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High-risk human papillomavirus (HPV): An emerging health issue for women and minorities

Dierdre O. Turner
University of Nevada, Las Vegas

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HIGH-RISK HUMAN PAPILLOMAVIRUS (HPV): AN EMERGING HEALTH ISSUE
FOR WOMEN AND MINORITIES

by

Deidré O. Turner

Bachelor of Science
California State University Dominguez Hills
2006

A thesis submitted in partial fulfillment
of the requirements for the

Master of Public Health
Department of Environmental and Occupational Health
School of Community Health Sciences
Division of Health Sciences

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May 2011

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THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Deidre O. Turner

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**High-risk Human Papillomavirus (HPV): An Emerging Health Issue
for Women and Minorities**

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Master of Public Health

Department of Environmental and Occupational Health

Shawn Gerstenberger, Committee Chair

Karl Kingsley, Committee Co-chair

Mark Buttner, Committee Member

Michelle Chino, Committee Member

Chad Cross, Committee Member

Connie Mobley, Graduate Faculty Representative

Ronald Smith, Ph. D., Vice President for Research and Graduate Studies
and Dean of the Graduate College

May 2011

ABSTRACT

High-Risk Human Papillomavirus (HPV): An Emerging Health Issue for Women and Minorities

by

Deidre O. Turner

Dr. Shawn Gerstenberger, Examination Committee Chair
Professor of Public Health
University of Nevada, Las Vegas - School of Community Health Sciences

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

The human papillomavirus (HPV) is the cause of nearly all cases of cervical cancers worldwide. HPV viral DNA is found in more than 99% of cervical cancers. In addition to cervical cancer, HPV is also associated with some breast and oral cancers. White women have been showing a decline in breast cancer rates while black women are continuously showing higher rates of mortality from both breast and cervical cancer. Minority women are also more likely to receive a late diagnosis and are showing increased incidence of oral cancer, which makes study of HPV in women and minorities significant.

To date, little evidence has been provided to estimate oral HPV prevalence among healthy adults in the US. A few select international studies have evaluated HPV prevalence in healthy adults using biopsy samples and these results show HPV prevalence ranging from 0 – 15%. More recently, new international studies have begun to report less invasive saliva-based testing methods to successfully screen for oral HPV infection among healthy adults, revealing prevalence rates of approximately 20%. To

date, there are no reports of saliva-based HPV screening studies of normal, healthy adults, rather than oral cancer patients, to screen for oral prevalence in the U.S.

The goal of this study was to collect saliva from the University of Nevada Las Vegas – School of Dental Medicine (UNLV-SDM) patient clinic and screen for the presence of the high risk strain, HPV 16, found in the majority of HPV-associated cervical, breast, and oral cancers. Two hundred participants were asked to provide a saliva sample for HPV screening. Demographic information such as age, gender, and race were also obtained for statistical analysis. DNA was isolated from the saliva sample in order to perform PCR to screen for HPV 16.

Analysis of the UNLV-SDM patient population revealed a higher percentage of females and minorities than in the local community, Clark County. Analysis of the demographic information from the saliva samples revealed that these samples were representative of the UNLV-SDM patient pool. Four (4) samples tested positive for HPV16 (all from women and minority participants) from more than one hundred samples screened (n=102). Although the prevalence of HPV16 in this study was relatively low (3.9%), it is comparable to other studies of oral HPV (range 0 -21%). This study is significant because it is the first saliva-based oral HPV screening on healthy adults to be completed in the U.S. and only the third study of its kind overall.

Future studies might incorporate larger sample sizes and provide alternative sites for screening other at-risk populations.

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isolate DNA, perform PCR analysis, and run a gel electrophoresis. Thank you for never giving up on me, and most importantly, thank you for answering ALL of my many, many emails. You have been exceptional. Just think, I am a part of a study that has NEVER been done in the US AND we published an article.

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CHAPTER 1

INTRODUCTION

Purpose of the Study & Specific Aim

The overall purpose of this research project was to screen University of Nevada Las Vegas-School of Dental Medicine (UNLV-SDM) patients for oral HPV. UNLV-SDM, with more than 90,000 active patients, is uniquely positioned to perform oral screening and oral HPV testing among this patient population. A UNLV Office of Research Integrity – Human Subjects protocol (#1002-3361- *The Prevalence of Oral Human Papilloma Virus in the UNLV School of Dental Medicine Clinic Population*) has been approved to sample and study HPV infection among these patients.

Background & Significance

HPV Background

HPV is a non-enveloped DNA virus. There are greater than 100 types of HPV which are classified into low and high risk types. Low risk types of HPV are more commonly associated with warts while high risk types of HPV are associated with cancers. HPV is associated with many cancers, but for the purpose of this study, cervical, breast, and oral cancers will be discussed. This study specifically focuses on HPV 16, a high-risk strain, due to its presence in cervical, breast, and oral cancers. Even though almost everyone gets infected with HPV, the immune system can rid itself of papillomaviruses, but persistent viruses can develop into warts and even lead to cancer (Kersiek , 2008). HPV 16 is the most prevalent strain in oral and breast cancers (Kingsley et al., 2009).

Cervical Cancer

Cervical cancer is a disease that is both preventable and curable. The papanicolaou cytological test (Pap smear) is a screening tool used to detect abnormalities in the cervix of women. If cervical cancer is detected early it can be cured. Screening is imperative for all women, despite socioeconomic status. “Cervical cancer is a disease most frequently found in poverty-stricken communities and reflecting a problem of equity at both levels: gender and regional, and this is not only due to social and economic development inequalities, but also due to the infrastructure and human resources necessary for primary care” (Chankapa et al., 2011).

