SEPTEMBER 2011 | VOLUME 4 No. 4 | Quarterly Publication

➤ ***Research & Development***
Cervical Cancer & HPV Infection
Continued ..... pg 2

➤ ***Journal Watch***
Summaries of recent topical publications in the medical literature
Full Article ..... pg 5

The Laboratorian SM

Cervical Cancer and HPV Infection

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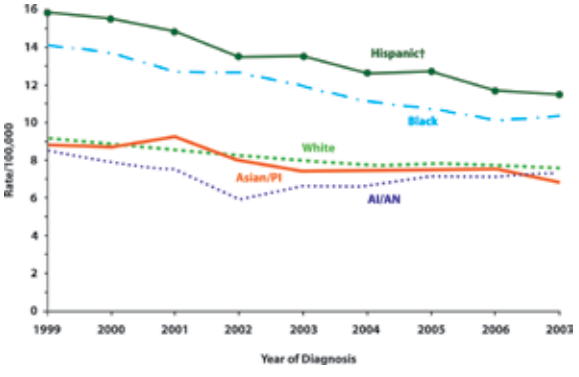
Cervical cancer is cancer that forms in the tissues of the cervix. It is a slow-growing cancer that may not have symptoms but is indicated by regular evaluation of cervical specimens via the Papanicolaou (Pap) smear. Seventy years ago, cervical cancer was a major cause of death among women of childbearing age in the US. However, the incidence of cervical cancer and death rate due to cervical cancer have declined by more than 60% since then. The decline is generally attributed to the introduction of the Pap smear in the 1950s. More recent trends shown in Figure 1 indicate continued decline in cervical cancer incidence and deaths of about 3% per year with variability among race and ethnicity. Today, cervical cancer ranks 14th in frequency with 12,710 new cases and 4,290 deaths estimated in the US in 2011 as reported by the National Cancer Institute (NCI).

Cervical Cancer
Incidence Rates by Race and Ethnicity, US: 1992-2007



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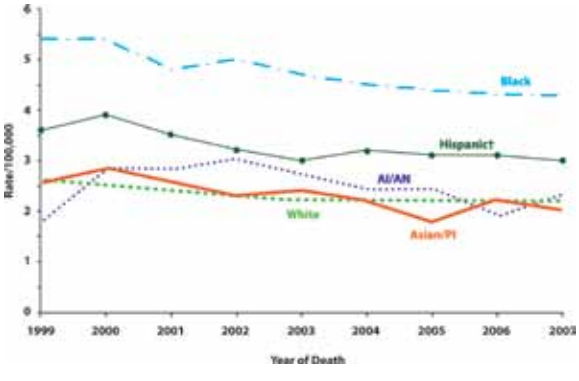

Figure 1. Cervical Cancer Incidence and Death Rates

Centers for Disease Control (http://www.cdc.gov/cancer/cervical/statistics/race.htm)

Genital human papillomavirus (HPV) is the most common sexually transmitted infection in the US. The Centers for Disease Control and Prevention (CDC) reports that at least 50% of sexually active individuals will have genital HPV during their lives. HPVs are a group of over 150 related viruses of which there are more than 40 types that can infect the genital area, mouth, and throat of males and females. HPV types are often referred to as "low-risk" and "high-risk" based on if they put a person at risk for cancer. Several types of cancer are associated with HPV. For example, about 40% of vulvar cancer, 70% of vaginal cancer, 40% of penile cancer, 85% of anal cancer 25% of mouth cancer, and 35% of throat cancer is associated with HPV. Cervical cancer is the most common HPV-associated cancer. Over 90% of all cervical cancers are caused by HPV.

Although genital HPV infections are very common, 90% of infections occur without symptoms and are effectively cleared by the immune system within 8 to 13 months. Sometimes the infection will persist for many years. The small percentage of persistent high-risk HPV (HR-HPV) infections is the primary cause of cervical cancer. On average cervical cancer takes decades to develop and follows a progressive histopathological pattern. Abnormalities in cervical cells called cervical intraepithelial neoplasia grade 1 (CIN1) can form after infection with an HR-HPV. A high proportion of CIN1 will spontaneously regress. However, if the infection persists, especially with HR-HPV types HPV-16 or HPV-18, CIN1 lesions may progress to CIN2 or CIN3. CIN2 and CIN3 lesions are commonly considered precancerous and are treated. If untreated, CIN2 and CIN3 may progress to invasive disease. Because very few HPV infections develop into cancer, detection of HPV infection alone has low specificity and severely limits the potential clinical application for early detection of cervical cancer.

