, and categorical variables were summarised by frequency and percentage. Continuous variables were summarised by the mean, standard deviation, median and interquartile range. The X^2 test was used to assess the relationship between gender, type of lesion (group)and HPV prevalence. The strength of the associations was measured by Cramer's V and the phi coefficient respectively. The relationship between age and HPV prevalence was assessed by the t-test (or ANOVA for more than two categories).

This study received institutional ethical clearance (SMUREC/P/86/2016) from the Sefako Makgatho Health Sciences University in South Africa.

Results:

A total of 159 formalin-fixed paraffin-embedded (FFPE) tissue specimens were included from the archives of the Department of Oral Pathology at the Sefako Makgatho Health Sciences University (SMU) spanning a ten-year time period (2006 to 2017). Of these lesions, 61 (38.4%) were from the mouth, and 97 (61%) were from the oropharynx anatomic regions. Only one lesion overlapped both anatomic locations. These descriptive data are summarized in table 1. These included 67 (42.1%) benign HPV-associated lesions such as verrucae, condyloma and papules, 21 (13.2%) oral/oropharyngeal SCC, and 71 (44.7%) tonsillar tissue specimens of tonsillar lesions not associated with HPV such as tonsillitis. The median age (n=158; 0.6% missing data) was 15y (IQR 8-43y; range 2-82 years). The distribution in Figure 1 shows that there were more samples from the under 20y especially less than 10y. For further analysis, age was categorised as paediatric (up to 18y) (51.9%) vs. adult (48.1%). There were more specimens from females (57,6) than males (42.4%)(Table 1).

Table 1: Summary for descriptive analysis of specimens

Variable	**Category**	**n**	**%**
n		**159**	
Age	0-17y	82	51,9
	18y+	76	48,1
	missing	1	
Gender	Male	67	42,4
	Female	91	57,6
	missing	1	
Type of lesion	benign HPV-associated	67	42,1
	malignant HPV-associated	21	13,2
	non-HPV related tonsillar	71	44,7
Location of specimen	Oral	61	38,4
	Oropharyngeal	97	61,0
	Oral and oropharyngeal	1	0,6

DNA sufficiency	DNA insufficient	5	3,1
	DNA sufficient	154	96,9
HPV status (detailed)*	HPV negative	138	86,8
	LR-HPV positive	11	6,9
	HR-HPV positive	6	3,8
	DNA insufficient - no result	4	2,5
In situ Hybridization (ISH)	ISH Positive	19	11.9
	ISH Negative	98	61.6
	ISH no result	42	26.4

*3 participants had multiple HPV types present.

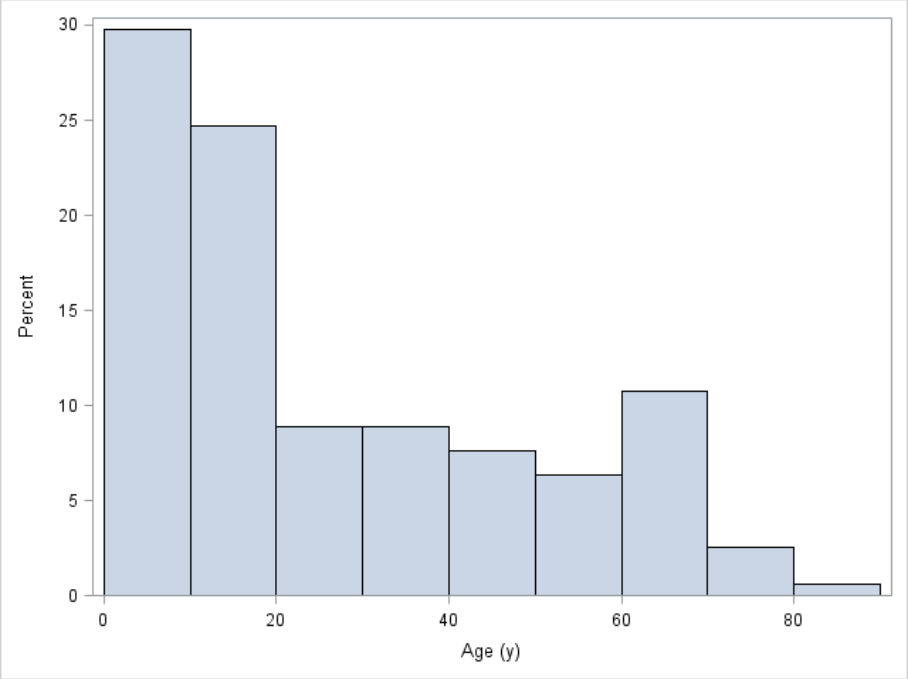


Figure 1: Age distribution for all FFPE tissue specimens

There were fewer specimens that were malignant (13.2%) with a slight difference in number between benign lesions (42.1%) and tonsillar tissue specimens (44.7%). A large proportion of the lesions were from the oropharynx (61%), while 38.4% were oral and 1 specimen was from both locations. The median DNA concentration was 0.6 (IQR 0.2-1.1; range 0.0-6.3 units) for qPCR. All but 5 specimens were found to have sufficient DNA for analysis. An HPV result

was nevertheless produced for one of these 5 specimens as described in the methodology, and this was included for all analyses.

Eleven percent of all tissue specimens were HPV positive. Only three specimens had two concomitant infections by different HPV-types: a gingival verruca vulgaris in an adult male presented with multiple infection of LR-HPV 11 and HR-HPV 56. A high-grade SCC from a 63-year-old female that spanned both the mouth and oropharynx had LR-HPV 12 and HR-HPV 33. A lymphoid hyperplasia of the tonsils from 11-year-old female had LR-HPV 12 and HR-HPV 18. However, all three cases were negative for ISH. All SCC specimens were also $p16^{INK4a}$ negative, including the only SCC specimen that demonstrated HPV-33 and HPV-12 positivity by qPCR. The most commonly detected HPV types were HPV-6 found in 8 benign specimens, followed by HPV-11 found in 2 benign lesions and 1 tonsillar control specimen. HPV-12 and HPV-58 were detected in two specimens each, and HPV-16, -18, -33, -56, and -67 had one detection each. This is a total of 20 positive qPCR results for 17 specimens.

Table 2 summarizes HPV detection within the specimens according to anatomic locations. HPV was detected in 19% all oral specimens and in 5% of all oropharyngeal specimens. Note however that the oropharyngeal specimens included the control non-HPV associated tonsillar tissue.

Table 2: HPV prevalence according to anatomic region for FFPE tissue specimens

	Overall		**Anatomic location**			
			Oral		**Oropharyngeal**	
	n	**%**	**n**	**%**	**n**	**%**
number included	154*		58	37.7	96	62.3
HPV prevalence (95% CI)	17	11.0% (6.5-17.%)	11	19% (10-31%)	5	5% (2-12%)

** DNA insufficient for 4 specimens, and one specimen overlapped both anatomical sites*

Table 3 demonstrates HPV detection in the three tissue-type categories. HPV was detected in 21% of all the benign HPV-associated lesions. Notably, 4% of all non-HPV associated tonsillar tissue specimens (n=71) had detectable HPV whereas only 1 HPV associated malignancy had detectable HPV (n=21). There was no significant difference in the prevalence of HPV in the Malignant and Benign groups (p=0.17) or between the Malignant and Tonsillar groups

(p>0.99). The larger confidence intervals are ascribed to the smaller sample size for this analysis.

Table 3: HPV prevalence for FFPE tissue specimen types

	Overall		**Tissue specimen type**					
			HPV-associated Benign		**HPV-associated Malignant**		**Non-HPV Tonsillar**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
number included	155*		63		21		71	
Prevalence (95% CI)	17	11.0% (6.5-17.%)	13	21% (11-33%)	1	5% (0-24%)	3	4% (1-12%)

** DNA insufficient for 4 specimens*

Between-group analysis of different tissue-types within gender categories shows a significant difference in HPV status in males (p=0.04), but not in females (p=0.061) (Table 4). This was influenced by the negative detection of HPV in malignant specimens from males. For males, the HPV prevalence was significantly higher in the Benign group (26%) compared to the Malignant and Tonsillar groups (0 and 6% respectively) (p=0.040; strength of association - phi=0.33; moderate effect size). There was no significant association between HPV status and tissue group for females.

Table 4: HPV status for tissue type, sub-categorized by gender

		Male							**Female**						
		Benign		**Malignant**		**Tonsillar**		p-value between-group test	**Benign**		**Malignant**		**Tonsillar**		p-value between-group test
Variable	**Category**	**n=19**	**%**	**n=13**	**%**	**n=34**	**%**		**n=43**	**%**	**n=8**	**%**	**n=37**	**%**	
HPV status	HPV negative	14	74	13	100	32	94	0.040 (phi=0.33)	35	81	7	88	36	97	0,061
	HPV positive	5	26	0	0	2	6		8	19	1	13	1	3	

Table 5 presents that analysis of the between-group comparisons of the different tissue specimen types stratified by age. For the 0-17y group, the HPV prevalence was significantly higher in the Benign group (24%) compared to the Tonsillar group (5%) (p=0.026; strength of association - phi=0.27; weak effect size). There were no cases in the malignant group. There was no significant association between HPV status and tissue group for age 18y+.

Table 5: HPV status for tissue type, sub-categorized by age

		Age 0-17y						**Age 18y+**					
		Benign		**Malignant**		**Tonsillar**	p-value between-group test	**Benign**		**Malignant**		**Tonsillar**	p-value between-group test
Variable	**Category**	**n=42**	**%**	**n=0**	**%**	**n=40**	**%**		**n=21**	**%**	**n=21**	**%**	**n=31**	**%**	
HPV status	HPV negative	32	76			38	95	0.026 (phi=0.27)	18	86	20	95	30	97	0,38
	HPV positive	10	24			2	5		3	14	1	5	1	3	

There was no significant association between HPV status and age or gender in either the benign tissue specimen group (Table 6), or in the non-HPV associated tonsillar tissue specimen group (Table 7).

Table 6: Between-group comparisons for Age and Gender of Benign HPV-associated lesions

		Benign								
		Age 0-17y		**Age 18y+**		p-value between-group test	**Male**		**Female**		p-value between-group test
Variable	**Category**	**n=41**	**%**	**n=21**	**%**		**n=19**	**%**	**n=43**	**%**	
n		**41**		**21**			**19**		**43**		
HPV status	HPV negative	32	78	18	86	0,74	14	74	35	81	0,51
	HPV positive	9	22	3	14		5	26	8	19	

Table 7: Between-group comparisons for Age and Gender of non-HPV-associated tonsillar specimens

		Non-HPV-associated Tonsillar								
		Age 0-17y		**Age 18y+**		p-value between-group test	**Male**		**Female**		p-value between-group test
Variable	**Category**	**n=40**	**%**	**n=31**	**%**		**n=34**	**%**	**n=37**	**%**	
n		**40**		**31**			**34**		**37**		
HPV status	HPV negative	38	95	30	97	>0.99	32	94	36	97	0,60
	HPV positive	2	5	1	3		2	6	1	3	

Due to the low prevalence of individual HPV types, no comparative analyses were undertaken on these.

Discussion:

Oral/oropharyngeal HPV infection can manifest clinically in immune-competent subjects. Once these lesions are cleared, viral persistence as an asymptomatic (subclinical) infection may occur due to HPV's sophisticated immune-evasion capacity (Egawa et al., 2015; Syrjänen, 2018). Development of latency following oral/oropharyngeal HPV infection in humans is not well established, and similarly, HPV persistence in the mouth and oropharynx without clinical symptoms is still not well defined (Kreimer et al., 2010; Sanders et al., 2012; Syrjänen, 2018). The association between HR-HPV and development of SCC of the oropharynx is well documented, and the subset of HPV-induced oropharyngeal cancers is well established (Egawa et al., 2015; Sekee et al., 2018; Syrjänen, 2018).

Human papillomaviruses are site-specific when eliciting lesions, and when the same subtypes are found in other anatomic regions, no such lesions develop which makes a "reservoir" or a "passenger-infection" theory more plausible (Evans et al., 2011; Paquette et al., 2013; Gaester et al., 2014; Egawa et al., 2015; Syrjänen, 2018). It is clear from the benign spectrum of HPV-associated oral lesions, that HPV infects and affects the oral lining mucosae. The oral-mucosal presence of various HPV-types is well established and manifests clinically as a spectrum of

benign oral lesions. The prevalence of oral HPV infection has been rising over time (Tam et al., 2018).

However, evidence for a similar causal relationship for HPV in the aetiopathogenesis of oral SCC is much less convincing. Whether or not the oral presence of oncogenic HPV-types is incidental (passenger-infection) or transformative, needs to be more clearly defined (Boy et al., 2006; Paquette et al., 2013; Syrjänen, 2018). Data from the very few South African studies support the notion that HR-HPV detection in the mouth is incidental, as opposed to HR-HPV infection of the oropharyngeal mucosa that plays a direct role in the aetiopathogenesis SCC of the oropharynx (Boy et al., 2006; Paquette et al., 2013). In contrast, HPV detection and any association with the aetiology of oral SCC is not well understood. Once the role of HR-HPV infection of the mouth is clarified and the epidemiology of oral HPV infection is better understood, the link between oral and oropharyngeal HPV infection and the development of SCC will become clearer.

