

5-1-2024

## Screening of Orthodontic Patients and Age-Matched Non-Orthodontic Samples for High-Risk Oncogenic Human Papillomavirus (HPV) Strains

Hunter Hinton

Follow this and additional works at: <https://digitalscholarship.unlv.edu/thesesdissertations>



Part of the [Virology Commons](#)

---

### Repository Citation

Hinton, Hunter, "Screening of Orthodontic Patients and Age-Matched Non-Orthodontic Samples for High-Risk Oncogenic Human Papillomavirus (HPV) Strains" (2024). *UNLV Theses, Dissertations, Professional Papers, and Capstones*. 5005.

<http://dx.doi.org/10.34917/37650828>

This Thesis is protected by copyright and/or related rights. It has been brought to you by Digital Scholarship@UNLV with permission from the rights-holder(s). You are free to use this Thesis in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself.

This Thesis has been accepted for inclusion in UNLV Theses, Dissertations, Professional Papers, and Capstones by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact [digitalscholarship@unlv.edu](mailto:digitalscholarship@unlv.edu).

SCREENING OF ORTHODONTIC PATIENTS AND AGE-MATCHED  
NON- ORTHODONTIC SAMPLES FOR HIGH-RISK  
ONCOGENIC HUMAN PAPILLOMAVIRUS  
(HPV) STRAINS

By

Hunter Hinton

Bachelor of Science – Biological Sciences  
Arizona State University  
2017

Doctor of Medicine in Dentistry  
Midwestern University  
2021

A thesis submitted in partial fulfillment  
of the requirements for the

Master of Science – Oral Biology

School of Dental Medicine  
The Graduate College

University of Nevada, Las Vegas  
May 2024

Copyright by Hunter Hinton, 2024

All Rights Reserved



## Thesis Approval

The Graduate College  
The University of Nevada, Las Vegas

February 20, 2024

This thesis prepared by

Hunter Hinton

entitled

Screening of Orthodontic Patients and Age-Matched Non- Orthodontic Samples for High-Risk Oncogenic Human Papillomavirus (HPV) Strains

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Oral Biology  
School of Dental Medicine

Karl Kingsley, Ph.D.  
*Examination Committee Chair*

Alyssa Crittenden, Ph.D.  
*Vice Provost for Graduate Education &  
Dean of the Graduate College*

Katherine Howard, Ph.D.  
*Examination Committee Member*

Brian Chrzan, Ph.D.  
*Examination Committee Member*

Erika Marquez, Ph.D.  
*Graduate College Faculty Representative*

Abstract

**SCREENING OF ORTHODONTIC PATIENTS AND AGE-MATCHED  
NON-ORTHODONTIC SAMPLES FOR HIGH-RISK  
ONCOGENIC HUMAN PAPILLOMAVIRUS  
(HPV) STRAINS**

By

Hunter Hinton, DMD

Dr. Karl Kingsley, Examination Committee Chair  
Professor of Biomedical Sciences  
University of Nevada, Las Vegas  
School of Dental Medicine

Introduction: The human papillomavirus (HPV) is known to cause cancer in several tissues and organs, including the cervix and the oral cavity. Several oncogenic or high-risk strains have been identified, which include HPV16 and HPV18. However, other research has demonstrated that additional oncogenic, high-risk strains exist - including HPV31, HPV33, HPV35, HPV52 and HPV58, although much less is known about their prevalence. Based upon this lack of knowledge, the primary objective of this project is to address the prevalence of these strains among pediatric and adult patients through screening of saliva samples from the UNLV-SDM and orthodontic clinic populations.

Methods: The protocol for this study was reviewed and approved by the UNLV Institutional Review Board (IRB #1619329-1) titled “Retrospective analysis of Oral Health Status of Dental Population”. Clinical samples from a saliva biorepository (N=253) were screened to provide age-matched pediatric and adult samples from the orthodontic and main patient clinics. Quantitative polymerase chain reaction (qPCR) screening of samples for high-risk HPV was performed using SYBR green master mix from Applied Biosciences and validated high-risk HPV primers.

Results: A total of N = 86 samples from the saliva biorepository met the quality and concentration standards and were screened for high-risk HPV. qPCR screening of adult samples revealed n = 10/45 or 22% were HPV31- or HPV33-positive. In addition, a total of n = 9/41 or 21.9% of pediatric samples were either HPV31- or HPV33-positive (or both). No samples harbored HPV35. Most samples were derived from patients within the recommended vaccination or catch-up age range (age 9-45 years).

Discussion and Conclusions: No previous studies from this institution have explored these high-risk HPV strains among this patient population. These results clearly demonstrate that a significant percentage of patients harbor additional high-risk HPV strains within the oral cavity, including HPV31 and HPV33. Although many studies promote the quadrivalent HPV vaccine (covering HPV6, 11, 16, and 18), these results suggest that oral healthcare providers may need to discuss the newer nine-valent vaccine, which includes HPV31 and HPV33. It might have a beneficial effect on this certain population to make a larger emphasis on this newer vaccine and the prevention potential to the general public.

Key words: Human papillomavirus (HPV); High-risk HPV; saliva screening; qPCR screening

## Acknowledgments

Thank you to Dr. Karl Kingsley for guiding me through this research project. I am also very grateful to Spencer Coleman, JR Salem, Lorena Herrera and Sofia Valenzuela for their commitment and support during this process. Additionally, I would like to extend a thank you to my committee members; Dr. Katherine Howard, Dr. Brian Chrzan, and Dr. Erika Marquez for their assistance and support.



## Table of Contents

Abstract	iii
Table of Contents	vii
List of Tables	ix
List of Figures	x
Chapter 1: Introduction	1
Background and Significance	1
Research Questions	2
Approval	3
Research Design	3
Anticipated Findings and Potential Impact	6
References	7
Chapter 2	9
Abstract	9
Introduction	10
Materials and Methods	12
Results	15
Discussion	25

Conclusions	26
References	29
Chapter 3	35
Summary and Conclusions	35
Limitations and Recommendations	37
Appendix A	38
Curriculum Vitae	39

## List of Tables

### Chapter 2

Table 1. Demographic analysis of adult study samples	16
Table 2. Demographic analysis of pediatric study samples	18
Table 3. Demographic analysis of adult HPV-positive and HPV-negative samples	21
Table 4. Demographic analysis of pediatric HPV-positive and HPV-negative samples	24

## List of Figures

### Chapter 2

- Figure 1. Heatmap analysis for qPCR screening of adult samples for high-risk HPV 19
- Figure 2. Analysis of heatmap for qPCR screening of pediatric samples for high-risk HPV 22

## Chapter 1: Introduction

### **Background and Significance**

Human Papillomavirus (HPV) is a non-enveloped, double stranded DNA virus that is epitheliotropic in nature [1]. There are between 150-200 different strains of HPV. Roughly 40 types cause cutaneous or mucosal infections. This includes infections of the skin, anogenital tract, and oral cavity - as HPV infects squamous cells in various cells and tissues. [1,2].

HPV can spread via skin-to-skin contact or through sexual intercourse [1-3]. It is estimated that there are over 42 million Americans who are currently infected with HPV [2].

With over 12% of the United States population currently infected, there are an estimated 13 million new HPV infections each year, just in the United States [2]. Most HPV infections (approximately 90%- 95%) are cleared by the immune system within 2 years [3]. However, some strains may cause infections that do not clear and can persist to cause precancerous abnormalities and eventual malignancy [1-4].

There are High Risk and Low Risk Strains of HPV based on their likelihood of causing cancer. The Low Risk HPV strains mostly do not cause disease and are often associated with warts, both anogenital and oral, including HPV6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73, and 81 [5]. The High Risk strains of HPV are associated with a variety of cancer HPV-related cancers including Cervical cancer, Oropharyngeal Cancer, Anal Cancer, Penile Cancer, and Vaginal Cancers. It is

well documented that HPV 16 and 18 are the main two strains relating to causing most of these cancers [6].

However, in addition to types 16 and 18, the International Agency for Research on Cancer (IARC) has identified 10 other High Risk strains: HPV31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. [7]. Due to the limited focus on two specific strains of HPV (found in the majority of both cervical and oral cancer cases), the true extent of oral high-risk HPV infection and exposure may be unknown. Although many additional HPV strains (listed above) are also known to infect the oral cavity, studies in this population are extremely limited [8]. In addition, the ability for cross protective antibodies against these strains cannot be determined until the incidence, prevalence and range of HPV strains is more clearly understood.

