DETECTION OF HPV IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

FJ Burt

T: +27(0)51 401 9111 | info@ufs.ac.za | www.ufs.ac.za

UNIVERSITY OF THE
FREE STATE
UNIVERSITEIT VAN DIE
VRYSTAAT
YUNIVESITHI YA
FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

DETECTION OF HUMAN PAPILLOMA VIRUS IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

FJ Burt[1], R Seedat, D Goedhals, T Sekee, Y Moonsamy, A Bulane, J Goedhals
A Hoosen

Medical Microbiology and Virology, UFS

Presenter: FJ Burt

Department of Medical Microbiology and Virology

NHLS Universitas

University of the Free State

What has been published regarding HPV and oral squamous cell carcinomas in South African patients

1995

van Rensburg et al., Detection of human papillomavirus DNA with in situ hybridisation in oral squamous carcinoma in a rural black population. SAMJ 1995 85 (9) 894-896.

Prevalence of HPV DNA in oral squamous carcinoma (OSCC) in Northern Transvaal (paraffin blocks)

0/66 ICC
1/66 ISH HPV-18 detected in normal tissue adjacent to tumour

1996

Van Rensburg et al., Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. Anticancer Res 1996 16 (2): 969-73.

PCR targeting E6 region, type specific 6, 11, 16 and 18
Tested +/- 140 samples from different age groups
2 samples positive for HPV-11 and -16

2006

Boy et al., HPV detection in primary intra-oral squamous cell carcinomas-commensal, aetiological agent or contamination? J Oral Pathol Med 2006 35: 86-90..

HPV DNA in OSCC archival tissue 1998-2003

7/59 real time PCR HPV-18
0/59 ISH (PanPath pan-HPV probes and DAKO GenPoint)

2002

Matsha et al., Human papillomavirus associated with oesophageal cancer. J Clin Path 55: 587-590.

Prevalence and HPV types in patients with oesaphageal cancer (Transkei)
23/50 paraffin embedded sections positive for HPV DNA using PCR MY 09/MY11 and GP5+/GP6+ nested PCR
11x HPV-11
7x HPV-39
2x HPV-16
1x HPV-52

2013

Paquette 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 7:361-372

Oropharyngeal squamous cell carcinomas (OPSC) on SA using PCR, chromogenic ISH, p16 ^{INK4a} IHC

55 cases OPSC in black South Africans 2005-2010
GP5+/GP6+ (at least 37 types, target L region) 140bp
PCR targeting E6/E7 of types 16, 18, 31, 33, 52, 58, 230-270 bp
PCR targeting E5 type 31 153 bp
CISH targeting 6, 11, 16, 18, 31, 33, 45, 51, 52
p16 ^{INK4a} IHC (DAKO)

2013
Paquette et al.,

41/55 pos GP5+/GP6+ 5x HPV-16, 4x HPV-18, 32x HPV-31

37/55 pos PCR targeting E6/E7 of types 34x HPV-16, 2x HPV-31, 1xHPV-33

PCR targeting E5 type -31 total of 29/29 tested had band and 14 confirmed by seq

CISH all negative

p16 ^{INK4a} IHC (DAKO) 26/51 specimens pos,
51% HPV pos samples were pos p16 staining
33% HPV neg samples were p16 pos

2013
Paquette et al.,

SA OPSC
HPV-16 and -31 (32%)
HPV-16 (32%)
HPV-31 24%
HPV-16 and -18 (8%)
HPV-18 (4%)

SA Cervical carcinoma (WHO data)
HPV-16 (52.1%)
HPV-18 (10.7%)
HPV-33 (9.1%)
HPV-31 (4.2%)
HPV-45 (3.3%)

HPV Research Group, Department of Medical Microbiology and Virology, Department of Otorhinolarngology, UFS.

What is the occurrence of HPV associated HNSCC in the Free State province?
What molecular assay can be used to detect HPV: considering cost , availability, sensitivity and specificity?
What HPV types are circulating in our region, prevaccination?
What is the significance of HPV in biospy tissues?

Projects

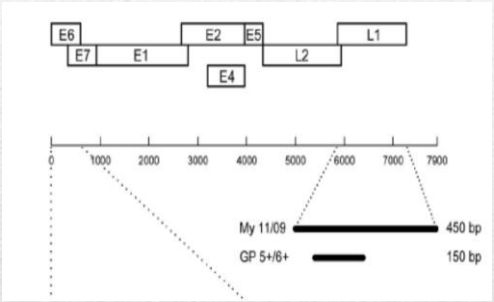
Molecular assays for detecting HPV DNA
Prevalence of HPV in head and neck squamous cell carcinomas in the Free State
Complete genome sequence analysis of HPV isolates
Molecular detection of replicating HPV
Assays for determining integration and integration sites
Biomarkers of HPV infection and indicators of enhanced prognosis

1. Molecular assays for detecting HPV DNA

Application of PCR for detection of HPV DNA in biopsy

DNA extracted using QIAGEN kit

PCR methods
1.Nested PCR: consensus primers targeting multiple types
MY09/MY11
PCR GP5+/GP6+
Targets the L gene



2.Multiplex in house PCR: two multiplex PCR reactions targeting high risk and low risk types
High risk (HR) group including types 16, 18, 31, 33, 58
Low risk (LR) group including types 6, 11,

3. Comparison of results from molecular assay with p16 detection (Ventana)

E6 Multiplex PCR

Primers were designed for the E6 region of the HPV based on the DNA sequence alignment for HPV types 6, 11, 16, 18, 31, 33 and 58 using data retrieved from GenBank. DNA sequences for each HPV type were aligned using Clustal Omega.

To confirm specificity for each primer designed BLAST analysis was performed.