The incidence of oral SCC has been increasing in developing countries. The increase in the incidence of oropharyngeal SCC is noticeably more than other head and neck malignancies (Van Rensburg et al., 1996; Mehanna et al., 2013). The prevalence rate of HPV in oropharyngeal SCC ranges from 35.9%-47% for the United States, 28% for Europe and Asia (Termine et al., 2008; Mehanna et al., 2013), and it has overtaken cervical cancer as the most common HPV-induced cancer in the United Sates (Tam et al., 2018). South African studies report an extremely wide HPV detection spectrum of 1.4% to 94.1% in oral/oropharyngeal SCC tissue specimens, with only six studies contributing to the pool of data on FFPE tissue specimens, none of which included the benign component of HPV-associated lesions. All of these included a mixed oral/oropharyngeal SCC specimen sample (Table 8). The sampling method, the population studied, detection methodology including sensitivity and specificity of the tests all contribute to the varying reports of HPV prevalence in these lesions (Adamopoulou et al., 2008; Richter et al., 2008; Gaester et al., 2014).

Table 8: South African reports of HPV detection in HNSCC tissue of the mouth and oropharynx.

Year	Study	N	Site represented	Overall detection	
				n	%
1995	*Van Rensburg et al.	66	Unclear	1	1,5

1996	*Van Rensburg et al.	146	Oral	2	1,4
2006	*Boy et al.	59	Oral and OP	7	11,9
2013	Paquette et al.	51	OP	48	94,1
2018	Sekee et al.	20	OP	1	5
2020	Bulane et al.	449	OP	33	7,3

*examined only HPV 16 and 18

Head and Neck Squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer in sub-Saharan Africa, and up to one third of these malignancies result from an infection (Bulane et al., 2020). However, when compared to the global contribution to HNSCC knowledge, data from Africa is sparse and presented in small series (Warnakulasuria, 2009; Vogt et al., 2013; Bulane et al., 2020; Wood et al., 2020). Mehanna and co-workers (2013) highlighted the need for research on HPV-associated head and neck SCC following their meta-analysis which did not include data from Africa. The same data-shortfall was mentioned in the preceding meta-analyses by Dayyani and colleagues (2010) and by de Martel et al., (2012). This sparsity continues even today.

Paquette et al., (2013) identified HPV in 37/55 Oropharyngeal SCC FFPE tissue specimens. This is the highest HPV-detection rate for any South African study involving malignant-tissue specimens. Of the 67% specimens identified with HPV, the subtype analyses identified 32% positive for both HPV 16 and 31, a further 32% positive for HPV-16, 24% positive for HPV-31, 8% positive for both HPV-16 and -18, and 4% positive for HPV-18.

This contrasts with an earlier report by Boy et al., (2006) who investigated 59 SCC's and found that 11.9% of specimens had detectable HPV DNA. The study focused on HPV-16 and -18 exclusively, with all the positive types identified as HPV-16. All the specimens were ISH negative which raised the question whether the mouth acts as a reservoir to propagate HPV infection. The authors also recommended, just like van Rensburg et al., in 1995, that different HPV-subtypes should be investigated in oral and oropharyngeal SCC's.

Two more recent papers investigated HNSCC with more inclusive specimen sampling (Sekee et al., 2018; Bulane et al., 2020). When the oral and oropharyngeal components are extracted from these papers, prevalence rates of 12.8% (Bulane et al., 2020) and 5% (Sekee et al., 2020) were found. Our study shows an overall prevalence of 11% (n=17) across the two anatomic locations (table 2). This falls within the global prevalence spectrum although a wide prevalence

variability is seen between different geographic regions (Tam et al., 2018). However, the relevance of the presence of HPV in either of these anatomic compartments must be interpreted with caution.

Since PCR will detect HPV in the tissue block regardless of site, the ISH allows for correlation of the particular cells associated with HPV, and therefore it can be deduced that HPV within the tumour cells are drivers of the tumorigenic process. (Paquette et al., 2013). However, clearance of HPV from lesional tissue may bias these types of interpretation. Boy et al., (2006) also emphasized the importance of demonstrating ISH positivity in addition to a positive PCR to conclude on an HPV-driven malignancy as opposed to a passenger infection. As pointed out by Syrjänen (2018), presence of HR-HPV should be excluded from biopsy specimens with suspect histomorphology using any commercially available ISH kit. Our study sample included 21 SCC specimens from the oropharynx. Only two of these were ISH positive, and both had negative PCR assay outcomes for any of the HPV-types targeted.

There are challenges in demonstrating oncogenic gene analyses in from FFPE tissue specimens due to RNA fragmentation and degradation (Prigge et al., 2017; Bulane et al., 2019). Of the 117 specimens chosen for ISH, 19 were ISH positive. None of these were $p16^{INK4a}$ positive, and only 5 were from the oropharynx. Five of the ISH positive specimens were HPV positive and all were from the mouth and associated with a benign HPV-associated lesion.

The use of $p16^{INK4a}$ positivity in tissue specimens serves as a surrogate marker for HPV-driven cellular transformation (Yang et al., 2016; Prigge et al., 2017). The cyclin-dependant kinase (CDK) inhibitor, $p16^{INK4a}$, is a cell-cycle regulatory protein that binds to, and inhibits, CDK-4 and CDK 6. The phosphorylation of the pRb-protein is then prevented, and the cell cycle is halted at the G_1 phase (Haller et al., 2010; Romagosa et al., 2011; Araldi et al., 2018). Although the $p16^{INK4a}$ stain is not routinely used for all oral/oropharyngeal SCC types we felt it prudent to assess the full spectrum of malignancies included in this study for $p16^{INK4a}$ status. This study had 9 SCC tissue specimens positive for $P16^{INK4a}$ all of these were negative for both ISH and for HPV (PCR). These specimens were all from the oropharynx and no malignancy from the mouth was $p16^{INK4a}$ positive.

It can therefore be safely interpreted that the malignant component of the tissue specimens from this South African sample is not driven by HPV. The combination of HPV-DNA detection,

positive $p16^{INK4a}$ staining, and ISH positivity has a 98.6% positive predictive value for HPV-driven oropharyngeal SCC (Jordan et al., 2012). This is not the case for this specimen group of head and neck malignancies.

Conclusion:

In this South African tissue specimen group, no HPV-driven head and neck malignancies could be identified, despite the 11% HPV overall prevalence for all specimen groups (n=155), which is itself within the global reported range. Of this total, 1.9% were from the tonsillar (non-HPV associated) tissue group, and 8.4% from the benign tissue group. Only 1 malignant tissue specimen (0.6%) had detectable HPV DNA but was negative for ISH and for $p16^{INK4a}$. Detection of HPV-DNA must be accompanied by selected biomarker detection to discern HPV-driven malignancies from those with a different aetiopathogenesis.

While no reports provide adequate answers whether HPV-16 vaccines are a requisite preventive measure for head and neck squamous cell carcinoma, it can be reasonably assumed that immunity will be conferred in those women receiving the vaccine, and therefore, protection against this SCC subset, albeit in a much smaller fraction of the affected population.

References:

Adamopoulou M, Vairaktaris E, Panis V, Nkenke E, Neukam FW, Yapijakis C. HPV detection rate in saliva may depend on the immune system efficiency. In Vivo 2008; 22:599-608.

Araldi RP, Sant'Ana TA, Módolo DG, de Melo TC, Spadacci-Morena DD, Stocco RD, Cerutti JM, de Souza EB. The human papillomavirus (HPV)-related cancer biology: An overview. Biomed & Pharmacother 2018; 106:1537-1556.

Benoy I, Xu L, Vanden Broeck D, Poljack M, Valenčak AO, Arbyn M, Bogers J. Using the VALGENT-3 Framework to Assess the Clinical and Analytical Performance of the RIATOL qPCR HPV Genotyping Assay. J Clin Virol 2019; 120:57-62.

Boy S, Janse van Rensburg E, Engelbrecht S, Dreyer L, van Heerden M, van Heerden E. HPV detection in primary intra-oral squamous cell carcinomas – commensal, aetiological agent or contamination? J Oral Pathol Med 2006; 35:86–90.

Bulane A, Goedhals D, Seedat RY, Goedhals J, Burt F. Human papillomavirus DNA in head and neck squamous cell carcinomas in the Free State, South Africa. J Med Virol 2020; 92:227-233.

Cubie HA. Diseases associated with human papilloma virus infection. Virology 2013; 445:21-34.

Dayyani F, Etzel CJ, Liu M, Ho C, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head & Neck Oncology 2010; 2:15.

Egawa N, Egawa K, Griffin H, Doorbar J. Human Papillomaviruses; Epithelial Tropisms, and the Development of Neoplasia. Viruses 2015; 7:3863-3890.

Evans MF, Matthews A, Kandil D, Adamson CS, Trotman WE, Cooper K. Discrimination of 'driver' and 'passenger' HPV in tonsillar carcinomas by the polymerase chain reaction,

chromogenic in situ hybridization, and p16(INK4a) immunohistochemistry. Head & Neck Pathol 2011;5:344-348.

Feller L, Khammissa RA, Wood NH, Marnewick JC, Meyerov R, Lemmer J. HPV-associated oral warts. SADJ 2011; 66:82-85.

Gaester, K. Fonseca LAM, Luiz O, Assone T, Fontes AS, Costa F, Duarte AJ, Casseb J. Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors. Sci Rep 2014; 4:6592.

Haeggblom L, Ramqvist T, Tommasino M, Dalianis T, Näsman A. Time to change perspectives on HPV in oropharyngeal cancer. A systematic review of HPV prevalence per oropharyngeal sub-site the last 3 years. Papillomavirus Research 2017; 4:1-11.

Haller F, Agaimy A, Cameron S, Beyer M, Gunawan B, Happel N, Langer C, Ramadori G, von Heydebreck A, Füzesi L. Expression of $p16^{INK4a}$ in gastrointestinal stromal tumours (GISTs): two different forms exist that independently correlate with poor prognosis. Histopathol 2010; 56:305-318.

IARC. Monographs on the evaluation of carcinogenic risks to humans, volume 100. A review of carcinogen—Part B: biological agents. Lyon: International Agency for Research on Cancer, 2011.

Jordan RC, Lingen MW, Perez-Ordonez B, He X, Pickard R, Koluder M, Jiang B, Wakely P, Xiao W, Gillison ML. Validation of methods for oropharyngeal cancer HPV status determination in United States cooperative group trials. Am J Surg Pathol 2012; 36:945.

Kang SYC, Kannan N, Zhang L, Martinez V, Rosin MP, Eaves CJ. Characterization of epithelial progenitors in normal human palatine tonsils and their HPV16 E6/E7-induced perturbation. Stem Cell Reports 2015; 5: 1210–1225.

Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giulliano AR. Oral Human papillomavirus in healthy individuals: A systematic review of the literature. Sex Transm Dis 2010; 37:386-391.

Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, Roberts S. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. Head and Neck 2013; 35:747-755.

Paquette C, Evans MF, Meer SS, Rajendran V, Adamson CS-C, Cooper K. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in black South Africans. Head and Neck Pathol. 2013; 7:361 – 372.

Piña AR, Fonseca FP, Pontes FS, Pontes HA, Pires FR, Mosqueda-Taylor A, Aguirre-Urizar JM, de Almeida OP. Benign epithelial oral lesions - association with human papillomavirus. Medicina Oral, Patologia Oral y Cirugia Bucal 2019; 24(3):e290-e295

Prigge E, Arbyn M, von Knebel Doeberitz M, Reuschenbach M. Diagnostic accuracy of p16INK4a immunohistochemistry in oropharyngeal squamous cell carcinomas: A systematic review and meta-analysis. Int J Cancer 2017; 140:1186-1198.

Prue G, Lawler M, Baker P, Warnakulasuriya S. Human papillomavirus (HPV): making the case for 'Immunisation for All'. Oral Dis 2017; 23:726-730.

Romagosa C, Simonetti S, López-Vicente L, Mazo A, Lleonart ME, CastellviJ, Ramon y Cajal S. p16INK4a overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors. Oncogene 2011; 30:2087-2097.

Sanders AE, Slade GD, Patton LL. National prevalence of oral HPV infection and related risk factors in the U.S. adult population. Oral Dis 2012; 18: 430–441.

Sekee TR, Burt FJ, Goedhals D, Goedhals J, Munsamy Y, Seedat RY. Human papillomavirus in head and neck squamous cell carcinomas in a South African cohort. Papillomavirus Res 2018; 6:58-62.

Syrjänen S. Oral manifestations of human papillomavirus infections. Eur J Oral Sci 2018; 126(Suppl. 1):49–66.

Tam S, Fu S, Xu L, Krause KJ, Liarson DR, Miao H, Sturgis EM, Dahlstrom KR. The epidemiology of oral human papillomavirus infection in healthy populations: A systematic review and meta-analysis. Oral Oncol 2018;82:91-99.

Termine N, Panzarella V, Falaschini S, Russo A, Matranga D, Muzio LL, Campisi G. HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988–2007). Ann Oncol 2008; doi:10.1093/annonc/mdn372

Van Rensburg EJ, van Heerden WF, Venter EH, Raubenheimer EJ. Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population. S Afr Med J 1995; 85: 894– 896.

Van Rensburg EJ, Engelbrecht S, Van Heerden WF, Raubenheimer EJ, Schoub BD. Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. Anticancer Res 1996; 16: 969– 973.

Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45:309-306.

Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken OM, Bogers J. Human Papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-positive cohort from Pretoria, South Africa. Adv in Virol 2020; https://doi.org/10.1155/2020/2395219.