## **Research Questions**

Research question: What is the incidence and prevalence of various “overlooked “ high risk oral strains of human papilloma virus?

- Null Hypothesis: Oral high risk HPV strains are uncommon and their prevalence are not statistically significant
- Alternative Hypothesis: Oral high risk HPV strains are common and more prevalent than previously thought and are statistically significant

Is there a relationship between age, race, and gender with these specific HPV strains?

- Null Hypothesis: There is no relationship amongst the given criteria and the HPV strains being investigated
- Alternative Hypothesis: There is a relationship/correlation with the given criteria and the HPV strains being investigated

## **Approval**

The appointment of an advisory committee was submitted for approval and approved on June 29, 2022. The prospectus for this study was submitted for approval and approved on September 2, 2022. This study involved a retrospective analysis of previously collected saliva samples stored in an existing biorepository. No human subjects were recruited in this study.

## **Research Design**

The methods used for sequencing the HPV strains will be replicated using materials and methods established in previous research studies here at UNLV [9,10]. The samples being used in this investigation are from previously collected saliva samples from voluntary participants (saliva repository).

### *Study approval*

This study was reviewed and approved by the UNLV IRB under protocol [1717625-1] Retrospective analysis of microbial prevalence from DNA isolated from saliva samples originally obtained from the University of Nevada, Las Vegas (UNLV) School of Dental

Medicine (SDM) pediatric and clinical population on March 3, 2021.

*Inclusion category*

Samples from Patients Ages 7-26. This is based on the CDC's Advisory Committee on Immunization Practices (ACIP) The current ACIP recommended age range for the HPV vaccine is from ages 11 to 26. The target age is 11 to 12 years, although it may start at 9 years of age and can be given above the age of 26, specifically from 27 to 45 years of age, but is not recommended.

*Exclusion category*

Any patient sample over the age of 80. Any samples from patients outside the school of dental medicine at University of Nevada, Las Vegas

*Saliva Samples*

The following are the methods for handling saliva samples, which will be used [8-10]. Saliva samples will be centrifuged for 10 minutes at 2,100g (RCF) and the cell pellet washed with 1X phosphate-buffered saline and re-centrifuged. The pellet will be resuspended in 5mL of 1X phosphate-buffered saline. HPV DNA was isolated from the sample using the Genomic Prep DNA isolation kit. DNA purity and quantity will then be calculated prior to qPCR using the NanoDrop spectrophotometer.



*Quantitative PCR (qPCR)*

qPCR will be used to provide more specificity and sensitivity for the various HPV strains being tested. The validated primers for this study include: [8-10].

HPV 31

Forward primer: ATTCCACAACATAGGAGGAAGGTG

Reverse primer: CACTTGGGTTTCAGTACGAGGTCT

HPV 33

Forward primer: ATATTTCTGGGTCGTTGGGCA

Reverse primer: ACGTCACAGTGCAGTTTCTCTACGT

HPV 35

Forward primer: TCGGTGTATGTCCTGTTGGAAAC

Reverse primer: CATAGTCTTGCAATGTAGTTATTTCTCCA

HPV52

Forward primer: GACATGTTAATGCAAACAAGCGAT

Reverse primer: CATGACGTTACACTTGGGTCACA

HPV 58

Forward primer: GGCATGTGGATTTAAACAAAAGGT

Reverse primer: TCTCATGGCGTTGTTACAGGTTAC

### *Statistical analysis*

Descriptive statistics will be provided for the patient demographics. A chi-square test will be used to compare demographic characteristics (age, race, gender) and various oncogenic HPV strains. The mean age, range, and variance will also be calculated.

### **Anticipated Findings and Potential Impact**

This investigation hopes to provide insight and shed light on the prevalence and incidence of these other oncogenic strains that are often omitted from HPV research. The research performed can have major implications, such as demonstrating “at risk” groups of individuals.

Additionally, this research project will provide information to determine which strains are present and prevalent, which will help others to evaluate if there may be cross protective antibodies against these strains with the current HPV vaccines available. Lastly, the researchers of this investigation hope that their findings are able to provide an update recommendation for HPV vaccination.

## References

1. Agelaki S, Boukovinas I, Athanasiadis I, Trimis G, Dimitriadis I, Poughias L, Morais E, Sabale U, Bencina G, Athanasopoulos C. A systematic literature review of the human papillomavirus prevalence in locally and regionally advanced and recurrent/metastatic head and neck cancers through the last decade: The "ALARM" study. *Cancer Med.* 2024 Jan 21. doi: 10.1002/cam4.6916. Epub ahead of print. PMID: 38247106.
2. Fonsêca TC, Jural LA, Marañón-Vásquez GA, Magno MB, Roza ALOC, Ferreira DMTP, Maia LC, Romañach MJ, Agostini M, Abrahão AC. Global prevalence of human papillomavirus-related oral and oropharyngeal squamous cell carcinomas: a systematic review and meta-analysis. *Clin Oral Investig.* 2023 Dec 30;28(1):62. doi: 10.1007/s00784-023-05425-0. PMID: 38158517.
3. Zhang H, Cai S, Xia Y, Lin Y, Zhou G, Yu Y, Feng M. Association between human herpesvirus infection and cervical carcinoma: a systematic review and meta-analysis. *Virol J.* 2023 Dec 4;20(1):288. doi: 10.1186/s12985-023-02234-5. PMID: 38049836; PMCID: PMC10696706.
4. Kumarswamy R, Volkmann I, Thum T. Regulation and function of miRNA-21 in health and disease. *RNA Biol.* 2011 Sep-Oct;8(5):706-13. doi: 10.4161/rna.8.5.16154. Epub 2011 Jul 7. PMID: 21712654; PMCID: PMC3256347.
5. Lin R, Jin H, Fu X. Comparative efficacy of human papillomavirus vaccines: systematic review and network meta-analysis. *Expert Rev Vaccines.* 2023 Jan-Dec;22(1):1168-1178. doi: 10.1080/14760584.2023.2287135. Epub 2023 Nov 27. PMID: 37990881.
6. Yokoji K, Giguère K, Malagón T, Rönn MM, Mayaud P, Kelly H, Delany-Moretlwe S, Drolet M, Brisson M, Boily MC, Maheu-Giroux M. Association of naturally acquired type-specific HPV antibodies and subsequent HPV re-detection: systematic review and meta-analysis. *Infect Agent Cancer.* 2023 Nov 8;18(1):70. doi: 10.1186/s13027-023-00546-3. PMID: 37941016; PMCID: PMC10631102.
7. Cui M, Cheng J, Cheng H, Zhao M, Zhou D, Zhang M, Jia J, Luo L. Characteristics of human papillomavirus infection among oropharyngeal cancer patients: A systematic review and meta-analysis. *Arch Oral Biol.* 2024 Jan;157:105830. doi: 10.1016/j.archoralbio.2023.105830. Epub 2023 Oct 31. PMID: 37924712.
8. Hinton H, Coleman S, Salem JR, Kingsley K. Screening for High-Risk Oral Human Papillomavirus (HPV31, HPV33, HPV35) in a Multi-Racial Pediatric and Adult Clinic

Patient Population. *Cancers (Basel)*. 2023 Sep 10;15(18):4501. doi: 10.3390/cancers15184501. PMID: 37760471; PMCID: PMC10527517.

9. Kornhaber MS, Florence T, Davis T, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in Pediatric and Adult Patients within a Multi-Ethnic Clinic Population. *Dent J (Basel)*. 2022 Apr 1;10(4):54. doi: 10.3390/dj10040054. PMID: 35448048; PMCID: PMC9031267.
10. Tiku V, Todd CJ, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in a Multi-ethnic Pediatric Clinic Population. *Compend Contin Educ Dent*. 2016 Nov/Dec;37(10):e1-e4. PMID: 27875050.