HPV-16: Oncogenicity

About 18 of the 40 HPV types that can infect the genital area are identified as high-risk for cervical cancer. Of the HR-HPV types, HPV-16 is the prevalent genotype associated with approximately 60% of cervical cancers. HPV-18 is prevalent in approximately 10% of cervical cancers. Other HR-HPV types (i.e., HPV-26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82) have less than 10% prevalence in cervical cancers. Due to it having the highest prevalence in cervical cancer, HPV-16 has garnered the most attention in research and medicine.

So why does HPV-16 cause cancer? To answer this one must learn about several aspects of HPV virology. HPV infection is limited to the basal cells or keratinocytes of the stratified epithelium, the only tissue in which the virus can replicate. HPV infects the basal keratinocytes through an exposed basement membrane as would occur

Continued.....pg 2

WHAT'S INSIDE ➤➤

UPCOMING EVENTS ➤➤

09/14-19	**PCOGS:** Pacific Coast OBGYN Society Annual Meeting, SunrIver, OR
09/22-24	**ACOG-DV:** American College of Obstetricians & gynecologists Annual District Meeting (District V), Detroit, MI
09/23-25	**ACOG-DVII:** American College of Obstetricians & gynecologists Annual District Meeting (District VII), Kansas City, MO
10/13-16	**ACOG-DIII & VI:** American College of Obstetricians & gynecologists Annual District Meeting (Districts III, & VI) and ACOOG Fall Meeting Philadelphia, PA
10/14-16	**TACOG:** Texas Association of Ob/Gyn - ACOG District XI Annual meeting, Plano, TX
10/23-26	**ACOG-AFD:** American College of Obstetricians & gynecologists Armed Forces District, San Diego, CA
10/26	**NEOG :** New England OBGYN Society Annual Fall Meeting, Sturbridge, MA
11/19	**MN_ACOG:** Minnesota Section of the American College of Obstetricians & gynecologists Annual Meeting, Minneapolis, MN
11/22	**WHP:** Women's Health / Physician's for Women's Health Annual Meeting, Waterbury, CT

Cervical Cancer and HPV Infection

Continued From.....pg 1

from micro-abrasions or sexual intercourse. Once inside the keratinocyte, the HPV virus high-jacks the host cell machinery to replicate and produce more virus. To retain the host keratinocyte in a state that is favorable for the replication of HPV, the HPV virus produces proteins that alter the host cell cycle. All HPVs can transiently induce this cellular alteration, but only HPV-16 and HPV-18 can immortalize cells in *in-vitro* experiments, suggesting that their particular proteins are outstandingly oncogenic.

The HPV-16 genome contains the viral oncogenes E6 and E7, which encode proteins of the same names capable of immortalizing and transforming normal cells. The E6 and E7 proteins interfere with host tumor suppressor genes p53 and the retinoblastoma protein (pRB), respectively. E6 in association with the cellular E6-associated protein (an E3 ubiquitin ligase) acts to modify (i.e., ubiquitinate) p53 and lead to its degradation. Without p53 cells no longer respond to apoptotic signals that would otherwise destroy the infected cell. E7 acts as the main transforming protein by competing for binding to pRB. Normally, pRB binds to and inhibits the activity of the transcription factor E2F that is responsible for initiating the transcription of genes that encode proteins necessary for pushing the cell cycle forward. When E7 binds pRB, E2F is liberated and drives the cell into S phase. The actions of E6 and E7 in concert enhance the replication of the otherwise dormant keratinocyte, setting the stage for cancer development. However, the levels of E6 and E7 protein necessary to allow HPV replication are usually not sufficient to lead to tumor formation. Further deregulation of their expression to increase E6 and E7 levels is often required.