HPV is the cause for cervical cancer and has been linked to more than 99% of cervical cancers (Andrews et al., 2008). According to the Centers for Disease Control and Prevention (CDC), cervical cancer is the most common cancer associated with HPV (CDC, 2009). Cervical cancer is the second most common cancer in women worldwide (Verhoeven et al, 2009; Confertini et al., 2010). In the U.S., Black and Hispanic women are at a higher risk for cervical cancer (Downs, 2010). Moreover, Black women may not receive the best care available and therefore are more likely to die from cervical cancer (Coker et al., 2009). According to a meta-analysis based on over fifty studies, individuals with low socioeconomic status (SES) compared to individuals with a high SES had a two-fold increased risk for cervical cancer (Benard et al., 2008). Risk factors for cervical cancer include: age of first sexual contact, number of sexual partners, history of sexually transmitted disease, diet, and environment. Gillison and Shah (2003) suggested that the sexual practices of women, sexual practices of their male partners, and the standard of

health care, in regards to an effective screening program, are the major factors related to cervical cancer.

Breast Cancer

While breast cancer is the second most common cancer in the world, affecting one in eight women, it is the leading type of cancer affecting women worldwide (Shukla, 2009). HPV is relevant in breast cancer since high risk types of HPV have been seen in approximately 50% of breast cancer biopsies (Kingsley et al., 2009). Mammography is the most effective method for detecting cancer of the breast(s). Often times minority women do not get screened or are infrequently screened which can lead to a late diagnosis and mortality risk. Even though mortality rates for breast cancer have been steadily declining, rates for American Indian and Alaska Natives have been increasing (Engelman et al., 2011). Possible barriers that may prevent screening are fear of pain, beliefs, embarrassment, lack of insurance, and the ability to pay.

Oral HPV

Twenty five percent of oral cancers may be linked to HPV (CDC, 2009) with HPV 16 being the most predominant type detected in oral cancers. Risk factors for oral cancer may include: alcohol consumption, tobacco use, HPV, oral hygiene, diet, family history, age, gender, and race. The main risk factors are alcohol and tobacco use, which is a concern for minorities since minorities are more likely to use alcohol and tobacco products. Cigarette smoking was first identified in 1957 as an independent risk factor for oral and oropharyngeal cancer (Ragin et al., 2007). In essence, tobacco products were confirmed, along with alcohol, as the two major risk factors for developing oral and oropharyngeal cancers (Ragin et al., 2007). Furthermore, diets that do not meet the

appropriate levels for fruit and vegetable consumption are also related to the risk of developing oral cancer. Oral cancer is a major concern because the number of women with oral and pharyngeal cancers is expected to increase well beyond cervical cancer (Lyda, 2010).

Experimental Design

This study involves a prospective, cluster study design. This proposal involves using a cluster sample, located at the UNLV School of Dental Medicine. The investigators selected patients for participation based upon the following criteria. The inclusion criteria required participants to: be a current patient at UNLV-SDM; be over eighteen years of age; agree to participate; and provide Informed Consent. The exclusion criteria excluded participants under the age of 18 and those who did not wish to participate.

Research Questions and Hypotheses

1. How does the UNLV-SDM patient population demographics (gender, race, age) compare to the population of Southern Nevada (Clark County)?

Null hypothesis: There is no difference between UNLV-SDM and Clark County population demographics

Alternative hypothesis: There is a difference between UNLV-SDM and Clark County

2. How does the HPV screening sample demographics (gender, race, age) compare with the UNLV-SDM patient population?

Null hypothesis: There is no difference between HPV sample and UNLV-SDM population

Alternative hypothesis: There is a difference between HPV sample and UNLV-SDM

3. What is the prevalence of high-risk HPV in the sample population?

No hypothesis: There is no current information for comparison

4. How does this study compare with published oral HPV prevalence?

Null hypothesis: There is no difference between UNLV sample and other oral HPV studies

Alternative hypothesis: There may be a difference between UNLV sample and other oral HPV studies; although it is not known whether this will be higher or lower.

Predictions

It is predicted that there is a difference between UNLV-SDM demographics and Clark County; There is no difference between HPV sample and UNLV-SDM population; Since no current information is available, no comparison can be made on the prevalence of high risk HPV in the sample population; and there is a difference between UNLV sample prevalence and other oral HPV publications.

Preliminary Results

To answer the first research question, comparing the UNLV-SDM patient population demographics (gender, race, age) to the population of Southern Nevada (Clark County), US Census data were analyzed to determine the percentage of females, minorities and those within specific age ranges. These results demonstrate that the UNLV-SDM population was similar to Clark County with respect to gender, with approximately equal percentages of females and males (Table 1.1). More specifically, the percentage of females at UNLV-SDM was 50.6%, which is statistically higher than the percentage in Clark County 49.1%, although this makes little practical significance. The percentage of minorities, Blacks and Hispanics, were greater at UNLV-SDM (15.3% and 41.4%) than in Clark County (10.6% and 29.3%). UNLV-SDM also had higher percentages of patients 18-64 and 65+ (70.5% and 12.2%) compared with Clark County (63% and 10.7%). These data demonstrate that UNLV-SDM has a significantly different

composition of patients than the surrounding community, therefore the alternative hypothesis can be accepted.