## Chapter 2

### Screening for High-Risk Oral Human Papillomavirus (HPV31, HPV33, HPV35) in a Multi-Racial Pediatric and Adult Clinic Patient Population

This chapter has been submitted to and published by MDPI *Cancers* and is presented in the style of that Journal. The complete Citation is:

Hinton H, Coleman S, Salem JR, Kingsley K. Screening for High-Risk Oral Human Papillomavirus (HPV31, HPV33, HPV35) in a Multi-Racial Pediatric and Adult Clinic Patient Population. *Cancers (Basel)*. 2023 Sep 10;15(18):4501. doi: 10.3390/cancers15184501. PMID: 37760471; PMCID: PMC10527517.

#### Role of Authors:

KK and KMH were responsible for the overall project design. HH, SC, and JRS were responsible for data generation and analysis. KK, KMH and HH contributed to the writing and editing of this manuscript. All authors have read and agreed to the published version of the manuscript.

#### **Abstract**

Many human papillomavirus (HPV) strains induce cancer in the cervix and the oral cavity. Although high-risk strains including HPV16 and HPV18 are common - additional high-risk strains including HPV31, HPV33 and HPV35 may induce carcinogenesis although much less is known about their prevalence. Using an approved protocol, samples from a salivary biorepository were screened to find pediatric and adult samples from a university-based patient clinic population. A total of N=86 samples from the saliva biorepository met the quality and concentration standards and were screened for high-risk HPV. qPCR screening of adult samples revealed n=10/45 or 22.2% were HPV31- or HPV33-positive. In addition, a total of n=9/41 or 21.9% of pediatric samples were either HPV31- or HPV33-positive (or both). No samples harbored HPV35. The majority of these samples were derived from patients within the recommended vaccination age range (age 9 - 26 years) or within the catch up age range (27 - 45

years). These results clearly demonstrate that a significant percentage of patients harbor additional high-risk HPV strains within the oral cavity, including HPV31 and HPV33. Although many studies have promoted the quadrivalent HPV vaccine (covering HPV6, 11, 16, and 18), these results suggest that oral healthcare providers may need to promote the newer nine-valent vaccine, which includes HPV31 and HPV33.

Key words: Oral screening, saliva, high-risk human papillomavirus (HPV), qPCR

## **Introduction**

Human Papillomavirus (HPV) is a non-enveloped, double stranded DNA virus that is epitheliotropic in nature and is known to cause disease in a variety of tissues [1,2]. There are between 150-200 different strains of HPV some of which are known to cause human disease, including cancer [3,4]. Approximately 40 types of HPV are known to cause cutaneous or mucosal infections within human hosts [5,6].

HPV infections can spread through skin-to-skin contact through both sexual and non-sexual transmission pathways [7,8]. Epidemiologic prevalence estimates suggest that more than 40 million people in the United States are currently infected with some form of HPV, with an incidence of more than 10 million new cases per year [9-11]. Although most HPV infections may be cleared by the immune system (90-95% by some estimates), some infections result in long-term infections that cause precancerous abnormalities and malignancies if left untreated [12-14]. The main clinical distinction between the many human papillomavirus (HPV) strains lies in their ability to mediate cellular transformation into cancer within various tissues, which are broadly

categorized into low-risk (LR) or high risk (HR) depending upon their most frequently associated clinical outcomes [15,16]. The low-risk HPV strains 6 and 11 are responsible for >90% of anogenital warts and lesions, but also include additional commonly identified strains such as 40, 42, 43, and 44 [17,18]. In addition, high-risk strains HPV 16 and 18 are responsible for the vast majority of cervical as well as the majority of HPV-associated oropharyngeal cancers, although there are many additional high-risk HPV strains commonly identified including HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 [19,20].

Although high-risk strains including HPV16 and HPV18 are the most frequently identified - additional high-risk strains found in the cervix and oral cavity including HPV31, HPV33 and HPV35 may induce carcinogenesis although much less is known about their prevalence [21-23]. Based upon this understanding of additional clinically-relevant strains of HPV, the new nine-valent HPV vaccine incorporates not only the most common high-risk (16 and 18) and low-risk (6 and 11) HPV strains, but also includes additional high-risk strains such as HPV 31, 33, 45, 52, and 58 [24-26]. Although several studies from this institution have evaluated the prevalence of high-risk oral HPV strains 16 and 18 among both adult and pediatric patient populations, no study to date has evaluated these additional high-risk strains, such as HPV31, 33 and 35 [27-30]. Based upon lack of evidence regarding these high risk strains, the primary objective of this project is to evaluate the prevalence of these strains among pediatric and adult patients through screening of clinical saliva samples.

## **Materials and Methods**

### *Study approval*

This study was reviewed and approved by the University of Nevada, Las Vegas (UNLV) Institutional Review Board (IRB) under protocol [1717625-1] Retrospective analysis of microbial prevalence from DNA isolated from saliva samples originally obtained from the University of Nevada, Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and clinical population on March 3, 2021.

### *Human Subjects and Informed Consent*

The original collection protocol was approved by the UNLV IRB under protocol OPRS#1305-4466M “The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population”. Inclusion criteria included patients between the ages of 5 to 45 years of age that agreed to provide Informed Consent (adult over 18 years of age) or Pediatric Assent with Informed Consent (children under 18 years of age with guardian or parental permission and consent). Exclusion criteria included any patients (or parents/guardians that refused to provide informed consent or pediatric assent) and any samples from patients outside the UNLV School of Dental Medicine.

### *Original sample collection protocol*

Original sample collection study patients were voluntary participants. Following informed consent and/or pediatric assent, patients were provided a sterile 50 mL saliva collection tube.



Patients were asked to provide up to 5.0 mL of unstimulated saliva. Each tube was labeled with a randomly-generated, non-duplicated number to prevent the collection or subsequent disclosure of any patient-specific identifying information. Demographic information including the patient sex, age, race or ethnicity and orthodontic status was noted. Samples were then transferred to a biomedical laboratory for storage at -80C.

#### *DNA isolation and analysis*

A total of N=253 samples from the biomedical sample repository were identified for potential inclusion in the current retrospective analysis. DNA was isolated from each sample using the phenol:chloroform extraction method. In brief, samples were thawed, vortexed, and then 500 uL was transferred to a sterile microcentrifuge tube and mixed with 500 uL of TRIzol DNA isolation reagent from Invitrogen (Waltham, Massachusetts, USA). To each sample 200 uL of molecular grade Chloroform from Invitrogen (Waltham, Massachusetts, USA) was added prior to incubation on ice for 15 minutes. Samples were then centrifuged at 12,000X relative centrifugal force (RCF) for 15 minutes at 4C in a refrigerated microcentrifuge (Model 5425) from Eppendorf (Hamburg, Germany).

The upper aqueous phase (approximately 400 - 500 uL) was transferred to a new, sterile microcentrifuge tube and mixed with molecular grade Isopropanol from Invitrogen (Waltham, Massachusetts, USA) to precipitate the DNA. Each sample was then centrifuged using the settings described above. The isopropanol was removed and the DNA-containing pellet was washed with molecular grade Ethanol from Invitrogen (Waltham, Massachusetts, USA) and centrifuged for an additional 10 minutes. The ethanol was removed and the DNA was

resuspended using 100 uL of nuclease-free water from Fisher Scientific (Waltham, Massachusetts, USA). Each sample was analyzed for DNA quantity and DNA quality using a NanoDrop 2000 spectrophotometer from Fisher Scientific (Waltham, Massachusetts, USA) at absorbances of A260 and A280 nm. Samples with sufficient quantity ( $> 10$  ng/uL) and sufficient quality (A260:A280 ratio  $> 1.65$ ) were subsequently screened using qPCR.