Yang H, Cao Y, Li Z, Li Y, Jiang W, Shi Y. The role of protein $p16^{INK4a}$ in non-oropharyngeal head and neck squamous cell carcinoma in Southern China. Oncol Letters 2018; 16:6147-6155.

CHAPTER 7:

General conclusion

CHAPTER 7:

General conclusion

This project followed a systematic approach to determine the prevalence of HPV infection in the mouth and oropharynx of the population residing in the North-western regions from Pretoria, Gauteng, South Africa, and also established an important baseline for the practice of oral sex, tobacco use and alcohol use in this population. This project therefore recorded the first reliable data for any South African population concerning high-risk behaviours associated with the development of head and neck SCC in association to regional HPV oral/oropharyngeal prevalence in all sampling types.

Valuable insight was gained into the combined practices of oral sex and tobacco use in a single population group, and the differences compared to developed countries as reported in the international literature. It was an important baseline contribution which assists in the understanding of the subsequent findings as outlined in this thesis. The bulk of the literature on HPV originates from developed countries and these important behavioural and social interactions are known to influence the development, natural course and progression of HPV infection, lesion development, and malignant transformation.

It was determined that in this population a baseline of 22.4% of individuals confirmed to practice oral sex. In a subsequent study as a follow up to the first chapter of this thesis, this figure was shown to increase to 40.3%, but from a much smaller sample size. One of the reasons for this is that the topic of oral sex practice is considered taboo within the community, and in the protected environments of the dental and HIV-clinics it is thought that the patients felt more comfortable and safer to disclose the sensitive information.

A much larger percentage of this population 54.8% confirmed to have consumed alcohol and up to 21.7% were smokers. Also, important to note is that the use of tobacco and alcohol and the practice of OS are independent of each other and are not associated risk factors but may act independently within this population. Black South Africans were also less likely to perform OS in this geographic region (22.8%). This lower rate of OS practice may play a role in the lower prevalence of HPV-associated oral and oropharyngeal cancer seen in this population group.

A systematic review and meta-analysis of published oral and oropharyngeal HPV infection from South African studies presented in chapter 2 of this thesis revealed a pooled prevalence of 11% for South Africa, but many limitations within the existing South African data were highlighted.

Overall HPV prevalence for each population/specimen group within this project was determined as follows:

HIV-seropositive patients	-	2.8%
Patients attending a dental clinic	-	1.4%
Men who have sex with men	-	6%
Benign and Malignant tissue specimens:	-	11%

Although the overall prevalence rate in South Africa reflects favourably to that reported in the international literature, the prevalence rates for HIV-seropositive patients, for dental clinic attendees and for MSM are on the lower end of the spectrum. Importantly, the clinical presence of any HPV-associated lesions was zero in those from the HIV management clinic, the dental clinic and the MSM participants. This is a significant finding that requires a reassessment for the role of HPV infection in the mouth and oropharynx of this particular South African population, perhaps as suggested: a passenger and transient infection.

HPV-positivity was observed for 11% for all tissue specimens, of which the benign tissue specimen group had the most substantial proportion (8.4%). HPV prevalence within the different groups were as follows: In the tonsillar tissue specimen group 4% was HPV positive, in the malignant tissue component it was 5%, and the HPV-positive benign lesions was 21% of all benign lesions. Three tissue specimens which had multiple HPV types detected. The most commonly detected HPV types were HR-HPV (7.7%). It is worth noting is that only 1 malignant tissue specimen (0.6%) had detectable HPV-DNA but was negative for ISH and for $p16^{INK4a}$. This leans strong credence to the postulate that the oral and oropharyngeal malignancies within this population group are not HPV-driven.

It is noteworthy that not a single participant in the clinical component of this project presented with a clinically manifest HPV-associated oral/oropharyngeal lesion.

The data from this project supports the proposal that HPV infection of the mouth represents a passenger infection and may propagate from this anatomic site, but it is not responsible for oral or oropharyngeal SCC in the South African population group involved. Furthermore, the prevalence of HPV-associated SCC in the tissue-specimen group examined in this project is undetermined as none of the SCC's from the mouth or the oropharynx were HPV-induced or maintained.

It could be reasonably proposed from the observations in the preceding chapters, that removal of the lingual tonsils before the onset of sexual activity may prove to be protective/preventive against the development of HPV-induced oropharyngeal SCC. Remarkably, none of the dental clinic attendees had lingual tonsils present, and only one participant from the HIV-seropositive cohort had tonsils but was HPV negative. This observation requires further investigation.

While no reports provide adequate answers whether HPV-16 vaccines are a requisite preventive measure for head and neck squamous cell carcinoma, it can be reasonably assumed that immunity will be conferred in those women receiving the vaccine, and therefore, protection against this SCC subset, albeit in a much smaller fraction of the affected population. In so doing, and in the context of head and neck SCC driven by HPV, vaccinating boys from this population group prior to their commencement of sexual activity may be questionable, and more investment into research focused in this field is required.

List of recent publications:

2015

Moodley A, Wood NH. HIV-associated oral lesions in HIV-seropositive patients at an HIV-treatment clinic in South Africa. J AIDS Clin Res 2015; 6:2.

Lutin A, Bulatov V, Jadwat Y, Wood NH, Feller L, Schechter I. Detection of indoor common contaminants on the surface of dental implants. Talanta 2015; 134:514-523.

2016

Wood NH, Moodley A. Oral Medicine Case Book 72: A rare case of nodular fasciitis affecting the mandibular gingiva. SADJ 2016; 71:78-80.

Khammissa RAG, Ballyram R, Wood NH, Lemmer J, Feller L. Glucocorticoids in the treatment of immune-mediated oral diseases. SADJ 2016; 71:62-67.

Ballyram R, Wood NH, Khammissa RAG, Lemmer J, Feller L. Oral diseases associated with human herpes viruses: aetiology, clinical features, diagnosis and management. SADJ 2016; 71:253-259.

Wood NH, Meer S, Khammissa RAG, Lemmer J, Feller L. Lower facial and cervical lymphadenopathy in the context of clinical dentistry. SADJ 2016; 71:509-512.

2017

Feller L, Wood NH, Khammissa RAG, Lemmer J. Review: Allergic Contact Stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol 2017; 123:559-565.

2019

Wood NH, Ayo-Yusuf OA, Gugushe TS, Boger J. Tobacco use and oral sex practice among dental clinic attendees. PLoS ONE 2019; 14(3): e0213729.

2020

Jonker CH, Van der Vyver P, Arnold S, Wood NH. A combined therapeutic approach to treating a challenging case of Dens Invaginatus. SADJ 2020; 75:81-86.

Koutras S, Govender S, Wood NH, Motloba PD. COVID-19 pandemic and the dental practice. SADJ 2020; 75:119-125.

Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken O, Bogers J. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-Positive cohort from Pretoria, South Africa. Advances in Virology 2020; https://doi.org/10.1155/2020/2395219.

List of HPV-associated publications:

2006

Feller L, Wood NH, Raubenheimer EJ. Complex manifestations of an HIV-seropositive patient. J Intl Acad Periodontol 2006;8:10-16.

2009

Feller L, Khammissa RAG, Wood NH, Lemmer J. Epithelial maturation and molecular biology of oral HPV. Infect Agents Cancer 2009;4:16.

2010

Wood NH, Khammissa RAG, Chikte UME, Meyerov R, Lemmer J, Feller L. The pathobiology and mechanisms of infection of HPV. SADJ 2010; 65;122-128.

Feller L, Khammissa RAG, Wood NH, Malema V, Meyerov R, Lemmer J. Focal epithelial hyperplasia (Heck disease) related to highly active antiretroviral therapy in an HIV-seropositive child. A report of a case, and a review of the literature. SADJ 2010; 65:172-175.

Feller L, Wood NH, Khammissa RAG, Lemmer J. Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. Part 1: Human papillomavirus-mediated carcinogenesis. Head and Face Med 2010; 6:14.

Feller L, Wood NH, Khammissa RAG, Lemmer J. Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. Part II: Human papillomavirus associated oral and oropharyngeal squamous cell carcinoma. Head and Face Med 2010; 6:15.

Feller L, Wood NH, Khammissa RAG, Chikte UME, Meyerov R, Lemmer J. HPV modulation of host immune responses. SADJ 2010; 65:266-268.

Feller L, Bouckaert M, Chikte UME, Wood NH, Khammissa RAG, Meyerov R, Lemmer J. A short account of cancer – specifically in relation to squamous cell carcinoma. SADJ; 2010: 65:322-324.

Feller L, Wood NH, Khammissa RAG, Chikte UME, Gugushe T, Meyerov R, Lemmer J. Epidemiology and transmission of HPV infection: vaccination for HPV infection. SADJ 2010; 65:478-481.

2011

Feller L, Khammissa RAG, Wood NH, Marnewick J, Meyerov R, Lemmer J. HPV-associated oral warts. SADJ 2011; 66:82-85.

2012

Moodley A, Wood NH. HIV-associated oral lesions as an indicator for HAART failure: a review. SADJ 2012; 67(7):348-352

Moodley A, Wood NH. Oral Medicine case book 43 – Focal epithelial hyperplasia in an HIV-seropositive child. SADJ 2012; 67(9):510-512

2020

Wood NH, Makua KS, Lebelo RL, Redzic N, Benoy I, Vanderveken O, Bogers J. Human papillomavirus prevalence in oral and oropharyngeal rinse and gargle specimens of dental patients and of an HIV-Positive cohort from Pretoria, South Africa. Advances in Virology 2020; https://doi.org/10.1155/2020/2395219.

PLOS ONE

RESEARCH ARTICLE

Tobacco use and oral sex practice among dental clinic attendees

Neil H. Wood[1]*, **Olalekan A. Ayo-Yusuf**[2], **Tshepo S. Gugushe**[3], **John-Paul Bogers**[4,5]

1 Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa, **2** Africa Centre for Tobacco Industry Monitoring & Policy Research, Sefako Makgatho Health Sciences University, Pretoria, South Africa, **3** School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa, **4** Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), University of Antwerp, Antwerp, Belgium, **5** Laboratory of Cell Biology & Histology, Antwerp, Belgium

* neil.wood@smu.ac.za, drnhwood@gmail.com

OPEN ACCESS

Citation: Wood NH, Ayo-Yusuf OA, Gugushe TS, Bogers J-P (2019) Tobacco use and oral sex practice among dental clinic attendees. PLoS ONE 14(3): e0213729. https://doi.org/10.1371/journal.pone.0213729

Editor: Mark Allen Pershouse, University of Montana, UNITED STATES

Received: May 11, 2018

Accepted: February 27, 2019

Published: March 13, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information file. Minimal anonymized data are available and the data file has been uploaded with the filename "Dataset" during submission of the revision.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Tobacco use and oral sex (OS) are important risk factors for oral and oropharyngeal Human papillomavirus (HPV) infection. Little is known about the prevalence of OS practice in South Africa. This study aimed to determine the prevalence of OS practice and tobacco use in a South African patient population. This cross-sectional study used a structured questionnaire to collect socio-demographic characteristics, tobacco use, betel nut use and OS practice data from consenting adults (≥18 years; n = 850). Oral sex practices were recorded for patients 18–45 years-old (n = 514). Data analysis included chi-square and multiple logistic regression analyses. Of the study population, 55.2% (n = 468) were female, 88% (n = 748) self-identified as black Africans and 45.1% (n = 383) were unemployed. Furthermore, 19.7% (n = 167), 6.4% (n = 54) and 2.1% (n = 18) were current smokers, snuff users and betel nut users, respectively. Out of the 514 who answered the questionnaire in relation to OS, 22.8% (n = 115) reported to practice it. Oral sex practice in the age group 18–45 years was most common among the self-identified white participants (41.9%); and among tobacco users than among non-tobacco users (30.9% vs. 20.5%; p = 0.022). A multivariable-adjusted regression model showed that white South Africans were more likely to use tobacco than black Africans (OR = 5.25; 95% CI = 2.21–12.47). The practice of OS was more likely among those 18–35 years-old (OR = 1.67; 95% CI = 1.01–2.74), but had no significant association with tobacco use (OR = 1.06; 95% CI = 0.62–1.83). The observed age and ethnic differences in both risk behaviours suggest a need for targeted population intervention in order to reduce the risk for oral HPV infection.

Introduction

The aetiopathogenesis of oropharyngeal squamous cell carcinoma (SCC) has been linked to high-risk human papillomavirus (HPV) infection [1–3].While the incidence of SCC of the head and neck is diminishing, that of HPV-related oropharyngeal SCC is increasing [4]. This implies that different aetiologic mechanisms may be at play [5] and support the postulate that HPV-associated SCC is a distinct and separate clinical entity from tobacco and alcohol-

associated SCC [6,7]. Earlier oral/oropharyngeal HPV studies were limited by the lack of a standardized meaning for the "oral" vs "oropharyngeal" anatomical compartments. This lead to ambiguity in some reports and care must be taken when interpreting results representative of these two distinct anatomic sites [8,9]. The oropharyngeal site is defined by Paquette and colleagues [9] as "...posterior one-third of the tongue, palatine and pharyngeal tonsils, bounded inferiorly by the epiglottis and superiorly by the soft palate.".

Oral and oropharyngeal SCC is the 6th most common cancer and also the 6th largest cause of cancer related deaths worldwide [10]. Patients diagnosed with oral SCC have a mean 5-year survival rate of about 50%. The most important risk factors of oral SCC are tobacco smoking, excessive alcohol intake, chewing betel quid and areca nut and a diet low in fresh fruits and vegetables [10].