Table 1.1 Demographic analysis of Clark County and UNLV-SDM

Variables	Clark County	Expected	Observed UNLV-SDM	<i>p</i> -value
<i>Gender</i>				
Female	N = 934,291 (49.1%)	(.491) 71,051 n = 34,886	50.6% n = 35,952	$X^2 = 61.3$ df = 1 p < 0.001
Male	N = 966,640 (50.8%)	(.508) 71,051 n = 36,093	49.4% n = 35,099	$X^2 = 61.3$ df = 1 p < 0.001
<hr/>				
<i>Race</i>				
White	N = 953,320 (50.1%)	(.501) 71,051 n = 35,597	40.8% n = 28,989	$X^2 = 2458.3$ df = 1 p < 0.001
Black	N = 201,700 (10.6%)	(.106) 71,051 n = 7,531	15.3% n = 10,871	$X^2 = 2458.3$ df = 1 p < 0.001
Hispanic	N = 557,530 (29.3%)	(.293) 71,051 n = 20,818	41.4% n = 29,415	$X^2 = 2458.3$ df = 1 p < 0.001
<hr/>				
<i>Age</i>				
18 - 64 years	N = 1,198,785 (63%)	(.63) 71,051 n = 44,762	70.5% n = 60,598	$X^2 = 6671.1$ df = 1 p < 0.001
65+	N = 190,283(10.7%)	(.107) 71,051 n = 7,602	12.2% n = 10,453	$X^2 = 6671.1$ df = 1 p < 0.001

The remaining three research questions were answered in a publication in the manuscript submitted to the journal BMC Oral Health, contained in Chapter 2.

CHAPTER 2

HPV SCREENING & DETECTION

This chapter has been submitted (and accepted) for publication to the peer-reviewed scientific journal BMC Oral Health and is presented in the style of that journal.

High-risk human papillomavirus (HPV) screening and detection in normal, healthy patient saliva samples: a pilot cluster randomized study.

Deidre O Turner¹, Shelley J Williams², Ryan Bullen³, Jeremy Catmull³, Jesse Falk³, Daniel Martin³, Jarom Mauer³, Annabel E Barber², Shawn L Gerstenberger¹, Karl Kingsley^{3*}.

¹ University of Nevada, Las Vegas – School of Community Health Sciences, Department of Environmental and Occupational Health, Las Vegas, Nevada, USA

² University of Nevada, Reno – School of Medicine, Department of Surgery, Las Vegas, Nevada, USA

³ University of Nevada, Las Vegas – School of Dental Medicine, Department of Biomedical Sciences, Las Vegas, Nevada, USA

Abstract

Background: The human papillomaviruses (HPV) are a large family of non-enveloped DNA viruses, mainly associated with cervical cancers. Recent epidemiologic evidence has suggested that HPV is an independent risk factor for oral cancers, suggesting HPV may modulate the malignancy process in some tobacco- and alcohol-induced oral cancers, but may also be the primary oncogenic factor for inducing carcinogenesis among non-smokers. Little evidence, however, is available regarding oral HPV prevalence among healthy adults in the US. The goal of this study was to perform a

non-invasive, saliva-based HPV screening of normal healthy adults to assess oral HPV prevalence.

Methods

Healthy, adult patients at a US dental school were randomly selected to participate in a clustered pilot study. DNA was isolated from saliva samples and screened for HPV16 using PCR. A small subset were subsequently screened using qPCR to confirm analytical sensitivity and specificity.

Results: Chi-square analysis revealed the random patient sample was representative of the general clinic population with respect to gender, race and age ($p > 0.05$). Four patient samples were found to harbor HPV16 DNA, representing 3.9% of the total ($n = 102$). Three of the four HPV16-positive samples were from patients under 65 years of age and all four were female and Hispanic.

Conclusions: The successful recruitment and screening of healthy, adult patients revealed HPV16 was present in a small subset of minority females. These results provide new information about oral HPV status, which may help to contextualize results from other studies that demonstrate oral cancer rates are increasing in the US among both females and minorities and in some geographic areas – and may be associated with risk factors other than tobacco and alcohol use. Although future studies may explore the role of other factors that influence oral HPV exposure, as well as the short- and long-term consequences of oral HPV infection, the results of this study may be of significant value to further our understanding of oral health and disease risk.

Background

The human papillomavirus (HPV) has been implicated as the cause of virtually all cervical cancers worldwide [1-3]. These represent a large family of non-enveloped DNA viruses that may be found integrated into the host genome, non-integrated or episomal, or as a combination or mixture of these types in infected tissues [4-9]. HPV viruses infect many types of epithelial cells, with intraepithelial neoplasias accounting for the overwhelming majority of HPV-related cancers [10,11].

More than one hundred types of HPV have been identified and classified. These HPV types may be the primary oncogenic or etiologic cause of cancer or are associated with other dermatologic disorders, including the development of warts [12,13]. The HPV strains determined to be oncogenic have been classified as high-risk, with HPV16 and HPV18 the most prevalent - accounting for the overwhelming majority of all HPV-associated cancers [14]. Other HPV strains, more commonly associated with genital and anal warts, or other skin and epithelial disorders, have been classified as low risk. These include HPV types 6 and 11, among many others [15]. Although the majority of these HPV strains were originally identified in cervical lesions, more recent evidence has demonstrated their presence in other tissues including colorectal, penile, breast, lung, and oral tissues [16-27].

Recent epidemiologic evidence has suggested that HPV is an independent risk factor for oral cancer, revealing HPV in three times as many pre-cancerous oral lesions, and almost five times as many oral cancers, compared with normal oral mucosa [28-30]. Although the traditional risk factors for developing oral cancer remain tobacco use and heavy alcohol consumption, these data suggest other risk factors, such as HPV, may play

significant roles in determining whether oral cancer develops and how quickly it may progress [31-34]. Of all HPV types, the high-risk strains HPV16 and HPV18 are the most commonly identified from biopsies of oral cancers [19-21], providing strong evidence that HPV may be an independent risk factor for oral cancer.

The role of HPV in the oral cavity, however, may differ by anatomic site and also by the particular strain of HPV infection [35]. For example, low-risk HPV strains 6 and 11 have been identified in benign laryngeal papillomas, common warts (*verruca vulgaris*), and condyloma acuminatum [36-38]. These strains have also been found in uncommon cancers, such as Ackerman's (verrucous carcinomas) [39] and Buschke-Lowenstein tumors [40]. Conversely, high-risk strains HPV-16, and to a lesser extent HPV-18, are found in nearly half of all oral squamous cell carcinomas and epithelial lesions [33,41,42].