#### *qPCR screening*

The study samples that met the minimum criteria for DNA quantity and DNA purity (N=86) were screened for high-risk HPV strains 31, 33 and 35 using quantitative polymerase chain reaction (qPCR). Each reaction consisted of 15 uL Fast SYBR Green Master Mix from Applied Biosystems (Waltham, Massachusetts, USA), 1.5 uL of forward primer, 1.5 uL of reverse primer, 2.0 uL of sample DNA and 5.0 uL of nuclease-free water. Reactions were performed using the QuantStudio 3 from ThermoFisher Scientific (Waltham, Massachusetts, USA) and the following validated primers:

HPV31 forward: ATCCACAACATAGGAGGAAGGTG; 24 nt, 45.8% GC,  $T_m=62.9C$

HPV31 reverse: CACTTGGGTTTCAGTACGAGGTCT; 24 nt, 50.0% GC,  $T_m=64.6C$

HPV33 forward: ATATTTTCGGGGTCGTTGGGCA; 20 nt, 50.0% GC,  $T_m=60.4C$

HPV33 reverse: ACGTCACAGTGCAGTTTCTCTACGT; 25 nt, 48.0% GC,  $T_m=64.6C$

HPV35 forward: TCGGTGTATGTCTGTTGGAAAC; 23nt, 47.8% GC,  $T_m=62.8C$

HPV35 reverse: CATAGTCTTGCAATGTAGTTATTTCTCCA; 29 nt, 34.5% GC,  $T_m=61.8C$

### *Statistical analysis*

Demographic variables for the study sample were compiled and presented as simple, descriptive statistics. Analysis of differences between the study sample and the overall clinic population with respect to categorical variables, such as sex and race or ethnicity, were done using Chi square statistics which is appropriate for non-parametric data analysis. Analysis of qPCR screening results were also presented as simple descriptive statistics, such as percentages, and the differences between HPV-positive and HPV-negative samples were also analyzed using Chi square statistics and the GraphPad Prism software, Version 8 (San Diego, CA, USA). Comparisons for parametric data, such as age, were completed using two-tailed Student's t-tests, using an alpha level of 0.05 for statistical significance.

### **Results**

A total of N=86 samples from an existing biorepository were identified for inclusion in this retrospective study, which were nearly equally divided between adults (52.3% or n=45/86, Table 1) and pediatric (47.7% or n=41/86) patients (Table 2). Analysis of the adults in the study sample revealed approximately half were derived from females 55.6% or n=25/45), which closely approximates the percentage of females in the overall clinic population (49.1%), p=0.1614 (Table 1). In addition, analysis of the demographic characteristics demonstrated that the majority of the adult study samples were derived from racial or ethnic minorities (55.6% or n=25/45), which was slightly lower but not significantly different from the percentages observed within the overall main clinic population (65.4%), p=.0592. Finally, the average age of the adult samples was found to be 41.5 years (range: 18 to 73 years), which closely

approximates the average age of the main patient clinic population of 42.3 years (range 18 to 89 years),  $p=0.7738$ .

Table 1. Demographic analysis of adult study samples.

<b>Demographics</b>	<b>Study sample Adults (n=45)</b>	<b>UNLV-SDM Clinic (Adult patient)</b>	<b>Statistical analysis</b>
<b>Sex</b>			
Adult - Females	(n=25) 55.6%	49.1%	$X^2=1.961$ , d.f.=1
Adult - Males	(n=20) 44.4%	50.9%	$p=0.1614$
<b>Race</b>			
White	(n=20) 44.4%	34.6%	$X^2=3.560$ , d.f.=1
Minority	(n=25) 55.6%	65.4%	$p=0.0592$
Hispanic	(n=13) 28.8%	58.6%	
Black	(n=6) 13.3%	10.2%	
Asian/Other	(n=6) 13.3%	6.6	
<b>Age</b>			
Mean	41.5 years	42.3 years	Two-tailed T-test
Range	18 - 73 years	18 - 89 years	$p=0.7738$

Analysis of the pediatric patients in the study sample revealed approximately half were derived from females 56.1% or n=23/41), which closely approximates the percentage of females represented in the overall pediatric clinic population (52.8%), p=0.5478 (Table 2). Analysis of the racial and ethnic distribution of these patients found that the overwhelming majority of the pediatric study samples were derived from racial or ethnic minorities (82.96% or n=34/41), which was higher but not significantly different from the overall percentages of minorities observed within the pediatric clinic population (75.34%), p=0.0647. Finally, the average age of the pediatric samples was 12.7 years (range 5 to 17 years), which closely approximated the average age of the overall pediatric patient clinic population of 10.4 years (range 0 to 17 years), p=0.2531.

Table 2. Demographic analysis of pediatric study samples.

<b>Demographics</b>	<b>Study sample Pediatrics (n=41)</b>	<b>UNLV-SDM Clinic (Pediatric clinic)</b>	<b>Statistical analysis</b>
<b>Sex</b>			
Pediatric - Females	(n=23) 56.1%	52.8%	$X^2=0.361$ , d.f.=1
Pediatric - Males	(n=18) 43.9%	47.2%	$p=0.5478$
<b>Race</b>			
White	(n=7) 17.1%	24.7%	$X^2=3.413$ , d.f.=1
Minority	(n=34) 82.9%	75.3%	$p=0.0647$
Hispanic	(n=23) 56.1%	52.1%	
Black	(n=4) 9.8%	11.8%	
Asian/Other	(n=5) 12.2%	11.4%	
<b>Age</b>			
Mean	12.7 years	10.4 years	Two-tailed T-test
Range	5 - 17 years	0 - 17 years	$p=0.2531$

Screening of the adult patients revealed that n=10/45 or 22.2% different samples harbored one

or more of the three high-risk strains of HPV analyzed, including 31, 33, and 35 (Figure 1). More specifically, n=4/10 or 40% of the HPV-positive samples harbored HPV31 while n=7/10 or 70% harbored HPV33 - including one that was also positive for HPV31. However, none of the samples evaluated harbored HPV35.

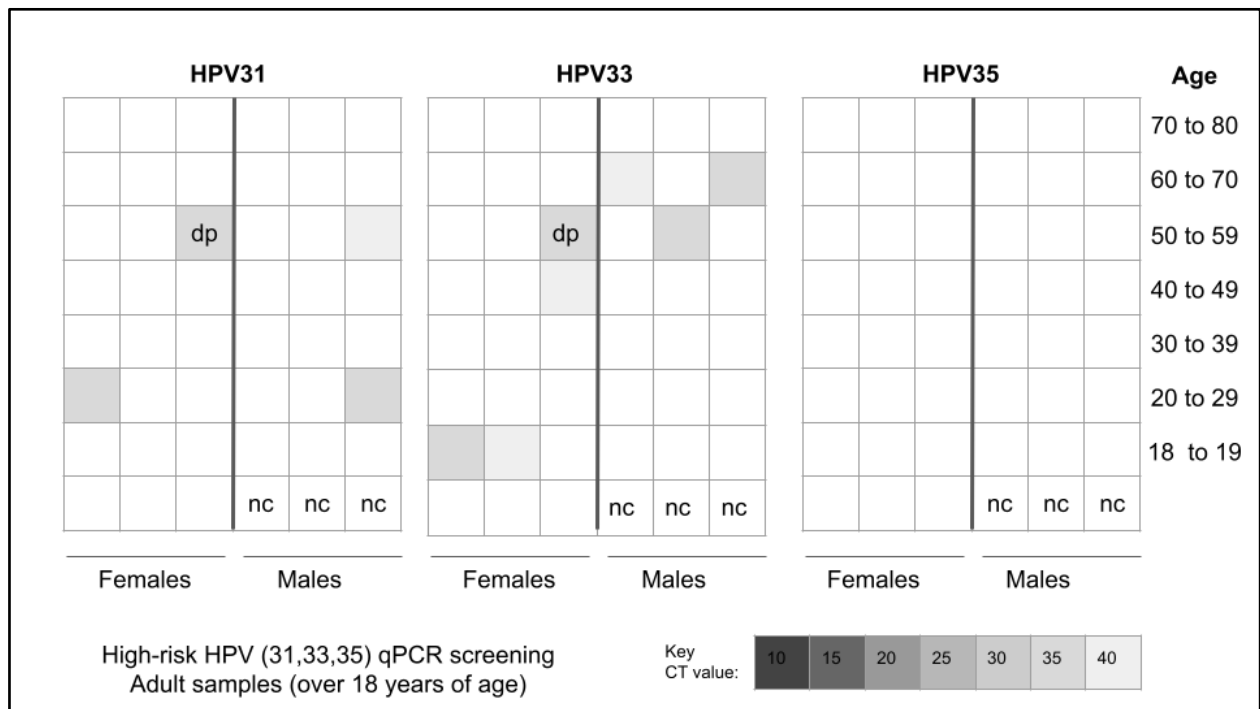


Figure 1. Heatmap analysis for qPCR screening of adult samples for high-risk HPV. A total of n=10 samples tested positive for HPV with n=4/10 or 40% testing positive for HPV31, n=6/10 testing positive for HPV33 and one sample tested positive for both HPV31 and HPV33. No samples tested positive for HPV35. (nc = negative control, dp=double positive)

More detailed analysis of the adult samples revealed an equal distribution of HPV-positive samples between males (n=5/10 or 50%) and females (n=5/10 or 50%), which closely matched the distribution of HPV-negative samples from males and females,  $p=0.5478$  (Table 3). In addition, the analysis of race and ethnicity revealed the majority of HPV-positive samples were derived from minority patients (60%), which closely matched the proportion of HPV-negative samples from minority patients (54.3%),  $p=0.2286$ . Finally, the proportion of HPV-positive samples and HPV-negative samples from patients within the catch up range (under 45 years) was nearly equal and not significantly different (40%, 42.9%, respectively),  $p=0.5445$ .



Table 3. Demographic analysis of adult HPV-positive and HPV-negative samples.

<b>Demographics</b>	<b>HPV-positive</b>	<b>HPV-negative</b>	<b>Statistical analysis</b>
Adult - Males	50% (n=5/10)	42.9% (n=15/35)	$X^2=0.361$ , d.f.=1
Adult - Females	50% (n=5/10)	57.1% (n=20/35)	p=0.5478
Total	22.2% (n=10/45)	77.8% (n=35/45)	
Adult - Non-Minority	40% (n=4/10)	45.7% (n=16/35)	$X^2=1.449$ , d.f.=1
Adult - Minority	60% (n=6/10)	54.3% (n=19/35)	p=0.2286
Total	22.2% (n=10/45)	77.8% (n=35/45)	
Below Catch-up Age (Under 45 years)	40% (n=4/10)	42.9% (n=15/35)	$X^2=0.367$ , d.f.=1
Above Catch-up Age (Over 45 years)	60% (n=6/10)	57.1% (n=20/35)	p=0.5445
Average Age	44.8 years	46.1 years	

Screening of the pediatric patient samples revealed that n=9/41 or 21.9% samples harbored one

or more of the three high-risk strains of HPV, such as 31, 33, and 35 (Figure 2). More specifically, n=6/9 or 66.7% of the HPV-positive samples harbored HPV31 while n=7/9 or 77.8% harbored HPV33 - including four that were double positive for both HPV31 and HPV33. However, none of the samples evaluated harbored HPV35.

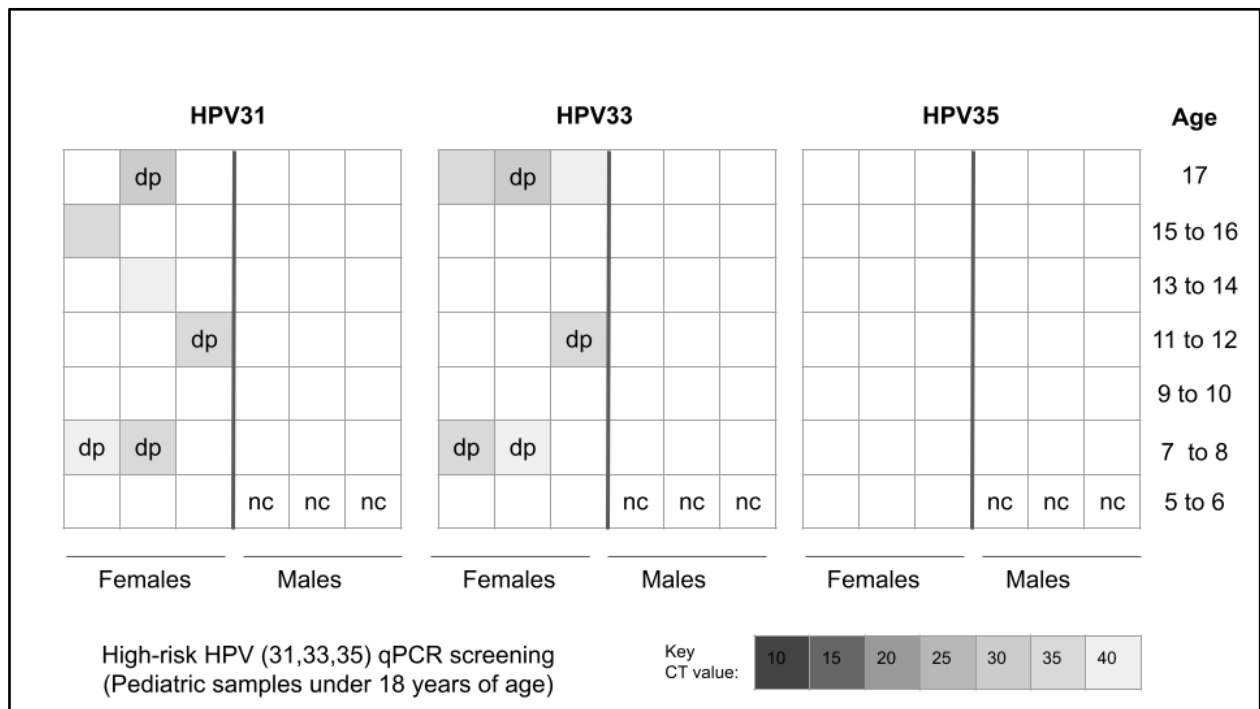


Figure 2. Analysis of heatmap for qPCR screening of pediatric samples for high-risk HPV. A total of n=9/41 or 21.9% of samples tested positive for HPV with n=6/9 or 66.7% testing positive for HPV31, n=7/9 or 77.8% testing positive for HPV33 and four samples testing positive for both HPV31 and HPV33. No samples tested positive for HPV35. (nc = negative control, dp=double positive)

More detailed analysis of the pediatric samples revealed an unequal distribution of HPV-positive samples between males (n=3/9 or 33.3%) and females (n=6/9 or 66.7%), which was significantly different from the distribution of HPV-negative samples from males and females, p=0.005 (Table 4). In addition, the analysis of race and ethnicity revealed the majority of HPV-positive samples were derived from minority patients (n=7/9 or 77.8%), which did not differ significantly from the proportion of HPV-negative samples from minority patients (n=27/32 or 84.4%), p=0.1017. Finally, the proportion of HPV-positive samples and HPV-negative samples from patients within the HPV vaccination age range (11 to 17 years) was significantly different (77.8%, 83.7%, respectively), p=0.0001.

Table 4. Demographic analysis of pediatric HPV-positive and HPV-negative samples.

<b>Demographics</b>	<b>HPV-positive</b>	<b>HPV-negative</b>	<b>Statistical analysis</b>
Pediatric - Males	33.3% (n=3/9)	56.9% (n=15/32)	$X^2=7.868$ , d.f.=1
Pediatric - Females	66.7% (n=6/9)	53.1% (n=17/32)	p=0.005
Total	21.9% (n=9/41)	78.1% (n=32/41)	
Pediatric - Non-Minority	22.2% (n=2/9)	15.6% (n=5/32)	$X^2=2.679$ , d.f.=1
Pediatric - Minority	77.8% (n=7/9)	84.4% (n=27/32)	p=0.1017
Total	21.9% (n=9/41)	78.1% (n=32/41)	
Within Vaccination Age Range (11 to 17 years)	77.8% (n=7/9)	93.7% (n=30/32)	$X^2=45.390$ , d.f.=1
Below Vaccination Age Range (Under 11 years)	22.2% (n=2/9)	6.3% (n=2/32)	p=0.0001
Average Age	13.4 years	12.7 years	

## **Discussion**

The primary goal of this study was to assess the prevalence of high-risk HPV strains 31, 33 and 35 using an existing biorepository including both pediatric and adult clinical saliva samples. The results of this study successfully demonstrated that HPV31 and HPV33 were found among both pediatric and adult samples in similar proportions (21.9%, 22.2% respectively), although no samples tested positive for HPV35. These data represent the first clinical descriptions of non-HPV16 and non-HPV18 high-risk HPV prevalence within this patient population [27-30].