Tobacco use has a long association with the development of head and neck malignancy and the use of alcohol and tobacco are well-known risk factors for the development of head and neck SCC [3,11,12]. Some association between smoking and prevalence of oral HPV infection exists, but more importantly, tobacco use has been associated with a reduced capacity for the clearance of oncogenic HPV-infection [13,14]. Although the biologic link responsible for increased prevalence of oral HPV in current smokers has not yet been fully defined, the rationale lies in the local oral/oropharyngeal mucosal pro-inflammatory milieu and the immune suppression induced by tobacco use, creating a favourable niche for HPV infection and persistence [15].

Infection by HPV is the most common sexually transmitted disease (STD) [16]. Although oral and oropharyngeal HPV infections are believed to be acquired by orogenital contact with an infected sexual partner, by mouth-to-mouth contact or by autoinoculation from another infected site [17], some studies report the majority of cases with oral HPV infection are not the result of sexual transmission [18,19]. Nevertheless, it is important to understand the demographic characteristics of OS practice in order to further research on its influence in oral health, especially in resource-poor settings such as this study's population.

HPV-infection and SCC of the mouth and oropharynx have been associated with patients becoming sexually active at a younger age, having numerous sexual partners, and with practicing orogenital sex (OS) [20–22]. While there is a strong association between HPV and oropharyngeal SCC with about 50% of all cases of HPV- cytopositive oropharyngeal SCC being caused by high-risk HPV genotypes, in the case of oral SCC there is limited evidence causally linking HPV infection of the mouth to oral SCC [23–25].

Within the limited scope of evidence, the apparently lower frequency of HPV infection in oral and oropharyngeal SCC of South African cohorts [8,26] could be because the practice of OS may be less common among South Africans than among Western and Asian populations; and may differ between different racial groups [27,28]. Reports on the ethnic distribution of OS practice are also very limited in the international literature, and when available, it presents different prevalence rates for OS practice according to the geographic region of the study [4,29,30]. While a number of studies have investigated the characteristics of tobacco use and to a lesser extent the practice oral sex [14], most have been done separately despite the fact that both risk behaviours may be related and co-exist. The practice of OS is a known high-risk sexual behaviour that facilitates oncogenic HPV transmission [31].

The purpose of this study was to investigate the prevalence of tobacco use and the practice of OS among the patients attending the Sefako Makgatho Health Sciences University Oral Health Centre located in a peri-urban area of South Africa.

Material and methods

This cross-sectional study involved consenting adults (≥18 years; n = 847) who attended for consultation at a university-based Oral Health Centre (OHC). Using a structured self-

administered questionnaire, socio-demographic characteristics that included age, gender, self-identified race/ethnicity (Black African; Coloured (Mixed ancestry); White; Indian/Asian), tobacco smoking and/or snuff use (some days or everyday), betel nut use and OS practices were recorded. Oral sex practices were recorded only for patients 18–45 years-old (n = 514). Oral sex practice was determined by asking participants whether they were currently engaged in oral sex practice, having their mouth in contact with a partner's genitalia.

Data analysis included chi-square and multi-variable adjusted logistic regression analyses. Two separate regression models were reported for OS and tobacco use. In both instances the independent effect of one as a predictor-variable of the other as an outcome-variable was controlled for age, gender, ethnicity and employment status. All tests were two-tailed and p values of 0.05 or less considered as significant. Ethical clearance for this project was obtained from the Sefako Makgatho Health Sciences University Research Ethics Committee (MREC/D/187/2010:IR).

Results

The study sample comprised 847 patients who visited the university-based Oral Health Centre. Study participants self-identified their race and of the 847 patients, 93% (748 patients) self-identified as black African, 6% (55 patients) were white, 2 patients (0.3%) were Indian, only 1 patient (0.1%) was of mixed race and 41 did not identify their race. Of the study population (n = 468), 55.2% were female and 26 participants did not indicate their gender on the questionnaire. Eighty-eight percent (n = 748) were Black and 45.1% (n = 383) were unemployed (Table 1). Owing to the small participation number, those participants who self-identified as 'unknown race', of Indian and of mixed race were excluded from further analysis.

Four hundred and seventy-five of the 748 black patients and 36 of the 55 white patients answered the question relating to their sexual behaviour; 21.6% (99) of the black patients and

Table 1. Socio-demographic characteristics of the study sample.

Characteristics		% (n)
Gender	Male	41.6 (n = 353)
	Female	55.2 (n = 468)
	Unknown	3.1 (n = 26)
Race	Black African	88.3 (n = 748)
	Whites	6.5 (n = 55)
	Indian/Asian	0.2 (n = 2)
	Mixed race	0.1 (n = 1)
	Unknown	4.8 (n = 41)
Age Group	18–35 years	44.2 (n = 368)
	36–45 years	22.5 (n = 187)
	>45 years	33.3 (n = 277)
Employment status	Employed	30.2 (n = 257)
	Retired/Student	7.6 (n = 64)
	Unemployed	45.1 (n = 383)
	Unknown	17.1 (n = 143)
Tobacco use	Current smoker	19.7 (n = 167)
	Current snuff user	6.4 (n = 54)
	Betel nut user	(n = 18)
Practice orogenital sex*	Current practice	22.8 (n = 115)

*Only among those 18–45 years old

https://doi.org/10.1371/journal.pone.0213729.t001

Table 2. Association between socio-demographic characteristics, oro-genital sex and tobacco smoking.

Characteristics		Practice oro-genital sex* %	p-value	Tobacco use %	p-value
		(n = 514)		(n = 847)	
Gender	Male	32.1 (69)		38.6 (135)	
	Female	16.2 (46)		12.0 (56)	
			<0.001		<0.001
Race	Black African	21.6 (99)		21.7 (162)	
	Whites	41.9 (13)		53.7 (29)	
	Other' (Excluded)	0.5 (3)		0.0 (0)	
			0,031		<0.001
Age group	>45 years	n/a		25.8 (71)	
	36–45 years	17.4 (28)		23.0 (43)	
	18–35 years	25.2 (89)		21.5 (79)	
			0,05		0,432
Employment	Unemployed	24.8 (50)		23.8 (91)	
status	Retired/student/unknown	19.6 (31)		18.7 (39)	
	Employed	23.4 (36)		27.0 (69)	
			0,503		0,108
Tobacco user	No	20.5 (83)			
	Yes	30.9 (34)			
			0,022		
Practice oro-	No			19.1 (76)	
genital sex*	Yes			29.1 (34)	
					0,022

*Only among those 18–45 years that responded to this question

https://doi.org/10.1371/journal.pone.0213729.t002

41.9% (13) of the white patients practiced OS (Table 2). Data for other race groups were excluded from analysis due to insufficient sample size (n = 3).

Of the participants, 19.7% (n = 167), 6.4% (n = 54) and 2.1% (n = 18) were current smokers, snuff users and betel nut users, respectively (Table 1). Out of the 514 who answered the questionnaire in relation to OS, 22.4% (n = 115) reported to practice OS (Table 2). Oral sex practice in the age group 18–45 years was significantly more common among white South Africans (41.9%) than among black South Africans; and among tobacco users than among non-tobacco users (30.9% vs. 20.5%; p = 0.022) (Table 2). A multivariable-adjusted regression model showed that compared to black South Africans, white South Africans were more likely to use tobacco (OR = 5.25; 95% CI = 2.21–12.47) and practice OS (OR = 2.38; 95% CI = 1.06–5.35). However, after controlling for confounding factors, the practice of OS was not significantly associated with tobacco use (OR = 1.06; 95% CI = 0.62–1.83) (Table 3).

Discussion

This study showed that about 1 in 5 clinic attendees in this dental training institution practice OS. Furthermore, almost a third of those who practice OS were also current tobacco users. Consistent with the literature, tobacco use were more common among men (38.6%) than among women (12%) and this ratio (3.2:1) is closer than that of the national prevalence (4:1) [32–33]. Our study indeed showed that tobacco use in this predominantly black African population of dental clinic attendees (21.7%) was slightly higher than the reported national prevalence of 17.7% for this population group [33,34].

Table 3. Multivariable-adjusted regression model of factors associated with tobacco use and practice of oro-genital sex among those 45 years old and younger.

Characteristics		Practice oro-genital sex* % (N = 514)	p-value	Tobacco use % (N = 847)	p-value
		OR (95% CI)		OR (95% CI)	
Gender	Male	1		1	
	Female	0.42 (0.27–0.67)	<0.001	0.15 (0.09–0.25)	<0.001
Race					
	Black African	1		1	
	Whites	2.38 (1.06–5.35)	0.035	5.25 (2.21–12.47)	<0.001
Age group					
	36–45 years	1		1	
	18–35 years	1.67 (1.01–2.74)	0.045	1.25 (0.74–2.13)	0.401
Employment status					
	Unemployed	1		1	
	Retired/Student/ unknown	1.15 (0.69–1.94)	0.593	0.50 (0.27–0.92)	0.026
	Employed	0.73 (0.41–1.31)	0.291	0.70 (0.40–1.23)	0.220
Tobacco user					
	No	1		-	
	Yes	1.08 (0.63–1.85)	0.776	-	
Practice oro-genital sex*	No	-		1	
	Yes	-		1.06 (0.62–1.83)	0.825

*Only among those 18–45 years that responded to this question

https://doi.org/10.1371/journal.pone.0213729.t003

Despite South African data showing that oropharyngeal cancer in white South African population occurs at a much older age than other ethnic groups [35], no reports on ethnic distribution of OS practice are available for the South African population. However, broader population based reports of OS practice demonstrate a wide variation between population groups.

Our finding of 32% prevalence of OS practice among males is comparable to 40% prevalence reported among high-risk male South African factory workers recently published [26]. However, the study by Vogt and colleagues [36] reports 84% of men and 82% of women in heterosexual couples practiced oral sex which was consistent with data from Canada (71%) [28] and the US (80%) [31]. Conversely, another South African study of heterosexual couples, but in a different geographic location, reported that only 8.7% of women and 6.2% of men reported to practice oral sex which is similar to that reported in China [37,38].

The differences in these reports could be due to different study designs, data collection methods, and analyses. The target population group also plays a role in the reporting of oral sex practice [28]. Conceivably, the practice of OS may be culturally inclined. The number of oral sex partners, the frequency of oral sexual events, and even the duration of each oral sexual event may all play a role in the extent to which OS practice is self-reported. However, these variables were not explored in detail due to the cultural and societal sensitivities surrounding this topic in this population group.

This study highlighted a significantly higher likelihood to practice OS among youth than older adults. This is consistent with the literature [28]. Furthermore, considering that OS is a significant source of exposure to HPV, OS may partly explain why HPV-associated oropharyngeal SCC is more common in younger people [10]. The practice of OS by younger adults has

been characterised as a normative social practice that is less intimate and others do this in an effort to avoid pregnancy [39] and as a "benefit-provisioning mate retention behaviour" [40]. A study of 410 younger heterosexual adult women reported that OS was performed as a way to express love and care to their male partner [40]. The higher risk for OS among youths support targeted interventions such as the promotion of condom and dental dam in the prevention of oral HPV infection [41]

There were significant racial differences in the practice of OS and tobacco use with white South Africans most likely to report both risk behaviours for oral and oropharyngeal cancer. On the one hand, OS increases the risk of HPV-exposure and on the other hand, smoking reduces the clearance of HPV, which means that white South Africans who are more likely to both smoke and practice OS may be at a higher risk to develop oral and oropharyngeal infection. It is nevertheless pertinent to note that in this study, smoking was not significantly associated with OS practice, therefore neither of these risk behaviours can be used as a risk behaviour marker for the other.

The practice of OS was twice more common among white than black South Africans in this study. This relatively low frequency of OS, in particular among black South Africans, may explain why despite the fact that in South Africa the prevalence of genital HPV infection is as high as 22.1% among women [42] with one study demonstrating a prevalence of 68% [43], the prevalence of oral HPV infection (3.5–8.4%) [38,44] is relatively low. In fact, only about 20% of HIV-seropositive black women with genital HPV infection have concurrent oral HPV infection, and in only half of this 20% can the genital HPV genotypes be detected in the mouth [8]. Self-inoculation via the genital-oral route has been suggested as a source of oral HPV infection in the South African setting [38].

Study limitations

Some caution in the interpretation of our study findings in relation to the study's limitations would include the fact that the OS and tobacco behaviour were self-reported. It may indeed be that respondents provided sociably desirable responses and that this may be an under-representation of OS practice and of tobacco use. The findings of this study are limited to dental clinic attendees therefore may not be generalized to the general South African population.

Due to cultural and societal sensitivities associated with the practice of OS in this population group, the nature of the OS practice, including frequency of practice, was not further investigated. We believe that forcing this sensitive topic on this population would have greatly reduced participation and this project sensitised many participants and non-participants in this population to a topic considered taboo.

Despite these limitations, this study provides useful information for prioritizing public health interventions and for further research, which may include more in depth demographic and epidemiological profile of those who practice OS and the presentations of signs and symptoms of related infection.

Conclusion

The study findings suggest that tobacco use and the practice of oral sex are not significantly associated risk behaviours and thus could be considered independent risks for oral and oropharyngeal infection. Furthermore, age and ethnic differences in both risk behaviours suggest need for targeted population intervention in order to prevent and reduce the incidence of oral and oropharyngeal infection. Community engagement and further investigation are required concerning perceptions of oral sex practice and tobacco use.

Supporting information

S1 File. Dataset submitted as per journal requirement.
(XLSX)

Acknowledgments

L Feller for contribution to the conceptualization of the study; RAG Khammissa and R Chandran for assistance in the data collection

Author Contributions

Conceptualization: Neil H. Wood.

Formal analysis: Olalekan A. Ayo-Yusuf.

Investigation: Neil H. Wood.

Methodology: Tshepo S. Gugushe, John-Paul Bogers.

Project administration: Neil H. Wood.

Resources: Tshepo S. Gugushe, John-Paul Bogers.

Validation: Olalekan A. Ayo-Yusuf.

Writing – original draft: Neil H. Wood, Tshepo S. Gugushe, John-Paul Bogers.

Writing – review & editing: Neil H. Wood, Olalekan A. Ayo-Yusuf, Tshepo S. Gugushe, John-Paul Bogers.

References

1. Petti S. Lifestyle risk factors for oral cancer. Oral Oncol. 2009; 45: 340–350. https://doi.org/10.1016/j.oraloncology.2008.05.018 PMID: 18674956
2. Shah JP, Gil Z. Current concepts in management of oral cancer surgery. Oral Oncol. 2009; 45: 394–401. https://doi.org/10.1016/j.oraloncology.2008.05.017 PMID: 18674952
3. Upile T, Jerjes W, Al-Khawalde M, Radhi H, Sudhoff H. Oral sex, cancer and death: sexually transmitted cancers. Head Neck Oncol. 2012; 4: 31. https://doi.org/10.1186/1758-3284-4-31 PMID: 22673108
4. Farsi NJ, El-Zein M, Gaied H, Lee YCA, Hashibe M, Nicolau B, et al. Sexual behaviours and head and neck cancer: A systematic review and meta-analysis. Cancer Epidemiol. 2015; 39: 1036–1046. https://doi.org/10.1016/j.canep.2015.08.010 PMID: 26372414
5. Shibosky CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 22–44 years. Cancer. 2005; 103: 1843–1849. https://doi.org/10.1002/cncr.20998 PMID: 15772957
6. Gillison M, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, et al. Distinct risk factor profiles for Human papillomavirus type 16-positive and Human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008; 100:407–420. https://doi.org/10.1093/jnci/djn025 PMID: 18334711
7. Gillison ML, Castelsagué X, Chaturvedi A, Goodman MT, Snijders P, Tommasino M, et al. Eurogin roadmap: Comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. Int J Cancer. 2014; 134:497–507. https://doi.org/10.1002/ijc.28201 PMID: 23568556
8. Richter KL, Janse van Rensburg E, van Heerden WFP, Boy SC. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. J Oral Pathol Med. 2008; 37: 555–559. https://doi.org/10.1111/j.1600-0714.2008.00670.x PMID: 18355174
9. Paquette C, Evans MF, Meer SS, Rajendran V, Adamson CS, Cooper K. Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in Black South Africans. Head and Neck Pathol. 2013; 7: 361–372.
10. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009; 45: 309–316. https://doi.org/10.1016/j.oraloncology.2008.06.002 PMID: 18804401

11. International Agency for Research on Cancer (IARC). Tobacco smoking. IARC Monogr Eval Carcinog Risk Chem Hum. 38, Lyon: IARC, 1986. PMID: 3460963
12. Johnson N. Tobacco use and oral cancer: a global perspective. J Dent Edu. 2001; 65: 328–339.
13. Giuliano AR, Sedjo RL, Roe DJ, Harri R, Baldwi S, Papenfuss MR, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes & Control. 2002; 13: 839–846.
14. Fakhry C, Gillison ML, D'Souza G. Tobacco use and oral HPV-16 infection. JAMA. 2014; 312: 1465–1467. https://doi.org/10.1001/jama.2014.13183 PMID: 25291584
15. Kreimer AR, Pierce Campbell CM, Lin H, Fulp W, Papenfuss MR, Abrahamsen M, et al. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. Lancet. 2013; 382: 877–887. https://doi.org/10.1016/S0140-6736(13)60809-0 PMID: 23827089
16. Mbulawa ZZ, Johnson LF, Marais DJ, Coetzee D, Williamson A. The impact of human immunodeficiency virus on human papillomavirus transmission in heterosexually active couples. J Infection. 2013; 67: 51–58.
17. D'Souza A, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviours associated with prevalent oral human papillomavirus infection. J Infect Dis. 2009; 199: 1263–1269. https://doi.org/10.1086/597755 PMID: 19320589
18. Rintala M, Grenman S, Puranen M, Syrjänen S. Natural history of oral papillomavirus infections in spouses: A prospective Finnish HPV family study. J Clin Virol. 2006; 35: 89–94. https://doi.org/10.1016/j.jcv.2005.05.012 PMID: 16112613
19. Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Oral mucosa as a reservoir of human papillomavirus: Point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. Eur Urol. 2012; 62: 1063–1070. https://doi.org/10.1016/j.eururo.2012.06.045 PMID: 22771267
20. Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, et al. Oral human papillomavirus infection in adults is associated with sexual behaviour and HIV serostatus. J Infect Dis. 2004; 189: 686–698. https://doi.org/10.1086/381504 PMID: 14767823
21. Stanley M. Prevention strategies against the human papillomavirus: the effectiveness of vaccination. Gynecol Oncol, 2007; 107: S19–S23. https://doi.org/10.1016/j.ygyno.2007.07.068 PMID: 17938013
22. Syrjanen S. Human papillomaviruses in the head and neck carcinomas. N Engl J Med. 2007; 365: 1993–1995.
23. Hobbs CG, Stern JA, Baily M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin Otolaryngol. 2006; 31: 461–462.
24. Van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. Oral Oncol. 2010; 46: 423–425. https://doi.org/10.1016/j.oraloncology.2010.02.016 PMID: 20308005
25. Smith EM, Rubenstein LM, Haugen TH, Pawlita M, Turek LP. Complex etiology underlies risk and survival in head and neck cancer Human Papillomavirus, tobacco, and alcohol: a case for multifactor disease. J Oncol. 2015; ID571862, https://doi.org/10.1155/2012/571862 PMID: 22315596
26. Davidson CL, Richter KL, van der Linde M, Coetsee J, Boy SC. Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: a pilot study. S Afr Med J. 2014; 104: 358–361. https://doi.org/10.7196/samj.7542 PMID: 25212204
27. Johnson EH, Jackson LA, Hinkle Y, Gilbert D, Hoopwood T, Lollis CM, et al. What is the significance of black-white differences in risky sexual behaviour? J Natl Med Assoc. 1994; 86: 745–749. PMID: 7807559
28. Vannier SA, O'Sullivan LF. Who gives and who gets: why, when, and with whom young people engage in oral sex. J Youth Adolescence. 2012; 41: 572–582.
29. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. J Clin Oncol. 2015; 33: 3235–3242. https://doi.org/10.1200/JCO.2015.61.6995 PMID: 26351338
30. Jelihovschi I, Bidescu AC, Tucaliuc SE, Iancu LS. Detection of human papillomavirus in head and neck squamous cell carcinomas: a literature review. Revista Medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 2015; 119: 502–509.
31. D'Souza G, McNeel TS, Fakhry C. Understanding personal risk of oropharyngeal cancer: risk-groups for oncogenic oral HPV infection and oropharyngeal cancer. Ann Oncol. 2017; 28: 3065–3069. https://doi.org/10.1093/annonc/mdx535 PMID: 29059337
32. Peer N, Bradshaw D, Laubscher R, Steyn N, Steyn K. Urban-rural and gender differences in tobacco and alcohol use, diet and physical activity among young black South Africans between 1998 and 2003. Glob Health Action. 2013; 6: 19216. https://doi.org/10.3402/gha.v6i0.19216 PMID: 23364100

33. Reddy P, Zuma K, Shisana O, Jonas K, Sewpaul R. Prevalence of tobacco use among adults in South Africa: Results from the first South African National Health and Nutrition Examination Survey. SAMJ. 2015; 105: 648–655. PMID: 26449697

34. Peer N, Bradshaw D, Laubscher R, Steyn K. Trends in adult tobacco use from two South African demographic and health surveys conducted in 1998 and 2003. S Afr Med J 2009; 99: 744–749. PMID: 20128274

35. Ayo-Yusuf OA, Lalloo R, Johnson NW. Trends and ethnic disparities in oral and oro-pharyngeal cancers in South Africa 1992–2001. SADJ. 2013; 68: 168–173. PMID: 23971297

36. Vogt SL, Gravitt PE, Martinson NA, Hoffman J, D'Souza G. Concordant oral-genital HPV infection in South Africa couples: evidence for transmission. Front Oncol. 2013; 3: 1–7. https://doi.org/10.3389/fonc.2013.00001

37. Ma Q, Pan X, Cai G, Yan J, Xu Y, Ono-Kihara M, et al. The characteristics of heterosexual STD clinic attendees who practice oral sex in Zhejiang province, China. Plos One. 2013; 8, e67092. https://doi.org/10.1371/journal.pone.0067092 PMID: 23825627

38. Mbulawa ZZ, Johnson LF, Marais DJ, Coetzee D, Williamson AL. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. J Infection. 2014; 68: 185–189.

39. Vannier SA, Byers ES. A qualitative study of university students' perceptions of oral sex, intercourse, and intimacy. Arch Sex Behav. 2013; 42: 1573–1581. https://doi.org/10.1007/s10508-013-0130-0 PMID: 23835846

40. Sela Y, Shackelford TK, Pham MN, Eule HA. Do women perform fellatio as a mate retention behaviour? Pers Individ Dif. 2015; 73: 61–66.

41. Gupta A, Perkins RB, Ortega G, Feldman S, Villa A. Barrier use during oro-genital sex and oral Human Papillomavirus prevalence: Analysis of NHANES 2009–2014. Oral Dis. 2018; 00:1–8. https://doi.org/10.1111/odi.12997

42. De Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Munoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007; 7: 453–459. https://doi.org/10.1016/S1473-3099(07)70158-5 PMID: 17597569

43. Mbulawa ZZ, Coetzee D, Marais DJ, Kamupira M, Zwane E, Allan B, et al. Genital human papillomavirus prevalence and human papillomavirus concordance in heterosexual couples are positively associated with human immunodeficiency virus coinfection. J Infect Dis. 2009; 199: 1514–1524. https://doi.org/10.1086/598220 PMID: 19392625

44. Marais DJ, Passmore JA, Denny L, Sampson C, Allan BR, Williamson AL. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. J Med Virol. 2008; 80: 953e9.

Hindawi
Advances in Virology
Volume 2020, Article ID 2395219, 10 pages
https://doi.org/10.1155/2020/2395219

Research Article

Human Papillomavirus Prevalence in Oral and Oropharyngeal Rinse and Gargle Specimens of Dental Patients and of an HIV-Positive Cohort from Pretoria, South Africa

Neil H. Wood,[1] **Koketso S. Makua**,[2] **Ramokone L. Lebelo**,[3] **Nina Redzic**,[4] **Ina Benoy**,[5] **Olivier M. Vanderveken**,[6] **and Johannes Bogers**[4]

[1]*Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa*
[2]*Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa*
[3]*HIV and Hepatitis Research Unit, National Health Laboratory Service, Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa*
[4]*Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium*
[5]*Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium*
[6]*Department of ENT, Head and Neck Surgery, Antwerp University Hospital, Edegem, Belgium*

Correspondence should be addressed to Neil H. Wood; neil.wood@smu.ac.za

Received 20 February 2020; Revised 27 May 2020; Accepted 24 June 2020; Published 26 August 2020

Academic Editor: Subhash C. Verma

Introduction. Studies on HPV prevalence in the head and neck region of South Africans are sparse. Of the available reports in the literature, there were no studies on the association between HPV-DNA presence in the mouth and oropharynx in relation to high-risk behaviours such as oral sex practice or tobacco and alcohol use. *Materials and Methods.* Following ethical clearance and informed consent, patients attending a regional HIV-management clinic and patients attending a dental hospital were recruited to this study. The participants completed an interview-based questionnaire obtaining demographic information, data on HIV serostatus, and behavioural data including sexual practices and tobacco and alcohol use, and a rinse-and-gargle specimen was taken. Specimens were analysed for HPV DNA on 3 separate PCR/qPCR platforms. Statistical analyses were performed for associations between the study group and categorical variables, HPV status, and data from the questionnaires. *Results.* Of 221 participants, 149 were from a general population and 72 from the HIV-management clinic. Smokers comprised 29.4% of the sample, and 45.2% of participants reported to have ever used alcohol. Open mouth kissing during teenage years was confirmed by 64.7% of participants, 40.3% have given oral sex with their mouth, and 44.8% confirmed to have received oral sex from their partner's mouth. Seven participants (3.2%) had detectable α-HPV DNA, and 1 (0.4%) had detectable β-HPV DNA in their rinse-and-gargle specimens. Two participants were from the HIV-management clinic and 6 from the general dental population (overall 3.6%). *Conclusion.* Five high-risk HPV, 2 low-risk HPV, and one β-HPV types were detected. The low prevalence of 3.6% compares well to similar studies in different cohorts studied in South Africa and falls within the global oral/oropharyngeal prevalence spectrum. Only 4 participants, all from the HIV-management clinic, had palatine tonsils. No significant relationships were found between HPV presence and demographic data or sexual, oral sexual, tobacco use, or alcohol use, and no associations were seen with numbers of sexual and oral-sex partners.

1. Introduction

The human papillomavirus (HPV) is epitheliotropic and requires access to the basal cells of epithelium to initiate a complex sequence of events that additionally relies on specific host reactions and interactions to successfully infect the basal keratinocyte [1]. Mucosal infection by high-risk (HR) HPV subtypes has an established association with an

increased risk to develop cervical, anal, penile, and oropharyngeal carcinoma [2, 3].

Taking into consideration the high HIV prevalence in South Africa and the bidirectional risks in acquisition of either infection, HIV or HPV, in cases where the one precedes the other, the importance of providing prevalence data on oral and oropharyngeal HPV infection becomes urgent [2, 4]. The "Human papillomavirus and related diseases" report [5] shows both a deficiency and a comparative reporting lag, in data from Africa when compared to other geographic regions. This is reflected in various systematic reviews conducted on this topic [5–7].

The practice of oral sex (OS), open mouth kissing, having multiple sexual partners, and a compromised immune system increases the risk of acquiring oral/oropharyngeal HPV infection in general population groups [8–12]. The virus spreads through direct contact with infected genital mucous membranes or with bodily fluids. In the South African context, the practice of oral sex, open mouth kissing, smoking, and alcohol consumption has not been adequately studied in relation to HPV infection.

Smoking has been shown to influence HPV clearance from the mouth, and in cases of HPV-positive SCC, prognosis is worsened with concomitant smoking [13, 14].

This study describes the prevalence of HPV-DNA detected in the mouths and oropharynges of a dental clinic population and of an HIV-seropositive clinic population. High-risk sexual behaviours and habits that also include tobacco and alcohol use are reported.

2. Materials and Methods

2.1. Inclusion of Patients. Patients who attended the University oral health centre and the HIV management clinic were recruited as a convenience sample. The dental patients attended the institution for treatment of oral health-related issues that were unrelated to HPV infection, and the patients attending the HIV clinic came for follow-up and treatment management visits, which were also unrelated to the study.

2.2. Data Collection. An interview-based questionnaire was completed prior to oral/oropharyngeal specimen collection. The questionnaire was developed from a previous project [15] and existing literature. Data collected included age, gender, smoking habits, alcohol use, oral sex (OS) contact/practice, and HIV serostatus among others. In the case of the HIV-seropositive cohort, the most recent CD4+ T cell count and HIV viral load were recorded from the patient file. The questionnaires were sequentially numbered to ensure anonymity and delinked from patient names, hospital file-numbers, or other possible identifiers.

2.3. Specimen Collection and Transportation. Participants rinsed for 15 seconds and then gargled for 15 seconds with 10 ml of saline and then spit the contents into a Thinprep® vial containing Preservcyt® solution. The specimens were stored at 4°C until they were transported to the laboratory. The material in the vial is fixed by Preservcyt® and can be preserved for 6 months at 15–30°C. For the purposes of this report, oral wash implies sampling of the oral and oropharyngeal mucosae. The vials were numbered with the corresponding questionnaire.

2.4. HPV Testing. All oral washes were tested for the presence of HPV with 3 different assays: Abbott HR-HPV assay and the RIATOL qPCR HPV genotyping assay, both focussing on the presence of α-papilloma viruses, and the AML β-human papillomavirus typing assay.

The Abbott *m*2000™ RealTime system was used for the qualitative HPV detection with the HR-HPV assay. This real-time multiplex PCR detection kit reports only limited HPV DNA typing results: presence of HPV 16, HPV 18, and other HR-HPV (pooled signal derived from the following HR-HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The assay is optimized using probe specificity design and an internal human beta-globin control, which is a sample validity control for cell adequacy, sample extraction, and amplification efficiency. The assay is clinically optimized for cervical cancer screening. This clinically based assay cut-off is also used in this study for the detection of HR-HPV in oral washes.

The Riatol qPCR HPV genotyping assay is an ISO certified, fully automated, clinically validated laboratory-developed test [16]. This qPCR HPV DNA test not only detects 14 HR-HPV types (HPV16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 56 E7, 58 E6, 59 E7, and 68 E7) but also reports selected potential high-risk HPV types (HPV 53 E6, 66 E6, and 67 L1) and two low-risk (LR) HPV types (HPV6 E6 and 11 E6). Cellularity control was performed on every sample, by amplification of the beta-globin gene. Based on the beta-globin standard curve, DNA concentration (ng/μl) was determined in every sample. Samples with a DNA concentration below 0.12 ng/μl are considered as invalid and reported as inconclusive. Type-specific HPV positivity is defined as having a positive amplification signal for that specific HPV type, independently of the beta-globin signal.

Specimens were also tested for a spectrum of β-papillomaviruses with the AML lab-developed assay. The assay involves a TaqMan real-time PCR containing type-specific primers and consensus probes capable of detecting multiple β-HPV types. In total, 5 multiplex consensus reactions are performed: three with double targets detecting HPV types 1/63, 3/10, and 7/41 and two with triple targets detecting HPV types 2/27/57 and 4/60/65.

2.5. Statistics. Sample size estimation was based on the estimation of the proportion of HPV-positive patients in each study group, taking the general global and locally reported prevalence rates into consideration. Based on an overestimated HPV prevalence of 20%, 5% precision, and the 95% confidence level, a sample size of 246 would be required for each group [17].

The X^2 test was used to assess the relationship between the study group and categorical variables. Fisher's exact test was used for 2×2 tables or where the requirements for the

X^2 test could not be met. The strength of the associations was measured by Cramer's V and by the phi-coefficient, respectively.

The relationship between study groups as well as between age and lifetime sex partners was assessed by the t-test (or ANOVA for more than two categories). Data analysis was carried out using SAS (version 9.4 for Windows). The 5% significance level was used.

2.6. Ethical Approval. This study received ethical clearance from the Sefako Makgatho Health Sciences University Research and Ethics Committee (SMUREC/P/86/2016). Individuals received an information sheet and provided informed consent prior to participation. Participants had the opportunity to ask questions and not one person was disadvantaged or prejudiced in any way in the event of opting not to participate in the study.

3. Results

A total of 221 participants were enrolled, 149 from the general population attending the dental clinic, and 72 attending the regional HIV-management clinic. The actual sample sizes of 72 (HIV clinic) and 149 (dental clinic) used in this study correspond to a precision of 9.2% and 6.5%, respectively (rather than 5.0%), which could be a limitation of the study. For a sample size of 221, this study has the resolution to determine differences between these two datasets. The power value informed the degree of confidence to which our sample size is sufficient in order to see differences between these datasets. A power value of 90% is deemed to be an acceptable level of confidence. Based on these analyses, for a sample size of 221 with a comparison difference of 0.018, the power value is 97.8%. The sample size is, therefore, sufficient to determine any differences between this dataset and global data.

Participants' ages ranged from 20 to 74 years with a mean age of 43.8 years. Majority of the participants were black and more than half were female (Table 1).

16.3% ($n = 36$) of all participants (29.4%) were current smokers with no significant difference between the clinic groups in the number of cigarettes smoked per day. 54.8% of participants confirmed to have consumed alcohol. Of those who confirmed to have consumed alcohol ($n = 121$), 36.4% consumed alcohol in the week prior to the study and 33.1% reported consuming alcohol during the four months before the study (Table 2). There was no significant difference between the HIV-management clinic and dental clinic participants on the amount of alcohol consumed. Thirteen participants used snuff tobacco (5.9%) (data not shown).

Majority (64.7%) of the participants had open mouth kissed during their teenage years (15–19 years) with a significant difference between the two groups where more dental clinic participants practiced open mouth kissing at a very early age (10–14 years) (Table 3). There was also a significant difference in the number of people who practiced open mouth kissing in the past 2 years between the two groups, with data showing that some HIV-positive people did not perform kissing in the past 2 years. Of all the participants, 65.2% ($n = 144$) confirmed they knew what OS was, and 40.3% ($n = 89$) reported to have given OS with their mouths and 44.8% ($n = 99$) reporting to have received OS. Only one of these men tested positive for oral/oropharyngeal HPV and was from the dental clinic group who indicated he did not give nor receive OS from his single OS partner during the past year. There was a significant association between age and number of lifetime OS partners within the dental clinic group ($n = 145$; $p = 0.006$) (Table 3). Interestingly, the mean age of those with no OS partners was older than that for those with OS partners.

The HIV-management clinic represents a population of exclusively HIV-positive patients, whereas 4.0% of the dental clinic attendees self-reported to be HIV positive. The HPV (α-papillomavirus) prevalence for the dental clinic population ($n = 149$) analysed by the Riatol assay only was 1.4% (95% CI: 0.2–4.9%) ($n = 2$) compared to the 2.8% (95% CI: 0.3–9.8%) ($n = 2$) for the HIV clinic ($n = 72$) with no significant difference between these groups. All specimens except 2 had a positive beta-globin control. Only 3 participants from the dental clinic and none from the HIV clinic had HPV-DNA detectable by the Abbott platform. The combined proportion of oral/oropharyngeal α-HPV-positivity for this study ($n = 221$) was 3.2% ($n = 7$) (Table 4). This compares favourably to international reports.

Palatine tonsils were absent in 100% of the dental clinic participants group and in 94.4% of the HIV-clinic participants group. None of the α-HPV-positive participants ($n = 7$) had tonsils. Not a single participant presented with HPV-associated lesions during clinical examination.

Table 5 shows the 8 participants that were positive for HPV, 2 from the HIV clinic, and 5 from the dental clinic had detectable α-HPV DNA (Table 5). One participant, from the dental clinic, had detectable β-HPV DNA. The discrepancy between these two HPV tests is generally seen in HPV assays where the overall concordance is rather low, especially in low-viral load samples (as here). No significant associations were found between HPV presence and any demographic factor or high-risk behaviour or with number of sex or of OS partners (supplementary file).

4. Discussion

There are limited data describing the spectrum of HPV genotypes infecting the oral and oropharyngeal mucosae of the general South African population, and there are similarly no reliable data describing the types of HPV present in oral/oropharyngeal HPV-associated benign and malignant lesions of South African populations. Previous studies from South Africa on oral/oropharyngeal HPV infection were further limited by smaller specific cohorts [18–22]. The authenticity of the lower overall prevalence rate (3.2%) found in this study is further supported by other independent studies from South Africa [18, 22, 23].

In general, HPV prevalence studies are centred around the α-papillomaviruses because of the strong oncogenic association and transduction potential of E6/E7

Table 1: Demographic data for the study population.

Variable	Category	Overall ($n = 221$)		Group: Dental clinic ($n = 149$)		Group: HIV clinic ($n = 72$)		p value for the between-group test
		n	%	n	%	n	%	
Gender	Male	101	46.5	67	45.0	34	47.2	>0.99
	Female	116	53.5	78	52.3	38	52.8	
	Unknown	4						
Ethnicity	Black	210	96.8	138	95.2	72	100.0	0.41
	Caucasian	4	1.8	4	2.8	0	0.0	
	Indian	2	0.9	2	1.4	0	0.0	
	Mixed heritage	1	0.5	1	0.7	0	0.0	
	Unknown	4						
Age	The age demographic represented by means, standard deviation, and range							
	n	$n = 219$		$n = 147$		$n = 72$		0.10
	Mean	43.8		42.7		46.0		
	Std deviation	14.0		15.0		11.5		
	Range	20–74		20–74		23–72		

oncoproteins. However, it is not strange to find HPV from other genera in the mouth and oropharynx and some even associated with benign lesions and more recently, potentially malignant lesions [24–26]. However, Agalliu and coworkers [27] suggested that HPV other than α-papillomaviruses may have a part to play in the aetiology of head and neck SCC, being the first to report a positive association between infection with β- and/or γ-HPV subtypes. Only one participant from this study had demonstrable β-HPV DNA, and we can only speculate that this was a passenger-infection (background infection) as described in the literature [19, 21].

Although this study did not interrogate multiple HPV-types in the individual specimens, it did look at HPV-16, HPV-18, and other HR-HPV types. The finding of multiple types infecting a single individual is not infrequently reported. It is entirely possible for one patient to have two HPV types infecting the same mucosal field and also possible for one patient to have one type affecting more than one mucosal field, e.g., orogenital as evident by numerous concordance studies for different anatomical locations lined by mucosa. No incidental finding of multiple HPV-type infections were made, and the 8 HPV-positive specimens in this study all had single HPV subtypes detected as determined by the three independent assays.

Oral and oropharyngeal HPV prevalence studies report prevalence rates of up to 10.0% for patients considered otherwise healthy. Many of these do not present data on HPV genotypes. In the USA, prevalence of oral HPV infection in a group of 14–69 years old participants was 6.9% and higher among men. The study showed a HR-HPV prevalence of 3.7% and a low-risk HPV prevalence of 3.1% [28]. Another US study demonstrated oral HPV infection prevalence of 2.4% in 18–30 year olds and confirmed the oral-to-oral and oral-to-genital transmission routes of HPV [29]. These fall within the range of a 2010 meta-analysis of oral HPV prevalence globally, which showed a prevalence of under 5.0% across different demographic strata [30]. The combined prevalence of 3.2% found in this study is within this spectrum.