The comparatively low presence of high-risk HPV in normal tissues and much higher prevalence in oral cancers may suggest that HPV preferentially infects already developing oral cancers [28-30]. HPV may then subsequently function to modulate the malignancy process in developing or establish oral cancers, as has been observed in studies of HPV infection in other developing cancers [23,43-45]. Recent epidemiologic and case-control studies have demonstrated that patients with HPV-positive tumors had significantly better response rates to chemotherapy and chemoradiation treatments when compared with HPV-negative tumors [28,46-48]. Several *in vitro* studies have investigated possible mechanisms that may account for these phenotypic changes in oral cancers [49,50]. Evidence that HPV infection in oral cancers correlates with increased

survival rates and better prognosis among some patients is now accumulating, possibly due to changes in cellular responsiveness [45,51,52].

Despite these findings, rates of oral cancer in the United States (US) have been rising among some subgroups within the population, and in specific geographic areas [34,53-56]. The steady decrease in the number of current smokers in recent years, combined with an ever-increasing percentage of never smokers in the US [33], suggests that other risk factors are likely responsible, in part, for these observed increases. One recent study found that although a majority of oral cancers in the US were linked with tobacco and alcohol consumption, a significant minority were not [57]. More importantly, this study found non-tobacco and non-alcohol related cases were six times more likely to harbor HPV infections than controls. Based upon this information, it is likely that HPV may modulate the malignancy process in some tobacco- and alcohol-induced oral cancers, thereby altering their phenotypes, but may also be the primary oncogenic factor for inducing carcinogenesis among non-smokers.

To date, little evidence has been provided to estimate oral HPV prevalence among healthy adults in the US. A few select international studies have evaluated HPV prevalence in healthy adults using biopsy samples, revealing prevalence rates that ranged from 0 – 15% [41,58-62]. More recently, new international studies have begun to report less invasive saliva-based testing methods to successfully screen for oral HPV infection among healthy adults, revealing prevalence rates of approximately 20% [63-65]. To date, there are few reports of saliva-based HPV screening studies of normal, healthy adults, rather than oral cancer patients, to screen for oral prevalence in the US. Based upon this information, the goal of this project was to perform a non-invasive, saliva-based HPV

screening of normal healthy adults. This screening was performed in Nevada, a state recently documented to have increasing rates of oral cancer – despite declining rates of oral cancer nationally and declining rates of tobacco and alcohol use in the state [34,56].

Methods

Human Subjects

The protocol for this study titled “The Prevalence of Oral Human Papilloma Virus (HPV) in the University of Nevada, Las Vegas - School of Dental Medicine (UNLV-SDM) Clinic Population” was filed, amended, and approved by the UNLV Office of Research Integrity – Human Subjects (OPRS#1002-3361) on April 9, 2010. In brief, subjects were recruited by members of the UNLV-SDM Clinic during their dental visit on one of fifteen (15) clinic dates. Informed Consent was required and was conducted onsite. Inclusion criteria: subjects had to be eighteen (18) years old or older and must agree to participate. Exclusion criteria: subjects younger than eighteen (18) years of age and subjects with prior diagnosis of oral cancer were excluded from participation.

Saliva Collection Protocol

In brief, healthy adults who agreed to participate were given a small saliva collection container, 50 mL sterile polypropylene tube, Fisher Scientific (Fair Lawn, NJ). Participants were then asked to chew on a small piece of paraffin wax for one (1) minute and then to expectorate. Each saliva sample was assigned a unique, randomly-generated number to prevent research bias. Demographic information regarding the sample was concurrently collected, which consisted of age, gender, and ethnicity only. Samples were stored on ice until transport to a biomedical laboratory for analysis.

DNA isolation and polymerase chain reaction (PCR)

To determine if any samples harbored the HPV virus, DNA was isolated from the saliva using the GenomicPrep DNA isolation kit (Amersham Biosciences: Buckinghamshire, UK), using the procedure recommended by the manufacturer. DNA from each sample was then used to perform PCR with the Fisher exACTGene complete PCR kit (Fisher Scientific: Fair Lawn, NJ) and a Mastercycler gradient thermocycler (Eppendorf: Hamburg, Germany) using the following primers for HPV16 and glyceraldehyde- 3- phosphate dehydrogenase (GAPDH), synthesized by SeqWright (Houston, TX):

HPV16 forward primer, ATGTTTCAGGACCCACAGGA;

HPV16 reverse primer, CCTCACGTCGCAGTAACTGT.

GAPDH forward primer, ATCTTCCAGGAGCGAGATCC;

GAPDH reverse primer, ACCACTGACACGTTGGCAGT;

One μg of template DNA was used for each reaction. The initial denaturation step ran for three minutes at 94°C . Thirty amplification cycles were run, consisting of 30 second denaturation at 94°C , 60 seconds of annealing at 58°C , and 30 seconds of extension at 72°C . Final extension was run for five minutes at 72°C . The PCR reaction products were separated by gel electrophoresis using Reliant 4% NuSieve® 3:1 Plus Agarose gels (Lonza: Rockland, ME). Bands were visualized by UV illumination of ethidium-bromide-stained gels and captured using a Kodak Gel Logic 100 Imaging System and 1D Image Analysis Software (Eastman Kodak: Rochester, NY).

Quantitative PCR assay

HPV16 primers were designed to amplify genomic DNA isolated from the saliva samples. The first pair targeted HPV16 in the E6 region, with the second pair targeting beta actin (β -actin). Real-time PCR conditions were conducted using the procedure recommended by the manufacturer. The DNA quantity based on HPV16 gene data was normalized using the DNA quantity of β -actin as a reference.

Statistical evaluation

Following the acquisition of saliva samples and HPV screening results, demographic information from each sample was compared with the overall demographic profile of the UNLV-SDM patient pool ($N = 71, 051$) using a chi-square (χ^2) test, to determine if any characteristic (gender, race, age) was different than expected among the patients evaluated in this study ($n = 102$). A probability level of alpha (α) = 0.05 was used to determine significance. Sensitivity and specificity were calculated as the proportion of true positives and true negatives (cutoff value >0.1 copies/genome), respectively.

Results

One hundred and fifty one (151) samples of saliva were collected from the UNLV-SDM patient clinic between June 2 and October 1, 2010. The patients from whom the randomly collected saliva samples were obtained were similar to the overall UNLV-SDM clinic population with respect to gender (Table 1). More specifically, the total number of females and males was roughly equal (52.3% and 47.7%, respectively) and not significantly different ($p > 0.05$). However, there were slightly more White patients in the study population (48.3%) than in the overall UNLV-SDM population

(40.8%), which approached statistical significance ($p < 0.1$, $p > 0.05$). In addition, there were also slightly fewer 18 – 64 year olds in the study population (80.8%) than in the overall clinic (85.3%), which also approached statistical significance ($p < 0.1$, $p > 0.05$).

DNA was successfully isolated from one hundred and two (102) of the collected saliva samples, which were subsequently screened for the presence of HPV-16 (Table 2). Of the total number tested, only four (4) patient samples were determined to be HPV-16 positive, which represented 3.9% of the total screened. All four samples were taken from females ($p < 0.01$) who were non-White ($p < 0.05$), a result significantly different than expected. Three of the four samples were from patients between 18 – 64 years of age, and one sample came from a patient over 65 years of age.

Ten HPV-negative samples were selected at random to be screened in duplicate, using quantitative PCR (qPCR) to provide quantitative assessment, as well as the four HPV-positive samples (Table 3). These data confirm the four HPV-positive samples were true positives, yielding normalized copy numbers significantly higher than previously established cutoff values (>0.1 copies/genome). In addition, all ten HPV-negative samples were confirmed to have detectable levels below this cutoff value (range: 0.0003 – 0.0000016 copies/genome). Using more stringent (>0.001 copies/genome) and less stringent (>0.1 copies/genome) cutoff values did not alter these results, providing evidence that no false positives or false negatives were among the qPCR-screened samples. The proportion of true positives (4/4 or 100%) and true negatives (10/10 or 100%) from this analysis suggested this analysis demonstrated sufficient sensitivity and specificity, respectively.

Graphic analysis of qPCR results revealed striking differences in HPV-16 copy numbers between HPV-negative and HPV-positive samples (Figure 1). Analysis of the range of copy number/genome for the housekeeping gene (β -actin) within HPV16-negative (range: 4 – 363 copies/genome) and HPV-positive samples (range: 75-1096) were similar and well above the cutoff value (>0.1 copies/genome). However, analysis of the range of copy number/genome for HPV-16 demonstrated clearly divergent values among the HPV16-negative (range: 0.0003 – 0.0000016 copies/genome) and HPV-positive (range: 70 – 111 copies/genome) samples, which were easily distinguished using the cutoff value (>0.1 copies/genome).

Discussion

The primary aim of this study was to perform a non-invasive, saliva-based HPV screening of normal healthy adults within the patient population at UNLV-SDM. More than one hundred samples were successfully collected and screened for HPV, revealing a prevalence rate in this population of 3.9% ($n = 102$). Secondary analysis and screening of these samples using qPCR demonstrated no false positives or false negatives, providing further quantitative evidence of sufficient sensitivity and specificity within these results.

These data suggest a prevalence rate that is slightly higher than the most recent evidence, which demonstrated oral HPV prevalence in a multinational study of healthy, cancer-free patients of approximately 1.3% ($n = 1680$) [66,67]. Over the past few decades, a few select international studies have also evaluated HPV prevalence in healthy adults using biopsy samples, which reported widely variable prevalence rates that ranged from 0 – 15% [41,58-62]. The few published reports to screen for oral HPV infection

among healthy adults using saliva-based testing methods, however, reported much higher prevalence rates of approximately 20% [63-65]. The results of this current study provide new data from a previously unscreened patient population (and geographic area) to complement the growing corpus of information about oral HPV prevalence among healthy adults.

This study also provides critically important information because it is among the first to evaluate the prevalence of oral HPV infection among a patient population in Nevada, one of the only US states to have increasing short-term oral cancer incidence and mortality rates [56]. Although an analysis of the primary risk factors for oral cancer revealed higher rates of tobacco use and smoking prevalence in Nevada than in neighboring states, these rates were found to be steadily decreasing over time [34] – suggesting other confounding variables or risk factors may also be important. Based upon this evidence, evaluation of other independent risk factors for developing oral cancer, including infection with high risk HPV, becomes crucial.

In addition, the results of this study found oral HPV infection only among patients who were also minority and female. Although the vast majority of female and minority patients in this study were found to have no evidence of oral HPV infection, recent epidemiologic studies have shown that rates of oral cancer haven risen sharply among females in the US despite declining rates among males [54]. Moreover, rates of oral cancer have also been rising among minority populations in the US [53], despite an overall decline among the general population, and the non-minority population, more specifically [34,56]. Although the sample size in this study is limited, the results suggest that further investigation may be warranted.

This study had several limitations to be considered. Although the study was able to recruit and screen a significant number of patients, due to the preliminary nature of this pilot study, the overall sample size was somewhat limited. Future studies that are able to allocate more significant time and resources, could significantly increase the overall sample size evaluated. In addition, detailed demographic and behavioral data were not designated as critical to the initial goals of this pilot study, however, the inclusion of smoking and tobacco use, as well as more detailed information about other behaviors, housing, education, income, and other socioeconomic indicators may provide additional insights for future investigations. Finally, screening for other high-risk HPV strains, including HPV18 or other oral infectious agents, may be possible in future studies with more significant resources and personnel.

Conclusions

The goal of this study was to evaluate prevalence of HPV16 from a patient sample in a pilot study at the UNLV-SDM. This study successfully recruited patients and screened samples that confirmed HPV16 was present in a small subset of the healthy, adult patients. Moreover, the patients with oral HPV16 infection were both female and minority. Although oral cancer has traditionally been associated with White males, recent studies have found that rates of oral cancer are increasing in the US among both females and minorities – and may be associated with risk factors other than tobacco and alcohol use. Although future studies may explore the role of other factors that influence oral HPV infections, as well as the short- and long-term consequences of oral HPV, the results of this study may be of significant value as other dental, medical, health care, and professional schools evaluate and integrate this evidence to further our understanding of

oral health and disease risk.

Abbreviations

Human papillomavirus (HPV); Deoxyribonucleic acid (DNA); United States (US); University of Nevada, Las Vegas - School of Dental Medicine (UNLV-SDM); polymerase chain reaction (PCR); glyceraldehyde-3- phosphate dehydrogenase (GAPDH); quantitative polymerase chain reaction (qPCR).

Competing interests

The authors declare they have no competing interests.

Author contributions

KK and SLG conceived, monitored, and coordinated the experimental design. RB, JC, JF, DM, and JM were responsible for recruiting patients, informed consent, collecting samples, and some biomedical analysis. DOT, KK, and SJW carried out the DNA extractions, PCR, and qPCR analysis. KK, SJW and DOT were responsible for the data analysis, as well as the writing and editing of this manuscript.

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Figures and Figure legends

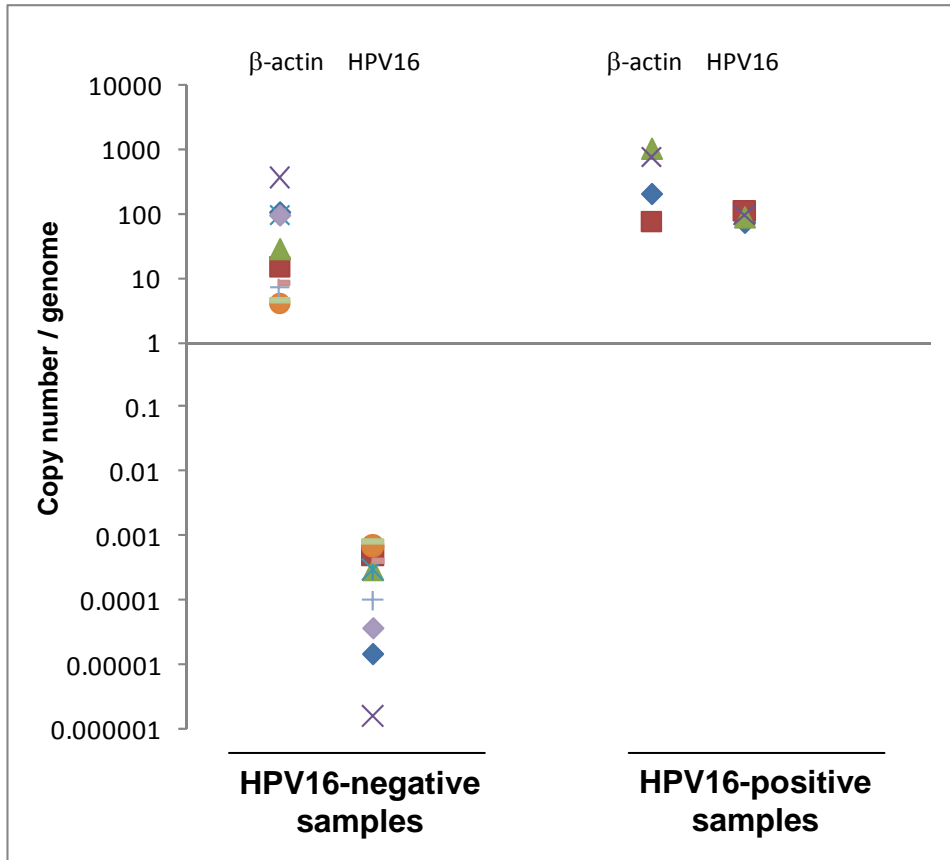


Figure 1. Graphic analysis of qPCR HPV screening results. Plotting of copy number/genome for housekeeping gene (β -actin) was similar from samples of HPV-positive (range: 75-1096) and HPV-negative samples (range: 4 – 363 copies/genome). Copy number/genome using qPCR was significantly above the cutoff value (>0.1 copies/genome), confirming the HPV-positive samples did harbor HPV DNA (range: 70 – 111 copies/genome). Values for HPV-negative samples were well beneath the cutoff value (range: 0.0003 – 0.0000016 copies/genome).

Tables

Table 1. Demographic analysis of study participants

Variables	UNLV-SDM	Study sample	<i>p</i> -value
<i>Gender</i>			
Female	n = 35,952 (50.6%)	n = 79 (52.3%)	<i>p</i> > 0.5
Male	n = 35,099 (49.4%)	n = 72 (47.7%)	
<i>Race</i>			
White	n = 28,989 (40.8%)	n = 73 (48.3%)	<i>p</i> > 0.10
Non-White	n = 42,062 (59.2%)	n = 78 (51.7%)	
<i>Age</i>			
18 - 64 years	n = 60,598 (85.3%)	n = 122 (80.8%)	<i>p</i> > 0.10
65 +	n = 10,453 (14.7%)	n = 29 (19.2%)	

Table 2. Analysis of HPV-16 DNA PCR screening

Variables	HPV-16 negative n = 98	HPV-16 positive n = 4
<i>Gender</i>		
Female	n = 58 (56.9%)	n = 4 (3.9%)
Male	n = 40 (39.2%)	n = 0 (0.0%)
<i>Race</i>		
White	n = 44 (43.1%)	n = 0 (0.0%)
Non-White	n = 54 (52.9%)	n = 4 (3.9%)
<i>Age</i>		
18 - 64 years	n = 81 (79.4%)	n = 3 (2.9%)
65 +	n = 17 (16.7%)	n = 1 (0.1%)