HPV-16: Integration

During its normal life cycle, the HPV-16 genome exists as a double-stranded DNA circle, or episome and upon infection of the basal cells of the squamous epithelium, remains distinct from the host genomic DNA. The HPV genome is composed of eight genes: six that encode the early proteins E1, E2, E3, E4, E6 and E7 and two that encode the late proteins L1 and L2. When in the episomal state, the expression of HPV E6 and E7 are tightly regulated by the protein encoded by the viral transcription factor E2. Cervical tumor progression requires elevated E6 and E7 expression throughout the epithelium as well as the basal keratinocytes. This can be achieved by disrupting E2 control of E6 and E7. In approximately 80% of HPV-16 positive cervical cancers, a truncated viral genome that has integrated into the host genomic DNA is found. This truncated integrated form of HPV-16 no longer expresses all the genes necessary for viral production and is therefore replication incompetent. In cervical tumors where HPV-16 has integrated into the host DNA, consistently the E2 gene is completely or partially deleted. The loss of E2 allows for increased expression of the viral oncogenes. In addition, the integration leads to stability of the viral E6 and E7 mRNA transcripts, therefore increasing production of the two viral proteins. In addition, HPV-16 E2 has been shown to bind p53 and induce apoptotic signals independently of E6 and E7. Therefore, the disruption of E2 further promotes oncogenesis via multiple mechanisms.

These basic biological findings regarding HPV-16 “status”, or whether the viral genome is episomal, integrated, or a mixture of the two, have relevant clinical implications. Multiple clinical studies have shown that the episomal form of HPV-16 was mostly found in non-progression of precancerous cervical lesions, whereas the integrated form was found mostly in progression of precancerous lesions. Thus, a decrease in the episomal form was associated with poorer outcome. Furthermore, clinical studies in several countries show that the integration of HPV-16 is accompanied by an increase in the grade of cervical lesions and is strongly associated with persistent HPV infection and progression of cervical lesions.

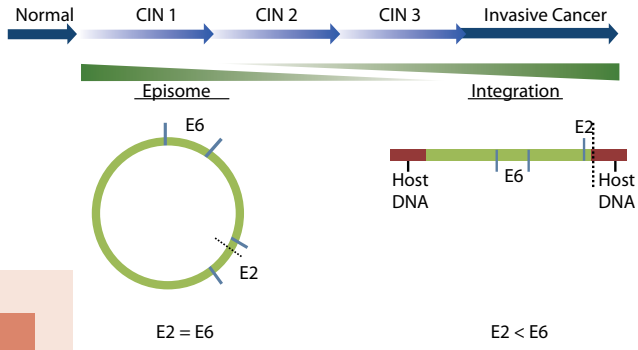

Figure 2. The HPV-16 Viral Genome Status in Cervical Cancer.

These clinically relevant changes in HPV-16 status are indirectly measured in the HPV-16 Status Test developed by Oncoveda Cancer Research Center™ and offered by Medical Diagnostic Laboratories, LLC (MDL). The HPV-16 Risk Assessment Status test is performed as a reflex to the HPV Type-Detect® 2.0 by Bio-Plex Analysis assay that can identify HPV-16 in **One*Swab*®** cervical samples. The HPV-16 Risk Assessment Status Test uses quantitative Real-Time PCR to measure the number of copies of HPV-16 E2, HPV-16 E6. As shown in Figure 2, the quantities of the two HPV-16 genes are compared in the E2/E6 Ratio, which is an estimate of the viral status. When HPV-16 is an episome the number of DNA copies of the E2 and E6 genes is expected to be equal; there is one copy of each gene in every viral episome. Therefore, the E2/E6 ratio is expected to be one (1). When HPV-16 is integrated and E2 is lost, the number of DNA copies of the E2 gene is less than that of the E6 gene that is still present. Therefore, the E2/E6 ratio diminishes, approaching zero (0). The actual E2/E6 values measured and their relationship to viral status was determined during the development and analytical validation of the HPV-16 Status Test and is summarized in Table 1.

Table 1. How the E2/E6 Ratio Relates to HPV-16 integration Status

E2/E6 Ratio	**Viral** Status
≥ 0.8	Episomal
≥ 0.2 and < 0.8	Mixed
< 0.2	Integrated

HPV-16: E6 Variants

Not only does the level of expression of HPV-16, E6, and E7 have an impact on oncogenesis; the exact nucleotide sequence of the E6 gene and the resulting amino acid sequence of the E6 protein also affect the ability of HPV-16 to immortalize and transform cells. Being the most prevalent high risk type, HPV-16 has been extensively sequenced to characterize its intratypic variants. The variants are defined as those in which the DNA sequence differs by no more than 2% in protein coding regions and 5% in non-coding regions. Over 200 variants of the HPV-16 E6 gene sequence have been identified and are commonly used to classify HPV-16 intratypic variants. Referencing the “European prototype” (EP), the first HPV-16 sequence identified from a German woman, the HPV-16 E6 variants can be clustered into six major groups: the European variant (EV), Asian (As), Asian-American (AA), African (Af1), African2 (Af2), and North American 1 (NA1).

Many of the genetic variations in E6 lead to changes in the amino acid sequence of the E6 protein. Therefore, there may be alterations in the structure and function of the variant E6 proteins. In fact several studies report differences in the ability of certain HPV-16 E6 variants to immortalize and transform cells. For example, the AA E6 variant protein, in conjunction with the prototypic E7 protein, more efficiently immortalized primary keratinocytes and induced faster cell division compared to EP E6 protein. Keratinocytes expressing the AA E6 protein also developed into larger cells with double the epithelial thickness, suggesting the AA E6 produces more hyperplastic keratinocyte populations than the EP E6. Finally, the AA E6 protein also promoted keratinocyte colony growth in soft agar whereas EP E6 did not, suggesting that in this in-vitro system only AA E6 was capable of transforming primary keratinocytes.

The variations in HPV-16 E6 sequence also have clinical relevance. A number of studies have reported HPV-16 intratypic variation to be an important predictor of progression to clinically relevant cervical neoplasia. Additionally, it was demonstrated that only naturally occurring variants of HPV-16 E6 are associated with the generation of invasive tumors from high-grade precancerous cervical lesions. Most HPV-16 variant groups harbor the T to G transition at nucleotide 350 of the E6 oncogene corresponding to amino acid 83 from L to V. Many epidemiological studies have suggested the importance of E6 amino acid 83 variants in invasive carcinomas and this variant also has been shown to have a role in human cervical tumorigenesis.

While there are conflicting studies in reporting the correlation of EV HPV-16 variant and disease, more consistent studies have been reported about the strong correlation seen between non-European variants (i.e., the As, AA, Af1, Af2, and NA1 variants) and disease. In addition, HPV-16 non-European variants are found more commonly in adenocarcinoma than squamous cell carcinoma of the cervix. This suggests that NE variants are more oncogenic and aggressive particularly in cervical tumors arising outside the transformation zone. Within the non-European variant group, the AA variant was reported by several

Cervical Cancer and HPV Infection

epidemiological studies to be up to 20-fold more prevalent than the prototypic EP variant in cervical cancer. Given the association of the HPV-16 intratypic variants with increased risk of cervical cancer progression, Oncoveda Cancer Research Center™ is developing a multiplex assay to identify the HPV-16 intratypic E6 variants as a reflex to HPV Type-Detect® 2.0 by Bio-Plex Analysis assay.

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Recent Publications

MDL: Research & Development

Oncoveda Cancer Research Center™ Peer-Reviewed Papers:

1. **Datta A, Adelson ME, Mogilevkin Y, Mordechai E, Sidi AA, Trama J.** 2011. Oncoprotein DEK as a tissue and urinary biomarker for bladder cancer. *BMC Cancer*. **11**(1): 234.

Abstracts:

1. **Huang L, Libby E, Trama J.** Identify and Evaluate Novel Biomarker CIP2A for Cervical Cancer Diagnosis. 102nd Annual Meeting of the American Association for Cancer Research (AACR), April 2-6, 2011, Orlando, Florida.

Femeris Women's Health Research Center™: Book Chapter:

1. **Hilbert D. 2011.** *Uropathogenic Escherichia coli*: the pre-eminent urinary tract infection pathogen. In M.C. Rogers and N.D. Peterson (Eds.), *E. coli Infections: Causes, Treatment and Prevention* (pp. 1-67). Nova Science Publisher Inc.

Abstracts:

Hilbert DW, Smith WL, Kaunitz AM, Mordechai E, Adelson ME, Trama JP, Gygax SE. 2011. Analysis of host antimicrobial protein production during bacterial vaginosis. 111th General Meeting of the American Society for Microbiology (ASM), May 21-24, 2011, New Orleans, Louisiana.

Venenum Biodesign Peer-Reviewed Papers:

1. **Villasmil ML, Ansbach A, Nickels JT, Jr.** 2011. The putative lipid transporter, Arv1, is required for activating pheremone-induced MAP kinase signaling in *Saccharomyces cerevisiae. Genetics*. **187**(2): 466-465.

2. **Nolt J, Rice LM, Gallo-Ebert C, Nickels JT, Jr.** 2011. PP2AC5c55 is required for multiple events using meiosis 1. *Cell Cycle*. **10**(9): 1420-34.

3. **Villasmil ML, Nickels JT, Jr.** 2011. Determination of the membrane topology of Arv1 and the requirement of the ER luminal region for Arv1 function in *Saccharomyces cerevisiae. FEMS Yeast Research*. **11**(6): 524-7.

Quality Assurance Q&A

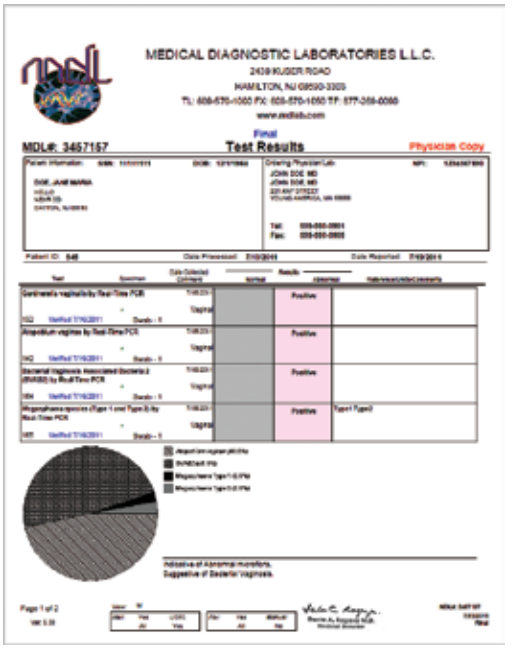
Q: All of a sudden we noticed a pie chart appearing at the bottom of the first page of our result reports. We have not been ordering anything differently so we are not sure what this means. Please help…!

A: MDL recently released a new assay as a component of the existing Bacterial Vaginosis (BV) Panel.

Test: 166 Bacterial Vaginosis Panel [*Atopobium vaginae*, BVAB2. *Gardnerella vaginalis, Megasphaera* species (Type 1 & 2)] (with Lactobacillus Profiling by qPCR)

This additional line of testing is run at no additional charge and provides the clinician with valuable information. This Panel now includes tests for four major vaginal *Lactobacillus* species: *L. crispatus, L. gasseri, L. jensenii,* and *L. iners,* in addition to the five BV-associated pathogens: *Gardnerella vaginalis, Atopobium vaginae, Megasphaera* Type 1 and Type 2, and BVAB2. Even though all of the qPCR tests in the panel can be ordered and performed separately, only the combination of the assays in a single panel allows for the accurate relative quantitative evaluation of the bacterial species composition in a given clinical sample.

The MDL Bacterial Vaginosis (with *Lactobacillus* profiling) qPCR Panel test results are reported in two formats: text-based and graphical. The text format has a standard layout of diagnostic qualitative test reporting. The graphic format is a representation of the results of all the quantitative tests included in the panel. The relative ratios of DNA species in the given sample in proportion to one another is a reflection of the relative concentrations of different bacteria in vaginal specimens. This user-friendly test report simplifies data interpretation and analysis. The single pie chart graph provides the physician with a snapshot of the vaginal bacterial microflora accompanied by a summary suggestive of vaginal microflora state: either normal or affected by BV.