However, some notable differences were found regarding the prevalence of these high-risk HPV strains compared with other previous studies of HPV strains HPV16 and HPV18. For example, this study found nearly one-quarter of adults (22.2%) harbored either HPV31, HPV33 or both. This is somewhat lower than the most recent study of HPV16 and HPV18 prevalence among adults within this patient population, which found an overall prevalence of 30.2% [27]. However, it is also much higher than the first description of HPV16 and HPV18 prevalence among adults from nearly a decade earlier that found an overall prevalence of only 2.6% within the same clinical patient population [30]. Moreover, these data also confirm other recent observations of HPV31 and HPV33 oral prevalence within other patient populations, which ranged between 5.7% and 14.3% [31-33].

In addition, these data also demonstrated that HPV31 and HPV35 were found in approximately one-fifth of pediatric patient samples (21.9%), which corresponds to similar prevalence levels of HPV16 and HPV18 found within these patients as recently as last year (19.5%) [27]. Although this represents the first non-HPV16 non-HPV18 screening within this patient population, the more aspect is the rise in pediatric oral HPV prevalence observed with HPV16 and HPV18, which was 2.5% in 2012, 9.2% in 2016, and 19.5% in 2022 [27-29]. As more studies confirm

oral prevalence levels of high-risk HPV among pediatric populations at similar levels, the case for screening and evaluating these additional HPV strains becomes more critical [34,35]. Despite the significance of epidemiological data regarding high-risk HPV prevalence as more and more studies are now screening for HPV strains other than HPV16 and HPV18, there are some limitations associated with this type of study that should also be considered [36,37]. More specifically, this was a retrospective study of previously collected saliva samples from an existing biorepository and may not reflect the most current oral prevalence, which may have shifted due to behavioral and vaccination practice changes following the onset of SARS-CoV-2 (COVID-19) pandemic [38,39]. In addition, due to the retrospective nature of this study - no other health information (oral or systemic) was available to determine if other factors, such as smoking, vaping or oral microbiota might have influenced the outcomes of this study [40-42]. Finally, due to the parameters of the original protocol these samples were part of cross-sectional studies involving one-time saliva collections and therefore have no information regarding the temporal nature of the HPV detected and whether this was a short- or long-term infection.

## **Conclusions**

The importance of these findings, including the high prevalence of high-risk strains HPV31 and HPV33 detected in this study, combined with previous data about the increasing prevalence of HPV16 and HPV18 within this patient population may suggest a more robust and focused effort on HPV vaccination and awareness of oral HPV infection may be warranted [27-30]. However, recent evidence regarding increasing levels of vaccine hesitancy also suggest that more evidence

may be needed to demonstrate the relevance of HPV prevention - particularly among this patient population [43,44]. This study may be among the first to provide this type of evidence through the assessment and evaluation of oral HPV infection outside of the conventional HPV strains of HPV16 and HPV18.

**Informed Consent Statement:** The original protocol for the collection of saliva samples from the UNLV SDM clinic was approved under OPRS#1305-4466M titled “The Prevalence of Oral Microbes in Saliva from the UNLV School of Dental Medicine Pediatric and Adult Clinical Population”. Under this protocol, saliva samples were collected from volunteer patients at the beginning of their clinic appointment. Informed Consent was collected from adult patients who chose to participate, while Pediatric Patients above the age of seven were also required to provide Pediatric Assent in addition to the Informed Consent and approval of the accompanying guardian or parent.

**Conflicts of Interest:** The authors declare no conflict of interest.



## References

1. Spurgeon ME. Small DNA tumor viruses and human cancer: Preclinical models of virus infection and disease. *Tumour Virus Res.* 2022 Dec;14:200239. doi: 10.1016/j.tvr.2022.200239. Epub 2022 May 27. PMID: 35636683; PMCID: PMC9194455.
2. Mukherjee AG, Wanjari UR, Gopalakrishnan AV, Kannampuzha S, Murali R, Namachivayam A, Ganesan R, Renu K, Dey A, Vellingiri B, Prabakaran DS. Exploring the Molecular Pathogenesis, Pathogen Association, and Therapeutic Strategies against HPV Infection. *Pathogens.* 2022 Dec 23;12(1):25. doi: 10.3390/pathogens12010025. PMID: 36678374; PMCID: PMC9865103.
3. Oyouni AAA. Human papillomavirus in cancer: Infection, disease transmission, and progress in vaccines. *J Infect Public Health.* 2023 Apr;16(4):626-631. doi: 10.1016/j.jiph.2023.02.014. Epub 2023 Feb 21. PMID: 36868166.
4. Zou K, Huang Y, Li Z. Prevention and treatment of human papillomavirus in men benefits both men and women. *Front Cell Infect Microbiol.* 2022 Nov 24;12:1077651. doi: 10.3389/fcimb.2022.1077651. PMID: 36506029; PMCID: PMC9729793.
5. La Rosa G, Fratini M, Accardi L, D'Oro G, Della Libera S, Muscillo M, Di Bonito P. Mucosal and cutaneous human papillomaviruses detected in raw sewages. *PLoS One.* 2013;8(1):e52391. doi: 10.1371/journal.pone.0052391. Epub 2013 Jan 14. PMID: 23341898; PMCID: PMC3544852.
6. Stanley M. Host defence and persistent human papillomavirus infection. *Curr Opin Virol.* 2021 Dec;51:106-110. doi: 10.1016/j.coviro.2021.09.010. Epub 2021 Oct 8. PMID: 34628358.
7. Petca A, Borislavski A, Zvanca ME, Petca RC, Sandru F, Dumitrascu MC. Non-sexual HPV transmission and role of vaccination for a better future (Review). *Exp Ther Med.* 2020 Dec;20(6):186. doi: 10.3892/etm.2020.9316. Epub 2020 Oct 13. PMID: 33101476; PMCID: PMC7579832.
8. Liu Z, Rashid T, Nyitray AG. Penises not required: a systematic review of the potential for human papillomavirus horizontal transmission that is non-sexual or does not include penile penetration. *Sex Health.* 2016 Feb;13(1):10-21. doi: 10.1071/SH15089. PMID: 26433493.

9. Rintala S, Dahlstrom KR, Franco EL, Louvanto K. A synthesis of evidence for cancer-specific screening interventions: A Preventive Medicine Golden Jubilee Review. *Prev Med.* 2023 Feb;167:107395. doi: 10.1016/j.ypmed.2022.107395. Epub 2022 Dec 21. PMID: 36565859.
10. Seay J, Matsuno R, Buechel J, Tannenbaum K, Wells N. HPV-Related Cancers: A Growing Threat to U.S. Military Health and Readiness. *Mil Med.* 2022 May 3;187(5-6):149-154. doi: 10.1093/milmed/usab443. PMID: 34697641.
11. Stenger MR, Baral S, Stahlman S, Wohlfeiler D, Barton JE, Peterman T. As through a glass, darkly: the future of sexually transmissible infections among gay, bisexual and other men who have sex with men. *Sex Health.* 2017 Feb;14(1):18-27. doi: 10.1071/SH16104. PMID: 27585033; PMCID: PMC5334461.
12. Wijnstma ES, Jongen VW, Alberts CJ, de Melker HE, Hoes J, Schim van der Loeff MF. Approaches to Estimating Clearance Rates for Human Papillomavirus Groupings: A Systematic Review and Real Data Examples. *Epidemiology.* 2023 Jan 1;34(1):119-130. doi: 10.1097/EDE.0000000000001550. Epub 2022 Sep 22. PMID: 36137191.
13. Andrei EC, Baniță IM, Munteanu MC, Busuioc CJ, Mateescu GO, Mălin RD, Pisoschi CG. Oral Papillomatosis: Its Relation with Human Papilloma Virus Infection and Local Immunity-An Update. *Medicina (Kaunas).* 2022 Aug 15;58(8):1103. doi: 10.3390/medicina58081103. PMID: 36013570; PMCID: PMC9415166.
14. Ntuli L, Mtshali A, Mzobe G, Liebenberg LJ, Ngcapu S. Role of Immunity and Vaginal Microbiome in Clearance and Persistence of Human Papillomavirus Infection. *Front Cell Infect Microbiol.* 2022 Jul 7;12:927131. doi: 10.3389/fcimb.2022.927131. PMID: 35873158; PMCID: PMC9301195.
15. Plotzker RE, Vaidya A, Pokharel U, Stier EA. Sexually Transmitted Human Papillomavirus: Update in Epidemiology, Prevention, and Management. *Infect Dis Clin North Am.* 2023 Jun;37(2):289-310. doi: 10.1016/j.idc.2023.02.008. PMID: 37105644.
16. Georgescu SR, Mitran CI, Mitran MI, Caruntu C, Sarbu MI, Matei C, Nicolae I, Tocut SM, Popa MI, Tampa M. New Insights in the Pathogenesis of HPV Infection and the Associated Carcinogenic Processes: The Role of Chronic Inflammation and Oxidative Stress. *J Immunol Res.* 2018 Aug 27;2018:5315816. doi: 10.1155/2018/5315816. PMID: 30225270; PMCID: PMC6129847.

17. Boda D, Neagu M, Constantin C, Voinescu RN, Caruntu C, Zurac S, Spandidos DA, Drakoulis N, Tsoukalas D, Tsatsakis AM. HPV strain distribution in patients with genital warts in a female population sample. *Oncol Lett*. 2016 Sep;12(3):1779-1782. doi: 10.3892/ol.2016.4903. Epub 2016 Jul 22. PMID: 27602111; PMCID: PMC4998207.
18. Chen X, Li L, Lai Y, Liu Q, Yan J, Tang Y. Characteristics of human papillomaviruses infection in men with genital warts in Shanghai. *Oncotarget*. 2016 Aug 16;7(33):53903-53910. doi: 10.18632/oncotarget.9708. PMID: 27270315; PMCID: PMC5288230.
19. Balmagambetova S, Tinelli A, Mynbaev OA, Koyshybaev A, Urazayev O, Kereyeva N, Ismagulova E. Human Papillomavirus Selected Properties and Related Cervical Cancer Prevention Issues. *Curr Pharm Des*. 2020;26(18):2073-2086. doi: 10.2174/1381612826666200422094205. PMID: 32321395.
20. Gravitt PE. The known unknowns of HPV natural history. *J Clin Invest*. 2011 Dec;121(12):4593-9. doi: 10.1172/JCI57149. Epub 2011 Dec 1. PMID: 22133884; PMCID: PMC3225991.
21. Arndt O, Johannes A, Zeise K, Brock J. HPV-Typen vom "high risk type" in oralen und laryngealen Papillomen und Leukoplakien [High-risk HPV types in oral and laryngeal papilloma and leukoplakia]. *Laryngorhinootologie*. 1997 Mar;76(3):142-9. German. doi: 10.1055/s-2007-997403. PMID: 9213402.
22. Wood ZC, Bain CJ, Smith DD, Whiteman DC, Antonsson A. Oral human papillomavirus infection incidence and clearance: a systematic review of the literature. *J Gen Virol*. 2017 Apr;98(4):519-526. doi: 10.1099/jgv.0.000727. Epub 2017 May 5. PMID: 28150575.
23. Kreimer AR, Bhatia RK, Messegue AL, González P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis*. 2010 Jun;37(6):386-91. doi: 10.1097/OLQ.0b013e3181c94a3b. PMID: 20081557.
24. Zhu K, Tian Y, Dong X, Akinwunmi BO, Zhang CJP, Huang J, Ming WK. The cost-effectiveness of bivalent, quadrivalent, and nine-valent HPV vaccination in Asia: a systematic review. *Arch Gynecol Obstet*. 2022 Jul;306(1):173-187. doi: 10.1007/s00404-021-06309-y. Epub 2022 Apr 5. PMID: 35380278.
25. Husein-ElAhmed H. Could the human papillomavirus vaccine prevent recurrence of anogenital warts?: a systematic review and meta-analysis. *Int J STD AIDS*. 2020

Jun;31(7):606-612. doi: 10.1177/0956462420920142. Epub 2020 May 21. PMID: 32438856.

26. Signorelli C, Odone A, Ciorba V, Cella P, Audisio RA, Lombardi A, Mariani L, Mennini FS, Pecorelli S, Rezza G, Zuccotti GV, Peracino A. Human papillomavirus 9-valent vaccine for cancer prevention: a systematic review of the available evidence. *Epidemiol Infect.* 2017 Jul;145(10):1962-1982. doi: 10.1017/S0950268817000747. Epub 2017 Apr 27. PMID: 28446260; PMCID: PMC5974698.
27. Kornhaber MS, Florence T, Davis T, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in Pediatric and Adult Patients within a Multi-Ethnic Clinic Population. *Dent J (Basel).* 2022 Apr 1;10(4):54. doi: 10.3390/dj10040054. PMID: 35448048; PMCID: PMC9031267.
28. Tiku V, Todd CJ, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in a Multi-ethnic Pediatric Clinic Population. *Compend Contin Educ Dent.* 2016 Nov/Dec;37(10):e1-e4. PMID: 27875050.
29. Flake C, Arafa J, Hall A, Ence E, Howard K, Kingsley K. Screening and detection of human papillomavirus (HPV) high-risk strains HPV16 and HPV18 in saliva samples from subjects under 18 years old in Nevada: a pilot study. *BMC Oral Health.* 2012 Oct 22;12:43. doi: 10.1186/1472-6831-12-43. PMID: 23088565; PMCID: PMC3532331.
30. Turner DO, Williams-Cocks SJ, Bullen R, Catmull J, Falk J, Martin D, Mauer J, Barber AE, Wang RC, Gerstenberger SL, Kingsley K. High-risk human papillomavirus (HPV) screening and detection in healthy patient saliva samples: a pilot study. *BMC Oral Health.* 2011 Oct 10;11:28. doi: 10.1186/1472-6831-11-28. PMID: 21985030; PMCID: PMC3200164.
31. Cho H, Kishikawa T, Tokita Y, Suzuki M, Takemoto N, Hanamoto A, Fukusumi T, Yamamoto M, Fujii M, Ohno Y, Inohara H. Prevalence of human papillomavirus in oral gargles and tonsillar washings. *Oral Oncol.* 2020 Jun;105:104669. doi: 10.1016/j.oraloncology.2020.104669. Epub 2020 Apr 4. Erratum in: *Oral Oncol.* 2021 Sep;120:105478. PMID: 32259682.
32. Saghravanian N, Ghazvini K, Babakoohi S, Firooz A, Mohtasham N. Low prevalence of high risk genotypes of human papilloma virus in normal oral mucosa, oral leukoplakia and verrucous carcinoma. *Acta Odontol Scand.* 2011 Nov;69(6):406-9. doi: 10.3109/00016357.2011.572560. Epub 2011 Apr 5. PMID: 21466259.

33. Kansky AA, Seme K, Maver PJ, Luzar B, Gale N, Poljak M. Human papillomaviruses (HPV) in tissue specimens of oral squamous cell papillomas and normal oral mucosa. *Anticancer Res.* 2006 Jul-Aug;26(4B):3197-201. PMID: 16886657.
34. Di Spirito F, Pantaleo G, Di Palo MP, Amato A, Raimondo A, Amato M. Oral Human Papillomavirus Benign Lesions and HPV-Related Cancer in Healthy Children: A Systematic Review. *Cancers (Basel).* 2023 Feb 8;15(4):1096. doi: 10.3390/cancers15041096. PMID: 36831439; PMCID: PMC9954073.
35. Pinheiro Rdos S, de França TR, Ferreira Dde C, Ribeiro CM, Leão JC, Castro GF. Human papillomavirus in the oral cavity of children. *J Oral Pathol Med.* 2011 Feb;40(2):121-6. doi: 10.1111/j.1600-0714.2010.00954.x. Epub 2010 Oct 24. PMID: 20969625.
36. Nemesio I, Cury F, Longatto-Filho A, Fregnani JH, Musselwhite L, Vazquez F, Peters AC, Oliveira C. Identification of human papillomavirus in oral rinse specimens from women with and without cervical intraepithelial lesions. *Sex Transm Infect.* 2020 Sep;96(6):408-410. doi: 10.1136/sextrans-2019-054359. Epub 2020 Feb 11. PMID: 32047004.
37. Walline HM, Komarck C, McHugh JB, Byrd SA, Spector ME, Hauff SJ, Graham MP, Bellile E, Moyer JS, Prince ME, Wolf GT, Chepeha DB, Worden FP, Stenmark MH, Eisbruch A, Bradford CR, Carey TE. High-risk human papillomavirus detection in oropharyngeal, nasopharyngeal, and oral cavity cancers: comparison of multiple methods. *JAMA Otolaryngol Head Neck Surg.* 2013 Dec;139(12):1320-7. doi: 10.1001/jamaoto.2013.5460. PMID: 24177760; PMCID: PMC4049419.
38. Grigolato R, Accorona R, Lombardo G, Corrocher G, Garagiola U, Massari F, Nicoli S, Rossi S, Calabrese L. Oral cancer in non-smoker non-drinker patients. Could comparative pet oncology help to understand risk factors and pathogenesis? *Crit Rev Oncol Hematol.* 2021 Oct;166:103458. doi: 10.1016/j.critrevonc.2021.103458. Epub 2021 Aug 27. PMID: 34461267.
39. Koskinen AI, Hemminki O, Försti A, Hemminki K. Incidence and survival in oral and pharyngeal cancers in Finland and Sweden through half century. *BMC Cancer.* 2022 Mar 2;22(1):227. doi: 10.1186/s12885-022-09337-2. PMID: 35236321; PMCID: PMC8889707.