Primers designed for HPV types based on the E6 region within the HPV genome.

Two multiplex PCRs developed: low risk ,LR, and high risk, HR.

Primer	Nucleotide sequence	T_m	%GC	Length	Amplicon size (bp)
HPV 6F	5'CCTCCACGTCGTCAACGACCA3'	61.9^{0}C	61.9	21bp	164 bp
HPV 6R	5'AGGCTGCATATGGATAGCCGG3'	59.8^{0}C	57.4^{0}C	21 bp	
HPV 11F	5'ATGGAAAGTAAAGATGCCTCCACGT3'	58.4^{0}C	44.0	25 bp	176 bp
HPV 11R	5'CAACAGGCACACGCTGCAAG3'	60.0^{0}C	57.1	20 bp	
HPV 16F	5'AGGACCCACAGGAGCGAC3'	59.6^{0}C	66.7	18 bp	130 bp
HPV 16R	5'TGCATAAATCCCGAAAAGCAAAGTC3'	56.4^{0}C	40.0	25 bp	
HPV 18F	5'ATGGCGCGCTTTGAGGATCC3'	60.8^{0}C	60.0	20 bp	172 bp
HPV 18R	5'GCAGCATGCGGTATACTGTCT3'	57.3^{0}C	52.4	21 bp	
HVP 31F	5'CGGCATTGGAAATACCCTACGA3'	57.0^{0}C	50.0	22 bp	122 bp
HPV 31R	5'GCACACACTCCGTGTGGTGTG3'	61.1^{0}C	61.9	21 bp	
HPV 33F	5'GAGAGGGAAATCCATTTGGAATATG3'	63^{0}C	40.0	25 bp	177 bp
HPV 33R	5'TCTTGAGGACACAAAGGTCTTTG3'	64^{0}C	43.0	23 bp	
HPV 58F	5'ATGTTCCACGCACAGAGGAGAAC3'	69^{0}C	54.0	23 bp	170 bp
HPV 58R	5'CACTTTACATACTGCAAATGGATTC3'	64^{0}C	38.0	25 bp	

UFS UV

1. Molecular assays for detecting HPV DNA (T Sekee, MMedSc)
Application of PCR for detection of HPV DNA in biopsy.
Determination of HPV type based on sequence analysis of amplicon
Results

35 samples from confirmed HNSCC (32 tested) DNA confirmed with beta globin primers

- Nested PCR MY09/11 and GP5+/6+

Larynx	1x HPV-6*
	2x HPV-11
	1x HPV- 31
Oropharynx	2x HPV-11
Tonsil	1x HPV-16*

7/32

- E6 multiplex
  HR PCR

Larynx	1x HPV-31
Tonsil	1x HPV-16

  LR (testing in progress)

- p16 (to be done)

2. Prevalence of HPV in head and neck squamous cell carcinomas (HNSCC) in the Free State (A Bulane, PhD)

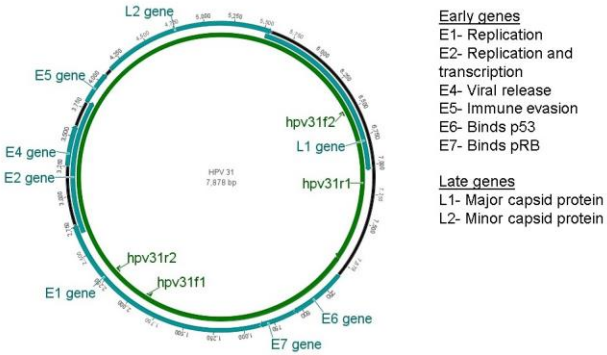
Paraffin embedded tissue from patients with confirmed OSCC submitted from 2004 to 2104 will be screened using PCR for HPV DNA.

Extraction of DNA will be confirmed using primers targeting a 260bp region of the b globin gene.
PCR method: in house E6 primers targeting regions of 122-172 bp in length.

Cohort includes to date
+/- 650 samples
Location: tongue, nasopharynx, larynx, oropharynx,

3. Complete genome sequence analysis of HPV isolates (Y Moonsamy, PhD)

On identification of positive samples from tissue biopsies, primers are identified amplify two overlapping regions encompassing complete genome. Amplicons submitted for NGS analysis.



Sequence data mapped against reference strains using GS Reference Mapper Roche Software and assembled using the GS De Novo Assembler Roche software. Gaps or incomplete regions of the genome determined using Sanger sequencing method and the BigDye.

4. Molecular detection of replicating HPV (T Sekee and Y Moonsamy)

HPV DNA positive tissue biopsies will be screened for replicating HPV, mRNA

Primers targeting E6 region will be used to detect mRNA.
All biopsy tissues are immediately stored in RNAlater until processing

- RNA extraction
- RT-PCR using E6 primers
- RT-PCR to be validated using transcribed RNA for each HPV type (LOD, specificity and sensitivity)

To summarize

Aim of our studies:
To develop in house molecular assays for diagnostic and surveillance purposes
To determine occurrence of HPV DNA in confirmed HNSCC in patients in the Free State
To determine the HPV types circulating, prevaccination data
Is there replicating HPV, mRNA?
Can we identify biomarkers that can be used for indicating patient outcome

Collaborators UFS
Prof Riaz Seedat, Head of Department of Otorhinolaryngology
Dr Nicki Goedhals, Head of Division of Virology, Department of Medical Microbiology and Virology,
Dr Jackie Goedhals, Head of Department of Anatomical Pathology

Current Postgraduate students
Tumelo Sekee
Yuri Moonsamy
Atang Bulane

Funders
NHLS Research Trust
Polio Research Foundation
NRF

Thank You
Dankie

T: +27(0)51 401 9111 | info@ufs.ac.za | www.ufs.ac.za

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE