The Effective Clinical Detection of Oral HPV in the Prevention of HPV Related Head and Neck Malignancies

Martin G. deGroot
Pacific University
The Effective Clinical Detection of Oral HPV in the Prevention of HPV Related Head and Neck Malignancies

Abstract

Background: Oral malignancies currently constitute the sixth most common malignancy globally. Recent studies have shown strong correlation between Human Papillomavirus (HPV) and head and neck cancers, indicating that approximately 50% of oropharangeal cancers in the western world can be attributed to HPV. Furthermore, the relationship between high risk HPV and head and neck squamous cell carcinoma (HNSCC) is strengthened by an approximately 14 fold increase in risk for individuals who are infected with HPV 16. Yet the implementation of a well-studied, effective clinical screening tool in the detection of oral HPV has not been established. The purpose of this systematic review is to investigate different screening methods for detection and typing of oral HPV and to establish the need for a large randomized control study to create new guidelines by which HPV screening can be initiated.

Methods: An exhaustive literature search was performed using MEDLINE, CINAHL, and Web of Science databases using the following search terms “human papillomavirus” or “human papillomaviruses,” combined with “oral mucosa” or “oral malignancies” limited to publications in English performed on humans. Excluded were studies that focused on HIV positive or immunocompromised patients, studies that focused on DNA processing, systematic reviews. There were no exclusions made by GRADE criteria.

Results: The two studies presented in this systematic review have showed that mouth rinse and superficial scrapings are more effective than biopsies in the harvesting of oral DNA and in the detection of HPV in the oral cavity.

Conclusion: Repeated, definitive, single protocol studies have yet to determine which technique is most accurate for the harvesting of oral DNA in the detection of HPV, but all studies presented in this systematic review showed that mouth rinse and superficial oral scrapings were more effective than biopsies.

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The Effective Clinical Detection of Oral HPV in the Prevention of HPV Related Head and Neck Malignancies

Martin G. deGroot

A Clinical Graduate Project Submitted to the Faculty of the School of Physician Assistant Studies Pacific University Hillsboro, OR For the Masters of Science Degree, August, 2012

Faculty Advisor: Mary Von
Clinical Graduate Project Coordinator: Annjanette Sommers MS, PA-C, MS
Biography

Martin G. deGroot was born and raised in Glide, Or where he spent the majority of his time rummaging the North Umpqua River in search for swimming holes and Steelhead. He graduated Glide High School in 1999, a three sport all-state athlete and continued his sports career at Southern Oregon University in Ashland, Or. After three years of undergraduate work at SOU he decided that the mountains of Montana was where he wanted to finish his undergraduate study. Amidst trout fishing, raft guiding, skiing, and playing bluegrass music in Bozeman, Mt he was somehow able to complete his undergraduate degree in Health Education/Community Health at Montana State University.

He moved back to Portland in 2010 to attend Pacific University and pursue his lifelong passion for medicine. In addition to family health, he has special interest in preventative medicine and lifestyle modification in the approach to disease.
Abstract

**Background:** Oral malignancies currently constitute the sixth most common malignancy globally. Recent studies have shown strong correlation between Human Papillomavirus (HPV) and head and neck cancers, indicating that approximately 50% of oropharangeal cancers in the western world can be attributed to HPV. Furthermore, the relationship between high risk HPV and head and neck squamous cell carcinoma (HNSCC) is strengthened by an approximately 14 fold increase in risk for individuals who are infected with HPV 16. Yet the implementation of a well-studied, effective clinical screening tool in the detection of oral HPV has not been established. The purpose of this systematic review is to investigate different screening methods for detection and typing of oral HPV and to establish the need for a large randomized control study to create new guidelines by which HPV screening can be initiated.

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**Conclusion:** Repeated, definitive, single protocol studies have yet to determine which technique is most accurate for the harvesting of oral DNA in the detection of HPV, but all studies presented in this systematic review showed that mouth rinse and superficial oral scrapings were more effective than biopsies.
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To the love of my life, Lindsay deGroot: the constant love and support that you share is immeasurable. To have shared this journey with you is a true blessing and I am grateful to have you in my life. Thank you for putting up with me.

To my parents: If it weren’t for you I wouldn’t be who I am today. Thank you for always supporting me in the decisions that I have made, and giving me the guidance to succeed.

To my brothers: Thank you for blazing the trail for me. I am blessed to have such amazingly talented siblings who will continue to do wonderful things in this world. You both have been amazing role models and I will continue to look up to you even though you are shorter than me.
# Table of Contents:

- Biography .................................................................................................................. 2
- Abstract .................................................................................................................... 3
- Acknowledgements .................................................................................................. 4
- Table of Contents .................................................................................................... 5
- List of Tables ........................................................................................................... 6
- List of Abbreviations ............................................................................................... 6
- Background ............................................................................................................. 7
- Methods ................................................................................................................... 12
- Results ..................................................................................................................... 12
- Discussion ............................................................................................................... 16
- Conclusion ............................................................................................................... 18
- References ............................................................................................................. 19
- Tables ..................................................................................................................... 22
List of Tables:

Table 1: GRADE summary of articles reviewed

Figure 1: DNA yield for Lawton et al study

Figure 2: HPV detection percentages for the Lawton et al study

List of Abbreviations:

HPV...............................................................Human Papillomavirus

SCC.............................................................Squamous Cell Carcinoma

HNSCC.................................Head and Neck Squamous Cell Carcinoma

CDC...........................................................Centers for Disease Control

FDA .........................................................Food and Drug Administration
The Effective Clinical Detection of Oral HPV in the Prevention of HPV Related Head and Neck Malignancies

BACKGROUND

Currently, oral malignancies constitute the sixth most common malignancy globally. More than 90% of these malignancies are found to be squamous cell carcinoma (SCC). Recent studies have shown strong correlation between Human Papillomavirus (HPV) and head and neck cancers, indicating that approximately 50% of oropharangeal cancers in the western world can be attributed to HPV. The Center for Disease Control (CDC) reports that every year in the United States 1500 women and 5600 men get HPV-associated oropharyngeal cancers (cancers of the back of throat including base of tongue and tonsils), the majority of which are squamous cell carcinoma.

HPV belongs to the Papillomaviridae family, small non-enveloped DNA virus that are known collectively as papillomaviruses. Several hundred genotypes have been recognized differing slightly in their DNA sequence in specific genomic regions. Papillomaviruses have been found to exclusively infect epithelial cells primarily in humans, with each genotype affecting a different epithelial surface that can either be mucosal or cutaneous. For example HPV 1 will primarily affect the soles of the feet while HPV 2 will affect the palms of the hands and the soles of the feet. The superficial cutaneous types of HPV that are commonly found to be associated with squamous cell carcinoma of the skin are HPV 5 and 8. Mucosal types of HPV are subdivided into two categories, low-risk and high-risk in relation to the potential oncogenic properties of
the genotype. The most common low risk types have been shown to be HPV 6 and 11 which are responsible for many mucosal benign lesions. The most common carcinogenic types of HPV include, HPV 16, 18, 31,33,and 45 which have been primarily found in squamous cell carcinoma of the cervix, anogenital malignancies, and head and neck carcinoma.\textsuperscript{14-17} These same high-risk strains of HPV have been targeted in recent vaccines that have been approved by the Food and Drug Administration (FDA).

Human papillomavirus infection is the most commonly diagnosed sexually transmitted disease in the United States.\textsuperscript{33} Risk increases with multiple sexual partners, and individuals with a compromised immune system. It has been found to be associated with condyloma acuminatum, squamous intraepithelial lesions and anogenital malignancy including cervical, vaginal, vulval, penile, and anal carcinoma. The majority (75-80\%) of sexually active individuals will acquire a genital tract HPV infection at some point in their lives.\textsuperscript{34} Most of the physical manifestations are found in cutaneous warts (hand and plantar), although a percentage of young adults will acquire mucosal types by sexual contact.\textsuperscript{9} Consequently, the majority of HPV infections go unnoticed and the infected individual has no indication that there has been a viral invasion. Like most viral infections, a healthy immune system will suppress the HPV virus. Fortunately, 80\% of all HPV infections will spontaneously resolve.\textsuperscript{9} More research is needed to determine why some HPV viruses will spontaneously resolve and why some progress to cervical cancers and HNSCC.

The correlation between HPV and cervical cancer has become widely accepted, not only in the medical community but in the general population. Therefore standardized
screening guidelines have been established for the effective detection of HPV of the cervix with high detection rates of cervical cell dysplasia. The CDC reports each year, about 12 000 women get cervical cancer in the U.S.\textsuperscript{35} High-risk HPV types have been identified as a significant risk factor for cervical cancer. HPV is detected in 95-100% of invasive cervical cancers.\textsuperscript{23} The FDA has approved testing for high-risk HPV DNA in conjunction with Pap smears for routine cervical screening of women aged 30 years and older. A negative HPV DNA test increases assurance that there is very little risk of a serious abnormality developing over the next several years. The US Preventative Services Taskforce reports that cervical cancer rates in the United States have decreased from 14.2 new cases per 100 000 women in 1973 to 3.0 cases per 100 000 women in 1998.\textsuperscript{28} Despite the falling incidence, cervical cancer remains the tenth leading cause of cancer death.\textsuperscript{28}

Given the presence of HPV is necessary for the development of most cervical cancer, it is likely that it is important in the oral cavity as well. This association is strengthened by the fact that it is the same HPV oncogenes (16, 18, 31, 33 and 45) that cause cervical carcinoma which are found to cause the majority of malignancies in the oral cavity.\textsuperscript{2} HPV 16 has been identified as the most common type that has been found in HNSCC, representing >95% of all HPV positive SCC.\textsuperscript{3} Other high-risk HPV types (18,31,33, and 45) represent the remaining 5%-10%. Furthermore, the relationship between high risk HPV and head and neck squamous cell carcinoma (HNSCC) is strengthened by an approximately 14 fold increase in risk for individuals who are infected with HPV 16.\textsuperscript{4}
In the past, health professionals and researchers have believed the etiology of SCC to be primarily due to exposure to tobacco and alcohol. This carcinogenic exposure currently accounts for a 4-6 fold increase in the prevalence of oral SCC. (10) However, as the rates of smoking continue to decline in the United States, the rates of oropharyngeal cancer have risen 3% annually from the years 1997-2003\textsuperscript{5} especially in young men, non-smokers and non-drinkers.\textsuperscript{25-27} This evidence shows that these risk factors may be independent of each other. It has been reported that 15-20% of all individuals who have HNSCC have never, or rarely been exposed to these carcinogens.\textsuperscript{6,7} With this changing demographic there is less specific relevant data to guide clinicians in making a diagnosis of HNSCC. In the past, a clinical history of chewing or smoking tobacco or heavy drinking would be an indication for concern regarding oral cancer. Oral HPV commonly presents without physical symptoms; as a result, clinical diagnosis is very difficult to make. Currently, clinicians are without guidelines to determine which patients need to be screened for HNSCC and early detection is vital to determining the mortality of these individuals.

Head and neck carcinomas are associated with significant morbidity and disfiguration which makes early detection of oncogenic viruses of great importance. A recent meta analysis\textsuperscript{8} found that there is a better prognosis for those who have HNSCC associated with HPV as opposed to HPV negative cancers. Knowing the better prognosis for these patients, oncologists are more recently implementing surgical treatment and observation only for patients with HPV positive HNSCC. Oncologists are taking into consideration the younger demographic that this disease is affecting and deciding that the side effects of radiation and chemotherapy create greater long-term health implications than the disease
itself. Patients with HPV positive tumors respond well to radiation and chemotherapy ultimately leading to lower risk of mortality (meta HR, 0.85; 95% CI, 0.7-1.0) and decreased recurrence rates (meta HR, 0.62; 95%CI, 0.5-0.8).⁸

Interestingly, HPV 16 is responsible for 28% of oral HPV infections while oropharangeal cancers caused by HPV 16 is approximately >95%.³ A recent study reported HPV16 accounts for approximately 25% of cervical infections among cytologically normal women.¹⁸ Furthermore, cervical cancers associated with HPV 16 are approximately 50%.²¹ This data suggests that HPV 16 in the oral cavity has a greater potential to turn malignant when compared to cervical cells.

Within the past twenty years there has been an unmistakable reduction in cervical cancer incidence, and mortality due to screening guidelines and treatment protocols. HPV should be considered a biomarker for head and neck squamous cell carcinoma, and it is clear that malignancies of the oral cavity would benefit equally from a similar course of action.

**Purpose**

The purpose of this systematic review is to investigate different screening methods for detection and typing of oral HPV, and to establish the need for a large randomized control study to create new guidelines by which HPV screening can be initiated.

**Clinical Question**

The clinical question that this systematic review would like to address is whether or not there is a Gold Standard screening method established in the detection of HPV in the oral cavity.
METHODS

An exhaustive literature search was performed using MEDLINE, CINAHL, and Web of Science databases using the following search terms “human papillomavirus” or “human papillomaviruses,” combined with “oral mucosa” or “oral malignancies” limited to publications in English performed on humans. Excluded were studies that focused on HIV positive or immunocompromised patients, studies that focused on DNA processing, systematic reviews. There were no exclusions made by GRADE criteria.

RESULTS

Results of the online search brought forth 103 possible journal articles. After reviewing the titles and abstracts of these articles, 15 were identified that appeared to assess the comparison of different cell harvesting techniques in the oral mucosa and the detection of HPV. After further review, two studies remained and are evaluated in this systematic review. A summary of these studies can be found in Table 1.

Lawton et al

Lawton et al\textsuperscript{29} explained that no studies had previously addressed the establishment of a standardized method for the harvesting of oral mucosal cells, therefore the purpose of this study was to compare three different methods for cell collection (mouth rinse, biopsy and superficial scrapings).\textsuperscript{29}

All 60 participants who enrolled in the study\textsuperscript{29} volunteered from the dental school at the University of Queensland, Australia. The participants were consenting adults attending dental extractions that had otherwise had normal oral mucosa with no complaints of oral lesions or leukoplakia.\textsuperscript{29}
Specimen Collection- One investigator performed all of the specimen collection. Specimens were collected by standardized method using a sterilized spoon shaped spatula and each mucosal site was scraped three times. The total number of scrapings collected were 53. Care was taken to avoid cross contamination. The specimen was placed in a transport medium and incubated at 37 degrees Celsius overnight. Biopsies were taken from the full thickness of the buccal mucosa and placed in the transport medium and stored at 37 degrees Celsius overnight. The total number of biopsies collected was 59. Participants were then asked to return for a follow up appointment to collect the mouth rinse samples. Of the total participants, 49 subjects returned and were asked to rinse 10ml of 3% sucrose solution for 30 seconds. These samples were subject to centrifuge for 10 min at 2000rpm and placed in the same transport medium and stored at 37 degrees Celsius.

DNA Extraction- DNA was purified by sequential phenol/chloroform extraction and salt/ethanol precipitation using automated 340A Nucleic Acid Extractor (Applied Biosystems Inc, Foster City, CA). HPV DNA amplification products were detected by dot/blot hybridization.

Results- The average DNA yield from the specimens concluded that the mouthwashes produced more DNA (median 15.5ug; range 1.0-149.8) than the biopsies taken (median 8.0ug; range 0.8 – 74.3) or superficial scrapings (median 3.1ug; range 0 – 74.3). This information is represented in Figure 1. There were 49 subjects that provided adequate mouthwash samples and 25 (51%) were shown to be HPV DNA positive by this technique. Fifty three subjects provided samples of oral superficial scrapings of these samples 24 (45%) were shown to be HPV DNA positive. Finally, of the biopsies only 7
(12%) were positive for HPV. HPV was significantly less likely to be detected using a biopsy when compared to each of the other two techniques (p<0.001). This information is represented in Figure 2. 29

**Furrer et al**

Furrer et al 24 focused on the specificity and sensitivity of biopsies, superficial scrapings, and physician’s clinical presumption in the detection of oral HPV. The intention of their study was to identify clinical features of HPV infection in the oral mucosa in potentially malignant and malignant lesions, in order to assist the clinician in the diagnosis of potentially malignant lesions that could evolve into HNSCC or verrucous carcinoma (VC). 24

Biopsies and cytological scrapings were collected from 59 consenting adults. The case participants included 33 patients with oral lesions that were either potentially malignant or malignant lesions. All potentially malignant lesions were considered, leukoplakias, plaques as well as atrophic lichen planus. Malignant lesions included previously diagnosed oropharangeal squamous cell carcinoma and verrucous carcinoma. The patients, before being selected for the case group were diagnosed clinically and by histopathology. The control group included 23 patients that had clinically normal oral mucosa. Clinical diagnosis of oral HPV infection was considered if the patient presented with bright, white, flat lesions, that showed slightly elevated plaques or patches with an erithematous base. 24

**Specimen Collection**- Samples were collected in the oral mucosa and contra lateral mucosa by a sterile metallic spatula. Each site was scraped five times and caution was taken to not cross contaminate between sites. Individual samples were analyzed
using the PCR-Southern Blot genotyping for cytological diagnosis. Each sample was stored in a transport medium at -80 degrees Celsius. 24

Keeping in mind the stated purpose of the study 24 was to evaluate the effectiveness of non-invasive sampling techniques, biopsies were only taken from patients with suspected malignant and malignant lesions. Each biopsy sample was taken from the clinically suspicious tissue of the oral mucosa, taking the full extension of the lesion. Biopsy samples were analyzed by PCR-Southern blot genotyping and also histopathological examination. 24

Results- Biopsies from nine patients (41%) with potentially malignant and malignant lesions were HPV positive by PCR-Southern blot analysis in the biopsy tissue. On the other hand, 21 (95%) and 22 (100%) of these lesions were HPV positive when superficial scrapes of the lesion's mucosa and the contralateral normal mucosa were, respectively, analyzed. There was no significant difference in HPV detection between lesion and contralateral normal mucosa scrapes (McNemar, \( P = 0.3938 \)), but there was a significant difference between biopsies and superficial scrapes either from the lesion or the contralateral normal mucosa (McNemar, \( P < 0.0001 \)). It is important to point out that \( \beta \) globin gene was normally amplified from all negative biopsy samples. Biopsy DNA (median 14 ng) was between four and 2000 times higher than scraping DNA (median 0.27 ng). In addition, the sensitivity of the PCR–Southern blot assay established for Hela cells was around 0.155 pg of Hela DNA. Hence, the lower HPV detection in biopsy tissues was not due to insufficient amount of DNA. 24

Of the potentially malignant and malignant lesions that were identified by clinical criteria, 67% were found to be HPV positive by either cytopathology or
histopathology. There were significant differences between cytology results and the clinical presumption results and the histopathological results.²⁴

Of the 23 control subjects, 91.3% were HPV negative, 4.3% were HPV 18 positive and 4.3% were HPV positive for other genotypes. Of the 18 subjects with previously confirmed oral malignancy, all were HPV positive with the largest representation of the infections being HPV 16 strain (39%).²⁴

**DISCUSSION**

The two studies presented in this systematic review have shown that mouth rinse and superficial scrapings are more effective than biopsies in the harvesting of oral DNA and in the detection of HPV in the oral cavity.²⁴,²⁹ Furrer et al.²⁴ concluded that the ability to detect HPV DNA in the oral mucosa is much more likely by superficial scrapings than biopsies. These findings are in agreement with Lawton et al.²⁹ who further concluded that oral mouth rinse is superior to biopsies and superficial scrapings in HPV detection in the oral cavity.

These results are in agreement with findings in a study performed by Syrjanen and Syrjanen on 102 cervical biopsies known to contain HPV. This study demonstrated that the presence of HPV was much more superficial than once believed. Showing that HPV in the epithelial layers increased progressively from the basal layer (5.8%) to the superficial layers (100%). This is the reason that pap testing is done with a cytological brushing of cervical mucosa and not biopsies.

In order to assess the quality of research GRADE criteria were used. Both studies initially received a moderate grade for being observational studies at their beginning and not randomized control trials. A point in both studies was subtracted due
to the process not being blinded. Another point was taken off for inconsistent results. One more point was subtracted from each study due to small sample size and lack of precision. This warrants a very low quality of evidence for recommendation for this specific intervention. According to the GRADE criteria, very low-grade means “signifies the effect is uncertain.” Future studies would have to be large randomized studies that are assessing one universal screening procedure to show a true benefit of this screening tool.

Validity

The lack of randomized control trial in the studies reviewed resulted in compromised validity. GRADE assessment performed on both studies\textsuperscript{24,29} determined, due to a total sample size is being lower than optimal, there was significant lack of precision. In turn this made it difficult to determine if the results would be reproducible in a clinical setting.

Limitations of the Study

Of the presented studies, limitations included the lack of large multi-centered randomized control trial, which would have given a larger sample size, and a more diverse population and reduce the risk of bias.

There were variances in the way that each study analyzed the collected sample of DNA. Lawton et al\textsuperscript{29} used a Dot/Blot hybridization that uses a specific standardized protocol for DNA amplification whereas Furrer et al\textsuperscript{24} used a Southern Blot hybridization that implements an entirely different method. One universal DNA analysis technique will reduce the variability of results. DNA amplification techniques have been studied to a large extent in the detection of cervical HPV and the prevention of cervical cancer.
Unfortunately, there has yet to be a well-established gold standard for the analysis of oral HPV. Without a well-studied gold standard for testing it makes the introduction and comparison of new testing modalities difficult.

**Recommendations**-

It is clear that more information is needed to make clinical recommendations that would be accepted in the medical community, but this is one step in the determination of these recommendations. A large multicentre randomized control study is essential in the development of these guidelines. This can be accomplished simply due to the fact that the population that is being studied is the general population, and this would be an easily administered mouthwash collection that is cheap, and non-invasive.

**CONCLUSION**

The studies presented show that the harvesting of oral mucosa from biopsies are less accurate in detecting the HPV virus when compared to superficial scrapings and mouthwashes. A simple oral mouth rinse given in the office to collect DNA samples would be an easily administered, inexpensive, non-invasive screening method for oral HPV detection for individuals who present with oral leukoplakia, potentially malignant lesions or have concerns regarding oral health. Furthermore, with this information clinicians and researchers will be able to track individuals who have a known high-risk oral HPV infection and collect data on progression of oral HPV to HNSCC. Although more information is needed to make clinical guidelines for oral HPV screening, there is a large potential for reduction in the incidence of and mortality from HNSCC.
References:


### Table 1: GRADE Assessment

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| *Small sample size used in this trial.

### Figure 1: Average DNA Yield (shown in ug)

![DNA Yield Chart](chart.png)
Figure 2: Percentage of HPV Detection

- HPV 16
- HPV 18
- HPV 31
- HPV 33
- HPV (any type)

Scraping (n=53)
Biopsy (n=59)
Mouthwash (n=49)