40. Zhang Y, D'Souza G, Fakhry C, Bigelow EO, Usyk M, Burk RD, Zhao N. Oral Human Papillomavirus Associated With Differences in Oral Microbiota Beta Diversity and Microbiota Abundance. *J Infect Dis*. 2022 Sep 21;226(6):1098-1108. doi: 10.1093/infdis/jiac010. PMID: 35038733; PMCID: PMC9492316.
41. Muzio LL, Ballini A, Cantore S, Bottalico L, Charitos IA, Ambrosino M, Nocini R, Malcangi A, Dioguardi M, Cazzolla AP, Brauner E, Santacroce L, Cosola MD. Overview of *Candida albicans* and Human Papillomavirus (HPV) Infection Agents and their Biomolecular Mechanisms in Promoting Oral Cancer in Pediatric Patients. *Biomed Res Int*. 2021 Nov 2;2021:7312611. doi: 10.1155/2021/7312611. PMID: 34765678; PMCID: PMC8577934.
42. Klawinski D, Hanna I, Breslin NK, Katzenstein HM, Indelicato DJ. Vaping the Venom: Oral Cavity Cancer in a Young Adult With Extensive Electronic Cigarette Use. *Pediatrics*. 2021 May;147(5):e2020022301. doi: 10.1542/peds.2020-022301. PMID: 33926987.
43. Maginot R, Esteves C, Kingsley K. Changing Perspectives on Pediatric Human Papillomavirus (HPV) Vaccination among Dental Students and Residents Reveals Recent Increase in Vaccine Hesitancy. *Vaccines (Basel)*. 2022 Apr 6;10(4):570. doi: 10.3390/vaccines10040570. PMID: 35455318; PMCID: PMC9029190.
44. Mann SK, Kingsley K. Human Papillomavirus (HPV) Vaccine Knowledge, Awareness and Acceptance among Dental Students and Post-Graduate Dental Residents. *Dent J (Basel)*. 2020 May 9;8(2):45. doi: 10.3390/dj8020045. PMID: 32397425; PMCID: PMC7345517.

## Chapter 3

### **Summary and Conclusions**

This study was the first to investigate and screen for the presence of high-risk HPV strains that are not HPV16 or HPV18 within this patient population. This study is also one of the only projects to date that has evaluated HPV31, HPV33 and HPV35 among oral samples from any patient population [1]. These findings therefore represent some of the earliest and most important indicators of high-risk HPV prevalence when combined with the numerous other studies from this institution and other groups that have found increasing HPV prevalence among both pediatric and adult populations over time [2].

In addition, the fact that the majority of the HPV-positive samples were derived from patient samples that were also within the recommended vaccination age range (9 - 26 years) or the updated catch up range (27 - 45 years) strongly suggests that these data may be extremely valuable to epidemiologists and public health professionals. Evidence-based research that can demonstrate the risk of HPV infection among patients within the recommended vaccination ranges could be an important tool to help combat the rising incidence of HPV hesitancy among young adults and their parents or guardians [3,4]. These data are part of a growing body of evidence that confirms the importance of prevention efforts, including the nine-valent HPV vaccine, which are demonstrated to reduce HPV-related diseases, including oral cancers [5].

Furthermore, this group has investigated other high-risk HPV strains, such as HPV52 and HPV58 and found that a total of  $n=4/45$  or 8.9% of adult saliva samples tested positive for high-risk HPV52 and  $n=2/45$  or 4.4% tested positive for high-risk HPV58. In addition, a total of  $n=6/42$  or 14.3% of the pediatric saliva samples tested positive for high-risk HPV, including  $n=5/42$  or 11.9% with HPV52 and  $n=3/42$  or 7.1% for HPV58.. These data demonstrated the presence of the high-risk oncogenic HPV52 and HPV58 strains among both adult and pediatric clinical patient samples.

Research question: What is the incidence and prevalence of various “overlooked “ high risk oral strains of human papilloma virus?

- Null Hypothesis: Oral high risk HPV strains are uncommon and their prevalence are not statistically significant
- Alternative Hypothesis: Oral high risk HPV strains are common and more prevalent than previously thought and are statistically significant

Based upon this research, the null hypothesis must be rejected and the alternative hypothesis should be accepted.

Is there a relationship between age, race, and gender with these specific HPV strains?

- Null Hypothesis: There is no relationship amongst the given criteria and the HPV strains being investigated



- Alternative Hypothesis: There is a relationship/correlation with the given criteria and the HPV strains being investigated

Based upon this research, the null hypothesis must be rejected and the alternative hypothesis should be accepted.

### **Limitations and Recommendations**

Although many limitations exist for any retrospective study, the primary limitation for this study was the sample size. In future studies, a larger sample size would be recommended to increase the ability to extrapolate results to a larger population - although the financial and other limitations would still likely present obstacles and challenges to overcome.

In addition, any prospective studies on these high-risk HPV strains may also want to record and evaluate whether the patients were already vaccinated for HPV. Almost half of younger adults are now receiving HPV vaccinations, although this number is highly variable and may be difficult to collect. If possible those studies may also include an evaluation of whether these patients already possess anti-HPV antibodies in the collected saliva, which is another variable that might provide more insight into the prevalence information already collected.

Appendix A

Permission to Use Copyrighted Material

University of Nevada, Las Vegas

I, **Karl Kingsley**, holder of copyrighted material entitled **Screening for High-Risk Oral Human Papillomavirus (HPV31, HPV33, HPV35) in a Multi-Racial, Pediatric and Adult Clinic Patient Population**, authored by **Hunter Hinton, Spencer Coleman, JR Salem, and Karl Kingsley** originally published in **MDPI *Cancers*, September 10, 2023**, hereby give permission for the author to use the above described material in total or in part for inclusion in a Master's thesis at the University of Nevada, Las Vegas.

I also agree that the author may execute the standard contract with ProQuest for storage and reproduction of the completed thesis, including the materials to which I hold copyright

Karl Kingsley

January 2024

---

Signature

Date

Karl Kingsley

Professor, Chair of Thesis Committee

---

Name (typed)

Title

Curriculum Vitae

Graduate College

University of Nevada, Las Vegas

Hunter Hinton

---

Email: [hintoh2@unlv.nevada.edu](mailto:hintoh2@unlv.nevada.edu), [dr.hunterhinton@gmail.com](mailto:dr.hunterhinton@gmail.com)

Degrees:

Bachelor of Science – Biology, 2017  
Arizona State University, Tempe, Arizona

Doctor of Dental Medicine, 2021  
Midwestern University, Glendale, Arizona

Thesis Title:

Screening Of Orthodontic Patients And Age-Matched Non Orthodontic Samples For High-Risk  
Oncogenic Human Papillomavirus (HPV) Strains

Thesis Examination Committee:

Chairperson, Karl Kingsley, Ph.D. M.P.H.  
Committee Member, Katherine Howard, Ph.D.  
Committee Member, Brian Chrzan, D.D.S., Ph.D.  
Graduate Faculty Representative, Erika Marquez, Ph.D.  
Graduate Coordinator, Brian Chrzan, D.D.S., Ph.D.