A Swedish study of oral HPV infection in youths between the ages of 15 and 23 years demonstrated higher HPV prevalence rates for males and females at 9.3% and 9.5%, respectively. However, females with concomitant cervical HPV infection had significantly higher oral HPV prevalence rates (17.1%) when compared to those without cervical HPV infection [31]. South African studies on HPV prevalence in the mouth and oropharynx are limited to 14. Of these, only 2 were conducted from oral rinse and gargle samples [22, 23], 6 used oral brushes to collect samples [18, 20, 32–35], and 6 were performed on formalin-fixed paraffin-embedded SCC samples [19, 21, 36–39]. This highlights the importance for data on HPV infection in the head and neck region of South Africans.

An investigation of oral/oropharyngeal HPV presence in 128 South African male factory workers revealed an oral/oropharyngeal HPV prevalence of 5.6% [23]. Chikandiwa and colleagues [22] recently demonstrated an oral/oropharyngeal HPV prevalence of 1.8% in a male population from Cape Town in South Africa. These compare favourably with the prevalence of 3.2% found in this study. Evidence for oral-genital HPV transmission from a South African study reveals oral/oropharyngeal HPV prevalence of 15.0% for the 34 adults examined but unfortunately did not analyse HPV subtypes detected [33]. A novel South African pilot study was in agreement with the Swedish data: when the oral and cervical mucosae were examined for HPV presence in 30 HIV-seropositive women, and Richter and coworkers found that oral mucosal HPV presence was higher (20.0%) in those HIV-positive females with concomitant cervical HPV infection [20]. The authors used a brush-biopsy technique to collect the samples. In contrast, Meyer et al. [40] found no significant difference in the prevalence rates of oral HPV infection when comparing women with cervical HPV infection (5.7%) to those without (5.1%).

The group led by Mbulawa [34] proposed that in the African setting, oral/oropharyngeal HPV is acquired from sexual partners, but in woman, it may also be due to autoinoculation. The authors reported oral HPV infection in

Table 2: Tobacco and alcohol use in both participant groups.

Variable	Category	Overall		Group				p value for the between-group test
				Dental clinic		HIV clinic		
		$n = 221$ (%)		$n = 149$ (%)		$n = 72$ (%)		
Ever used tobacco	No	156	70.6	104	69.8	52	72.2	0.75
	Yes	65	29.4	45	30.2	20	27.8	
Current smoker	No	185	83.7	121	81.2	64	88.9	0.18
	Yes	36	16.3	28	18.8	8	11.1	
Number of cigarettes per day-grouped (if current smoker; $n = 36$)	1–3	11	31	8	29.6	3	37.5	0.60
	4–8	14	40	10	37.0	4	50.0	
	9 or more	10	29	9	33.3	1	15.5	
	Unknown	1						
Ever used alcohol	No	100	45.2	69	46.3	31	43.1	0.67
	Yes	121	54.8	80	53.7	41	56.9	
Last alcoholic drink-grouped (if use alcohol; $n = 121$)	1–7 days	44	36.4	28	35.0	16	39.0	0.049 ($V = 0.22$)
	1 week–3 months	37	30.6	30	37.5	7	17.1	
	4 months or more	40	33.1	22	27.5	18	43.9	
Number of alcoholic drinks per week-grouped (if use alcohol; $n = 121$)	1	33	29.7	18	25.7	15	36.6	0.46
	2–3	38	34.2	26	37.1	12	29.3	
	4 or more	40	36.0	26	37.1	14	34.1	
	Unknown	10						

Table 3: Distribution of kissing and sexual behaviour in both participant groups.

Variable	Category	Overall		Group				p value for the between-group test
				Dental clinic		HIV clinic		
		$n = 221$ (%)		$n = 149$ (%)		$n = 72$ (%)		
Age at first open mouth kiss	10–14 years	20	9.7	17	12.6	3	4,2	0.039 (phi = 0.22)
	15–19 years	134	64.7	81	60.0	53	73.6	
	20–24 years	39	18.8	24	17.8	15	20.8	
	25–30 years	6	2.9	6	4.4	0	0.0	
	30 years or older	8	3.9	7	5.2	1	1.4	
	Unknown	14						
Number of people kissed with open mouth in last 2 years	0	21	10.4	0	0.0	21	29.2	<0.0001 ($V = 0.46$)
	1	85	42.3	61	47.3	24	33.3	
	2–3	52	25.9	36	27.9	16	22.2	
	4 or more	43	21.4	32	24.8	14	15.3	
	Unknown	20						
Age of first sexual encounter	10–14 years	19	9.1	12	8.8	7	9.7	0.81
	15–19 years	126	60.3	81	59.1	45	62.5	
	20 years or older	16	30.6	44	32.1	20	27.8	
	Unknown	12						
Number of OS partners (lifetime)	0	112	51.6	76	52.4	36	50.0	0.64
	1–2	61	28.1	38	25.5	23	31.9	
	3 or more	44	20.3	31	20.8	13	18.1	
	Unknown	12						
Number of partners given OS to (lifetime)-grouped (if lifetime OS partners ≥ 1; $n = 105$)	1	41	43.2	31	46.3	10	35.7	0.59
	2–3	34	35.8	22	32.8	12	42.9	
	4 or more	20	21.1	14	20.9	6	21.4	
	Unknown	10						

Table 4: HPV results of oral wash specimens for dental clinic and HIV clinic.

Overall for dental clinic and HIV clinic populations			
Test platform*	Category	$n = 221$	%
Abbott HR HPV result	Negative	202	91.4
	Positive (HPV 16 and 2x HPV other)	3	1.4
	Unknown (not run)	16	7.2
Riatol qPCR HPV genotyping result	Negative	213	96,3
	Low-risk HPV positive (HPV 53 and 67)	2	0.9
	High-risk HPV positive (HPV 16 and 35)	2	0.9
	DNA insufficient run	2	0.9
	Nonevaluable (technical error)	2	0.9
AML β-HPV genotyping result	Negative	216	97.7
	Positive (HPV group 4/40/65)	1	0.5
	DNA insufficient	2	0.9
	Nonevaluable (technical error)	2	0.9

*All specimens were tested on the three different platforms.

Table 5: Summary of the HPV-positive participants from both populations ($n = 221$).

Age	Gender	Type identified	Test platform	Population	Smoking current	OS given**	Alcohol use*
31	F	HPV-16	Abbott	Dental clinic		Yes	
66	F	HR-HPV	Abbott	Dental clinic		Yes	
35	M	HR-HPV	Abbott	Dental clinic			
28	M	HR-HPV 35	Riatol	Dental clinic	Yes	Yes	Yes
49	M	HR-HPV 16	Riatol	HIV clinic		Yes	
36	M	LR-HPV 53	Riatol	Dental clinic	Yes		Yes
46	M	LR-HPV 67	Riatol	HIV clinic			
66†	M	β-HPV 4/60/65	Riatol	Dental clinic			Yes

*During the 30 days preceding specimen collection. **OS given with participant's mouth, and †only participant where β-papillomavirus was detected as either HPV-4, HPV-60, or HPV-65.

8.4% of study participants. Furthermore to this, it is conceivable that when using saliva as a lubricant, autoinoculation and cross infection with HPV may occur [41].

High-risk sexual practices such as unprotected sexual intercourse, having multiple sexual partners, and the practice of oral sex/anal sex need to be investigated concomitantly with oral/oropharyngeal HPV prevalence because these are classic risk factors for HPV transmission. However, data on oral-sexual practice from South Africa are very limited [17], and no information relating this behaviour to different types of HPV infecting the oral and oropharyngeal mucosae in South Africans exists [23]. The proportion of participants who reported to have ever practiced oral sex were 46.3% for the dental clinic attendees and 50.0% for the patients attending the HIV management clinic (47.5% combined). This is much higher than a previous report published from the same area, which revealed that 22.4% of participants practiced oral sex [15]. In this current study, no link between the practice of oral sex and HPV presence could be demonstrated following analysis of the data in part because of the extraordinary low prevalence of HPV detected.

Global data suggests a much higher oral/oropharyngeal HPV prevalence for HIV-seropositive patients; and HPV infection rates of these mucosal sites are proportional to the level of immune suppression [33]. This study does not align with these global data in that only 2/72 (2.7%) HIV-positive participants had detectable HPV, and none had any clinically evident HPV lesions. This was no different when compared to the general dental participants, in which 6/149 (4%) had detectable HPV (Table 5). Oropharyngeal HPV infection rates are up to 3 times higher in HIV-seropositive patients than in patients with oral SCC [42]. Conversely, it is also reported that the risk for HIV infection is increased in instances of a preceding HPV infection [4]. The natural course and progression of HPV infection also appears to be different to that in HIV-negative individuals due to the HIV-induced immune alteration [43].

It is still unknown whether early or later HPV infection plays any significant role in the mouth/oropharynx nor has latency of HPV in humans been established conclusively [44]. However, several reports of immune reconstitution inflammatory syndrome following highly active antiretroviral treatment have been described with clinical manifestations of HPV infection that include rampant proliferation of oral and perioral papules and wart-like projections [43, 45, 46]. In the HIV-seropositive cohort of this study, no HPV-associated oral lesions were identified, and an oral/oropharyngeal HPV prevalence of 2.8% (2/72) in this particular cohort was found.

Tobacco and alcohol use are considered the traditional risk factors in the development of oral/oropharyngeal squamous cell carcinoma. However, a slow decline in tobacco use globally has also been accompanied by a rise in oropharyngeal cancer incidence [47]. A distinct subset of oropharyngeal HPV-induced carcinomas is already recognized and is increasing in prevalence with HPV-16 seemingly responsible for 40–60% of these SCC's [48–50]. The odds of developing oropharyngeal SCC rises more than 13 times when HPV 16 is found in keratinocytes shed from the oral/oropharyngeal mucosa [49]. Oral SCC incidence in South Africa is reported as 2.7/100000 and SCC of the pharynx as 2.4/100000 [33].

In a report on trends regarding tobacco use in South Africa, the prevalence of smoking among black South Africans was 22.7% and 36.6% among Caucasians; and the trend reflected a decrease in smoking [21, 51]. Another South African study from a different geographic area reported 19.7% of participants to be smokers, and 6.4% used snuff tobacco [15]. This study reports 29.4% of participants who indicated to have ever used tobacco and 16.3% to be current smokers. Thirteen participants (5.9%) used snuff. Aside from delaying HPV clearance, it is also known that smokers with concomitant HR-HPV infection in oropharyngeal SCC have a significantly higher risk of disease recurrence than nonsmoking counterparts [13].

HPV infection has a stronger association with tonsillar SCC than SCC of the mouth [20]. The dispersed reticular network of epithelial cells found in the tonsillar crypts expresses a primitive differentiation. These CD44-positive and nerve growth factor receptor- (NGFR-) positive cells have been shown to have a higher proliferative potential than regular mucosal counterparts. These proliferating cells also express CK5, which is a basal cell marker. The microstructural arrangement of these cryptal epithelial reticulations leads to the cells being exposed to the external environment, making it easier for HPV-access. No microlaceration or wounding is required. The proof of principle for this was published by Kang and colleagues [52]. However, the influence of cryptal surface progenitor cells has not been well-studied with regards to maintenance of cryptal epithelial cells. Similarly, very little information exists on the influence of HPV on the different epithelial cell population found in the tonsil [52].

An interesting incidental finding from this study's population groups showed that 98.2% of participants had their palatine tonsils removed. None of the oral/oropharyngeal HPV prevalence studies we interrogated included data on the presence or absence of palatine tonsils. It is, therefore, conceivable that the absence of tonsils could play a preventive role in HPV-associated SCC of the head and neck. This needs further investigation.

It could be that the oral cavity serves the passenger infection model as a reservoir to inoculate the tonsils and other more distant mucosal sites [19, 21]. The "oral HPV reservoir theory" was also postulated in the context of HIV-seropositive adults by Fatahzadeh and colleagues [53]. In order to further advance the knowledge frontier on the natural course and impact of oral/oropharyngeal HPV infection, the importance of early detection for prevention, persistence studies cannot be overlooked and must be standardized methodologically. Females receiving the HPV vaccine are believed to receive protection for HR-HPV types currently associated with head and neck cancer. This must be performed in parallel to cost-vs-benefit analyses and determination of optimal vaccine administration time in these cohorts [30]. It remains to be seen if this herd immunity will result in coverage of other high-risk populations such as men who have sex with men and HIV-positive cohorts practicing high-risk behaviours.

5. Conclusion

The overall prevalence of oral/oropharyngeal HPV DNA in dental clinic attendees and in attendees of an HIV-management clinic is low in this report. This data compares favourably to other South African papers in different cohort studies and also falls within the expected global prevalence range. One participant presented with β-papillomavirus DNA present in the rinse and gargle specimen.

Oral/oropharyngeal mucosal infection by HPV is not implied by the detection of any HPV-DNA in these participants. The possibility that these represent passenger infections that serve to inoculate other anatomical sites lined by mucosa must be considered. Single-site coinfection with multiple HPV types may be detected only as a singular HPV positivity using conventional PCR platforms and should be individually identified through sequencing [21]. A significant finding was that a large portion of the population did not have palatine tonsils, which raises the question whether tonsillectomy is prophylactic in the South African population; however, with such limited data on HPV-positive oral and oropharyngeal SCC, this is a question that remains to be answered. Similarly, the value of vaccination in order to prevent a very distinct subset of head and neck cancer must be studied.

Data Availability

The dataset used to support this study are included in the supplementary file, without restrictions, and named "dataset.xlsx."

Disclosure

The funding agency had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was funded by the Sefako Makgatho Health Sciences University Research Development Grant. This work

received additional funding from the WAKA-HPV Network and from the Flemish Government (IUC-VLIR-OUS).