Table 3. Comparison of HPV screening methods

Sample ID	PCR screening	qPCR screening	True positive	False positive
2527	Positive (+)	70 copies/genome	+	-
2430	Positive (+)	111 copies/genome	+	-
2819	Positive (+)	87 copies/genome	+	-
2718	Positive (+)	96 copies/genome	+	-
Sample	PCR screening	qPCR screening > 0.001 copies/genome	True negative	False negative
2858	Negative (-)	0.0000148	+	-
2108	Negative (-)	0.0005	+	-
2424	Negative (-)	0.0003	+	-
2003	Negative (-)	0.0000016	+	-
2208	Negative (-)	0.0003	+	-
2739	Negative (-)	0.0007	+	-
2051	Negative (-)	0.00001	+	-
2809	Negative (-)	0.00004	+	-
2782	Negative (-)	0.00008	+	-
2821	Negative (-)	0.000036	+	-
Change in threshold value		True negatives	True positives	
Cutoff copies/genome > 0.1		10/10 (100%)	4/4	(100%)
Cutoff copies/genome > 0.01		10/10 (100%)	4/4	(100%)
Cutoff copies/genome > 0.001		10/10 (100%)	4/4	(100%)

CHAPTER 3

DISCUSSION & CONCLUSION

General Discussion

Socioeconomic status (SES) is a predictor of health which reflects social influence and individual factors, environmental exposures, stressors, and health behaviors in populations (Benard et al., 2008). SES includes income, education, occupation, location of residence, poverty level, and race. Disparities of race and gender are differences in patterns and exposure to risk factors. Many types of barriers to screening and education exist when health disparities are present.

The health belief model fits the realm of this study because it can help explain why one would or would not use available preventive services. In this case, the reasoning of why women and minorities infrequently receive pap smears, breast exams, or oral cancer screening is measurable. Perceived susceptibility, perceived severity, perceived benefits, and perceived barriers are the four constructs that outline the health belief model. Self-efficacy evaluates the individual's confidence in their ability to perform the action. The health belief model can assist in answering why a particular behavior occurs and identify ways to change the behavior. Perceived susceptibility asks the question whether women and minorities believe that they are at risk for developing HPV, cervical cancer, breast cancer, and even oral cancer. Perceived severity is whether at risk individuals are aware that HPV is the primary cause of cervical cancer, seen in 50% of breast cancer biopsies, and now seen in oral cancer patients. The perceived benefit of screening is potential early detection for cervical, breast, and oral cancers, thus

preventing cancer. Perceived barriers are what prevent at-risk individuals from getting screened.

Barriers include access to health care, refusal to access care, perceived risks, follow up diagnosis of cancer, cultural beliefs, and most importantly lack of communication and education regarding preventive care options. Down's et al., (2010) identified five barriers to health. These five include availability (volume and type of service); accessibility (location of service vs location of clientele); accommodation (ease of obtaining it); affordability (cost and perceived ability to pay); and acceptability (perceptions about practice characteristics). Minorities may be less likely to have health insurance, are likely to face screening barriers, and are less likely to receive treatment. Low income, low education, and being a minority woman are risk factors for developing HPV. Although access to health care may exist, it may be refused. Women in poverty tend to only access health care in urgent or emergency situations. In addition, minorities with a native language other than English may have difficulties communicating due to language barriers.

Healthy People 2020 identified disparity as a particular type of health difference that is closely linked with social, economic, and/or environmental disadvantage (Healthy People 2020). The CDC (2009) referred to health disparities as differences in health outcomes and their determinants between segments of the population, as defined by social, demographic, environmental, and geographic attributes. Health disparities are important indicators of community health and provide information for decision making and potential intervention strategies used to reduce preventable morbidity and mortality (CDC, 2009).

Health disparities need to be addressed in order to increase knowledge among women and minorities about risks, screening, treatment, and diagnosis. These communities need to be educated about HPV and its links to cervical, breast, and oral cancers and may be unaware of its association with different types of cancers. According to the CDC (2009), the Department of Health and Human Services has recognized cancer screening and management as one of six focus areas in which minorities face racial disparities in access to health and outcomes. “African American (Black) women are more than twice as likely to die of cervical cancer than white women and are more likely to die of breast cancer than are women of any other racial or ethnic group” (CDC, 2009).

Media, including television, radio, periodicals, and the internet, are potential sources of information, but more specific information and awareness projects could be conducted to improve health literacy and health education for women and minorities. Media and public health campaigns, for example, might consider inclusion of several types of information, including some background information about HPV, modes and methods of transmission, prevention strategies, treatment, as well as the risk of developing cancer. In addition, individuals also need to be informed that HPV can be asymptomatic. Anhang et al., (2004) suggested that six points be emphasized when providing pertinent information about HPV to individuals. The six points included: (1) HPV is transmitted sexually; (2) HPV is very common; (3) most women with HPV will not develop cervical cancer; (4) HPV’s most common prognosis is infection clearing itself prior to treatment; (5) purpose of a Pap smear is to detect HPV-related lesions in the cervix suggestive of precancerous or cancerous conditions; and (6) most women who test positive for high risk HPV will not be diagnosed with cervical cancer or a precursor on

further evaluation (Anhang et al., 2004). In Healthy People 2020 (2010), the goal for health disparities is to achieve health equity, eliminate disparities, and improve health of all groups (Healthy People 2020, 2010).

As the principal investigator of this project, HPV became an interesting and important topic following the presentation of Dr. Karl Kingsley's group (class project) on immunizations. Although some prior knowledge and background has been circulated through commercial advertisement for the new HPV vaccine, Gardasil, little information regarding any other aspects of HPV has been available from the popular media and press. The presentation advised us of the links that HPV had to cervical, breast, and oral cancers and how minorities and women were most affected. As a woman and a minority, these issues represent distinct priorities. Based upon a first-hand experience, it may be a strong possibility that these at-risk communities have similar barriers to information. As a public health graduate student, an addition goal for this paper may be to serve as an educational resource for women and minorities with respect to HPV, its risks, and prevention.

Although there are many issues regarding HPV and women/minority health (cervical, breast, oral health), as the principal investigator, there was a major opportunity to take part in one specific project that targeted oral HPV. This allowed the chance to explore a population that is representative of minorities and females while discovering something new that nobody else has done in the US, an oral HPV screening of healthy adults in the US.

Minorities tend to be underrepresented in research studies, possibly due to barriers such as fear, lack of trust, and lack of education. Overcoming these barriers could allow minorities to participate in more research studies allowing researchers to establish

community specific education and awareness. Eliminating barriers could improve screening and lower morbidity and mortality rates in minorities and women. Screening is a preventive measure that can lower incidence rates in minorities and women and extend their duration of life. Screening, education, and eliminating barriers are essential for lowering risks in women and minority patients.

Future studies

Future studies conducted between the School of Community Health Sciences and Dental Medicine should continue the oral HPV screening while testing for HPV 18, another high risk strain, in addition to HPV 16. Also, a learning module should be created for females and minorities to educate about HPV and its relation to cervical, breast, and oral health. In addition, future students should see how many UNLV-SDM patients (female) have pap smears and dental screenings (male/female) regularly.

Conclusions and Significance

Analysis of the UNLV-SDM patient population revealed a higher percentage of females and minorities than in the local community, Clark County. Analysis of the demographic information from the saliva samples revealed that these samples were representative of the UNLV-SDM patient pool. Four (4) samples tested positive for HPV16 (all from women and minority participants) from more than one hundred samples screened (n=102). This study is significant because it is the first saliva-based oral HPV screening on healthy adults to be completed in the U.S. and only the third study of its kind overall.

In conclusion, although this study provides valuable information, it also had several limitations. These include the limited screening for only one HPV type, HPV16.

HPV16 is the most common HPV strain found in the oral cavity and was therefore selected for this pilot study, although future studies could evaluate other high-risk HPV strains, such as HPV18. In addition, detailed demographic information were not obtained in this study, although future studies could evaluate other demographic variables, including tobacco and alcohol use, education, income, and even knowledge of HPV. Even though the prevalence of HPV16 in this study was relatively low (3.9%), it is comparable to other studies of oral HPV (range 0 -21%). Future studies might incorporate larger sample sizes and provide alternative sites for screening other at-risk populations.

APPENDIX
IRB APPROVAL



Biomedical IRB – Expedited Review Approval Notice

NOTICE TO ALL RESEARCHERS:

Please be aware that a protocol violation (e.g., failure to submit a modification for any change) of an IRB approved protocol may result in mandatory remedial education, additional audits, re-consenting subjects, researcher probation suspension of any research protocol at issue, suspension of additional existing research protocols, invalidation of all research conducted under the research protocol at issue, and further appropriate consequences as determined by the IRB and the Institutional Officer.

DATE: April 9, 2010
TO: Dr. Karl Kingsley, School of Dental Medicine
FROM: Office of Research Integrity - Human Subjects
RE: Notification of IRB Action by Dr. Charles Rasmussen, Co-Chair **CRIC**
Protocol Title: **The Prevalence of Oral Human Papilloma Virus (HPV) in the UNLV School of Dental Medicine Clinic Population**
Protocol #: 1002-3361

This memorandum is notification that the project referenced above has been reviewed by the UNLV Biomedical Institutional Review Board (IRB) as indicated in regulatory statutes 45 CFR 46. The protocol has been reviewed and approved.

The protocol is approved for a period of one year from the date of IRB approval. The expiration date of this protocol is March 30, 2011. Work on the project may begin as soon as you receive written notification from the Office of Research Integrity - Human Subjects (ORI - Human Subjects).

PLEASE NOTE:

Attached to this approval notice is the official **Informed Consent/Assent (IC/A) Form** for this study. The IC/A contains an official approval stamp. Only copies of this official IC/A form may be used when obtaining consent. Please keep the original for your records.

Should there be any change to the protocol, it will be necessary to submit a **Modification Form** through ORI - Human Subjects. No changes may be made to the existing protocol until modifications have been approved by the IRB.

Should the use of human subjects described in this protocol continue beyond March 30, 2011 it would be necessary to submit a **Continuing Review Request Form** 60 days before the expiration date.

If you have questions or require any assistance, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 895-2794.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway • Box 451047 • Las Vegas, Nevada 89154-1047

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VITA

Graduate College
University of Nevada, Las Vegas

Deidre O. Turner

Degrees:

Bachelor of Science, Health Science 2006
California State University Dominguez Hills

Special Honors and Awards:

Diversity City Media Scholarship Recipient
Edwin Harris Memorial Scholarship Recipient
Daniel L. Morgan Scholarship Recipient
American Legion Scholarship Recipient

Publications:

Turner DO, Cocks SW, Bullen R, Catmull J, Falk J, Martin D, Mauer J, Barber AE, Gerstenberger SL, Kingsley K. High-risk human papillomavirus (HPV) screening and detection in patient saliva samples: a pilot study. *Infectious Agents and Cancer*.

Thesis Title: High-risk human papillomavirus (HPV): An emerging health issue for women and minorities

Thesis Examination Committee:

Chairperson, Shawn Gerstenberger, Ph.D.
Co-Chairperson, Karl Kingsley, Ph.D.
Committee Member, Mark Buttner, Ph.D.
Committee Member, Michelle Chino, Ph.D.
Committee Member, Chad Cross, Ph.D.
Graduate Faculty Representative, Connie Mobley, Ph.D.