Supplementary Materials

(1) Raw dataset for the population groups included in the study. (2) Supplementary statistical analyses of data regarding high-risk behaviors in the study population. (*Supplementary Materials*)

References

[1] T. Letian and Z. Tianyu, "Cellular receptor binding and entry of human papillomavirus," *Virology Journal*, vol. 7, p. 2, 2010.

[2] S. Syrjänen, "Oral manifestations of human papillomavirus infections," *European Journal of Oral Sciences*, vol. 126, no. S1, pp. 49–66, 2018.

[3] S. Yete, W. D'Souza, and D. Saranath, "High-risk Human papillomavirus in oral cancer: clinical implications," *Oncology*, vol. 94, no. 3, pp. 133–141, 2018.

[4] C. F. Houlihan, N. L. Larke, D. Watson-Jones et al., "Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis," *AIDS*, vol. 26, pp. 2211–2222, 2010.

[5] L. Bruni, G. Albero, B. Serrano et al., *Human Papillomavirus and Related Diseases in the World. Summary Report*, https://www.hpvcentre.net/statistics/reports/XWX.pdf, ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre), Lyon, France, 2019, https://www.hpvcentre.net/statistics/reports/XWX.pdf.

[6] A. R. Kreimer, G. M. Clifford, P. Boyle et al., "Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review," *Cancer Epidemiology Biomarkers & Prevention*, vol. 14, no. 2, pp. 467–475, 2005.

[7] C. De Martel, J. Ferlay, S. Franceschi et al., "Global burden of cancers attributable to infections in 2008: a review and synthetic analysis," *The Lancet Oncology*, vol. 13, no. 6, pp. 607–615, 2012.

[8] G. D'Souza, Y. Agrawal, J. Halpern, S. Bodison, and M. L. Gillison, "Oral sexual behaviors associated with prevalent oral human papillomavirus infection," *Journal of Infectious Diseases*, vol. 199, pp. 1263–1269, 2009.

[9] L. Feller, R. A. Khammissa, N. H. Wood, and J. Lemmer, "Epithelial maturation and molecular biology of oral HPV," *Infectious Agents and Cancer*, vol. 4, no. 1, p. 16, 2009.

[10] M. Adamopoulou, E. Vairaktaris, E. Nkenke et al., "Prevalence of human papillomavirus in saliva and cervix of sexually active women," *Gynecologic Oncology*, vol. 129, no. 2, pp. 395–400, 2013.

[11] J. Betiol, L. L. Villa, and L. Sichero, "Impact of HPV infection on the development of head and neck cancer," *Brazilian Journal of Medical and Biological Research*, vol. 46, no. 3, pp. 217–226, 2013.

[12] H. A. Cubie, "Diseases associated with human papillomavirus infection," *Virology*, vol. 445, no. 1-2, pp. 21–34, 2013.

[13] J. H. Maxwell, B. Kumar, F. Y. Feng et al., "Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence," *Clinical Cancer Research*, vol. 16, no. 4, pp. 1226–1235, 2010.

[14] K. Gaester, L. A. M. Fonseca, O. Luiz et al., "Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors," *Scientific Reports*, vol. 4, no. 1, p. 6592, 2014.

[15] N. H. Wood, O. A. Ayo-Yusuf, T. S. Gugushe, and J. Bogers, "Tobacco use and oral sex practice among dental clinic attendees," *PLoS One*, vol. 14, no. 3, Article ID e0213729, 2019.

[16] I. Benoy, L. Xu, D. Vanden Broeck et al., "Using the VALGENT-3 framework to assess the clinical and analytical performance of the RIATOL qPCR HPV genotyping assay," *Journal of Clinical Virology*, vol. 120, pp. 57–62, 2019.

[17] W. W. Daniel, *Biostatistics: A Foundation for Analysis in the Health Sciences*p. p191, 10th edition, John Wiley & Sons, New York, NY, USA, 2013.

[18] D. J. Marais, C. Sampson, A. Jeftha et al., "More men than women make mucosal IgA antibodies to Human papillomavirus type 16 (HPV-16) and HPV-18: a study of oral HPV and oral HPV antibodies in a normal healthy population," *BMC Infectious Diseases*, vol. 6, p. 95, 2006.

[19] S. Boy, E. J. V. Rensburg, S. Engelbrecht, L. Dreyer, M. Heerden, and W. Heerden, "HPV detection in primary intra-oral squamous cell carcinomas - commensal, aetiological agent or contamination?" *Journal of Oral Pathology and Medicine*, vol. 35, no. 2, pp. 86–90, 2006.

[20] K. L. Richter, E. J. Van Rensburg, W. F. P. van Heerden, and S. C. Boy, "Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy," *Journal of Oral Pathology & Medicine*, vol. 37, no. 9, pp. 555–559, 2008.

[21] C. Paquette, M. F. Evans, S. S. Meer, V. Rajendran, C. S.-C. Adamson, and K. Cooper, "Evidence that alpha-9 human papillomavirus infections are a major etiologic factor for oropharyngeal carcinoma in black South Africans," *Head and Neck Pathology*, vol. 7, no. 4, pp. 361–372, 2013.

[22] A. Chikandiwa, P. T. Pisa, M. F. Chersich, E. E. Muller, P. Mayaud, and S. Delany-Moretlwe, "Oropharyngeal HPV infection: prevalence and sampling methods among HIV-infected men in South Africa," *International Journal of STD & AIDS*, vol. 29, no. 8, pp. 776–780, 2018.

[23] C. L. Davidson, K. L. Richter, M. van der Linde, J. Coetsee, and S. C. Boy, "Prevalence of oral and oropharyngeal human papillomavirus in a sample of South African men: a pilot study," *South African Medical Journal*, vol. 104, no. 5, pp. 358–361, 2014.

[24] D. Bottalico, Z. Chen, A. Dunne et al., "The oral cavity contains abundant known and novel human papillomaviruses from the betapapillomavirus and gammapapillomavirus genera," *The Journal of Infectious Diseases*, vol. 204, no. 5, pp. 787–792, 2011.

[25] F. Paolini, C. Rizzo, I. Sperduti et al., "Both mucosal and cutaneous papillomaviruses are in the oral cavity but only alpha genus seems to be associated with cancer," *Journal of Clinical Virology*, vol. 56, no. 1, pp. 72–76, 2013.

[26] E. Martin, J. Dang, D. Bzhalava et al., "Characterization of three novel human papillomavirus types isolated from oral rinse samples of healthy individuals," *Journal of Clinical Virology*, vol. 59, no. 1, pp. 30–37, 2014.

[27] I. Agalliu, S. Gapstur, Z. Chen et al., "Associations of oral α-, β-, and γ-human papillomavirus types with risk of incident head and neck cancer," *JAMA Oncology*, vol. 2, no. 5, pp. 599–606, 2016.

[28] M. L. Gillison, T. Broutian, R. K. L. Pickard et al., "Prevalence of oral HPV infection in the United States, 2009-2010," *JAMA*, vol. 307, no. 7, pp. 693–703, 2012.

[29] R. K. L. Pickard, W. Xiao, T. R. Broutian, X. He, and M. L. Gillison, "The prevalence and incidence of oral human papillomavirus infection among young men and women, aged

18-30 years," *Sexually Transmitted Diseases*, vol. 39, no. 7, pp. 559–566, 2012.

[30] A. R. Kreimer, R. K. Bhatia, A. L. Messeguer, P. González, R. Herrero, and A. R. Giuliano, "Oral Human papillomavirus in healthy individuals: a systematic review of the literature," *Sexually Transmitted Diseases*, vol. 37, no. 6, pp. 386–391, 2010.

[31] J. Du, C. Nordfors, A. Näsman et al., "Human papillomavirus (HPV) 16 E6 variants in tonsillar cancer in comparison to those in cervical cancer in stockholm, Sweden," *PLoS One*, vol. 7, Article ID e36239, 2012.

[32] D. J. Marais, J.-A. S. Passmore, L. Denny, C. Sampson, B. R. Allan, and A.-L. Williamson, "Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa," *Journal of Medical Virology*, vol. 80, no. 6, pp. 953–959, 2008.

[33] S. L. Vogt, P. E. Gravitt, N. A. Martinson, J. Hoffmann, and G. D'Souza, "Concordant oral-genital HPV infection in South Africa couples: evidence for transmission," *Frontiers in Oncology*, vol. 3, no. 303, pp. 1–7, 2013.

[34] Z. Z. A. Mbulawa, L. F. Johnson, D. J. Marais, D. Coetzee, and A.-L. Williamson, "Risk factors for oral human papillomavirus in heterosexual couples in an African setting," *Journal of Infection*, vol. 68, no. 2, pp. 185–189, 2014.

[35] E. E. Muller, K. Rebe, T. F. Chirwa, H. Struthers, J. McIntyre, and D. A. Lewis, "The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa," *BMC Infectious Diseases*, vol. 16, p. 440, 2016.

[36] E. J. Van Rensburg, W. F. van Heerden, E. H. Venter, and E. J. Raubenheimer, "Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population," *South African Medical Journal*, vol. 85, pp. 894–896, 1995.

[37] E. J. Van Rensburg, S. Engelbrecht, W. F. Van Heerden, E. J. Raubennheimer, and B. D. Schoub, "Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample," *Anticancer Research*, vol. 16, pp. 969–973, 1996.

[38] T. R. Sekee, F. J. Burt, D. Goedhals, J. Goedhals, Y. Munsamy, and R. Y. Seedat, "Human papillomavirus in head and neck squamous cell carcinomas in a South African cohort," *Papillomavirus Research*, vol. 6, pp. 58–62, 2018.

[39] A. Bulane, D. Goedhals, R. Y. Seedat, J. Goedhals, and F. Burt, "Human papillomavirus DNA in head and neck squamous cell carcinomas in the Free State, South Africa," *Journal of Medical Virology*, vol. 92, no. 2, pp. 227–233, 2020.

[40] M. F. Meyer, C. U. Huebbers, O. G. Siefer et al., "Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection," *Oral Oncology*, vol. 50, no. 1, pp. 27–31, 2014.

[41] L. M. Butler, D. H. Osmond, A. G. Jones, and J. N. Martin, "Use of saliva as a lubricant in anal sexual practices among homosexual men," *JAIDS Journal of Acquired Immune Deficiency Syndromes*, vol. 50, no. 2, pp. 162–167, 2009.

[42] M. Adamopoulou, E. Vairaktaris, V. Panis, E. Nkenke, F. W. Neukam, and C. Yapijakis, "HPV detection rate in saliva may depend on the immune system efficiency," *In Vivo*, vol. 22, pp. 599–608, 2008.

[43] J. E. Cameron and M. E. Hagensee, "Oral HPV complications in HIV-infected patients," *Current HIV/AIDS Reports*, vol. 5, no. 3, pp. 126–131, 2008.

[44] N. Egawa, K. Egawa, H. Griffin, and J. Doorbar, "Human papillomaviruses; epithelial tropisms, and the development of neoplasia," *Viruses*, vol. 7, no. 7, pp. 3863–3890, 2015.

[45] L. Feller, R. A. Khammissa, N. H. Wood, V. Malema, R. Meyerov, and J. Lemmer, "Focal epithelial hyperplasia (Heck disease) related to highly active antiretroviral therapy in an HIV-seropositive child - a report of a case, and a review of the literature: case study," *SADJ*, vol. 65, pp. 172–175, 2010.

[46] A. Moodley and N. H. Wood, "HIV-associated oral lesions as an indicator for HAART failure: a review," *SADJ: Journal of the South African Dental Association = Tydskrif van die Suid-Afrikaanse Tandheelkundige Vereniging*, vol. 67, no. 7, pp. 348–352, 2012.

[47] E. E. Vokes, N. Agrawal, and T. Y. Seiwert, "HPV-associated head and neck cancer," *Journal of the National Cancer Institute*, vol. 107, no. 12, p. djv344, 2015.

[48] K. Zumbach, M. Hoffmann, T. Kahn et al., "Antibodies against oncoproteins E6 and E7 of human papillomavirus types 16 and 18 in patients with head-and-neck squamous-cell carcinoma," *International Journal of Cancer*, vol. 85, no. 6, pp. 815–818, 2000.

[49] G. D'Souza, A. R. Kreimer, R. Viscidi et al., "Case-control study of human papillomavirus and oropharyngeal cancer," *New England Journal of Medicine*, vol. 356, no. 19, pp. 1944–1956, 2007.

[50] E. M. Sturgis and P. M. Cinciripini, "Trends in head and neck cancer incidence in relation to smoking prevalence," *Cancer*, vol. 110, no. 7, pp. 1429–1435, 2007.

[51] C. Van Walbeek, "Recent trends in smoking prevalence in South Africa: some evidence from AMPS data," *South African Medical Journal*, vol. 92, pp. 468–472, 2002.

[52] S. Y. C. Kang, N. Kannan, L. Zhang, V. Martinez, M. P. Rosin, and C. J. Eaves, "Characterization of epithelial progenitors in normal human palatine tonsils and their HPV16 E6/E7-induced perturbation," *Stem Cell Reports*, vol. 5, no. 6, pp. 1210–1225, 2015.

[53] M. Fatahzadeh, N. F. Schlecht, Z. Chen et al., "Oral human papillomavirus detection in older adults who have human immunodeficiency virus infection," *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, vol. 115, no. 4, pp. 505–514, 2013.