MEDICAL DIAGNOSTIC LABORATORIES L.L.C.

Test Results

Generally, molecular diagnostic tests for BV are focused on the detection of recognized pathogenic markers of the disease. Incorporation of the *Lactobacillus* qPCR assays makes our test considerably more comprehensive and greatly extends the diagnostics available for assessment of vaginal health. The new MDL Bacterial Vaginosis (with *Lactobacillus* profiling) qPCR Panel is a significant advancement beyond the qualitative identification of BV-associated microorganisms since it now covers microbial markers of the normal vaginal environment. It can be used successfully for the determination of relative vaginal microflora composition and bacterial loads, which might facilitate monitoring of the response to antibiotic therapy.

If you have a question you would like addressed in future issues, please email your question(s) to QAQ&A@mdlab.com

e-Quiz

1. **True of False**. The integrated form of HPV-16 is mostly found in progression of precancerous lesions.

2. What is the most prevalent High Risk type of HPV?

3. What is the most common HPV associated Cancer?

   A. Throat Cancer C. Vulvar Cancer

   B. Cervical Cancer D. Penile Cancer

4. **True of False**. HPV-16 is the prevalent genotype associated with approximately 60% of cervical cancers. HPV-18 is prevalent in approximately 10% of cervical cancers

5. According to the National Cancer Institute (NCI), today cervical cancer ranks ____ in frequency with 12,710 new cases and 4,290 deaths estimated in the US in 2011.

For results to the electronic Epidemiology Quiz,
please visit www.mdlab.com and click on the e-Quiz link.

JOURNAL WATCH

Schmitt M, Dalstein V, Waterboer T, Clavel C, Gissmann L, Pawlita M. 2011. The HPV16 transcriptome in cervical lesions of different grades. *Mor Cell Probes* May 25. [Epub ahead of print] PMID: 21664454

Infections with high-risk human papillomaviruses (HPV), mainly HPV type 16, can cause malignant transformation of the human cervical epithelium and cervical cancer (CxCa). Very little is known about the quantitative expression of HPV16 transcripts in cervical lesions of different grades. The authors have analysed the viral transcriptome in 80 HPV16 DNA positive cervical smears including lesions of different cytological grades, using nucleic acid sequence-based amplification (NASBA)–Luminex hybridisation assays quantifying spliced and unspliced HPV16 transcripts. Based on the quantitative analysis of single transcripts, highly significant changes in transcript levels were observed between different grades of cervical lesions. In conclusion, quantitative expression changes of HPV16 transcript markers may be involved in tumour progression. This study provides a basis for selection of candidate RNA markers for diagnostics of HPV16-related disease.

The quantitative expression data from clinical specimens in this study confirmed the already known upregulation of early oncogene transcripts during cancer progression. In contrast to oncogene transcripts, L1 fl and E1^E4 RNA were downregulated. Based on the analysis of a single transcript, highly significant changes in transcript levels were observed between different grades of cervical lesions. However, the use of a single transcript for diagnostic purposes is limited. The exception of this rule was E1C that was almost exclusively detected in cervical lesions with a frequency of 30e57%. Future studies are needed to elucidate the role of E1C during cancer progression and to analyze whether analogous E1C transcripts do also exist in other high-risk HPV types.

Fakhry C, Rosenthal B, Clark DP, Gillison ML. 2011. Associations between oral HPV16 infection and cytopathology: evaluation of an oropharyngeal "Pap-test equivalent" in high-risk populations. *Cancer Prev Res.* Aug 11 [Epub ahead of print] PMID: 21836021

Human papillomavirus (HPV) is responsible for the rising incidence of oropharyngeal squamous cell cancers (OSCC) in the United States, and yet, no screening strategies have been evaluated. Secondary prevention by means of HPV detection and cervical cytology has led to a decline in cervical cancer incidence in the US. Here, the authors explored an analogous strategy by evaluating associations between HPV16 infection, cytopathology, and histopathology in two populations at elevated risk for OSCC. In the first, a cross-sectional study population (PAP1), cytology specimens were collected by means of brush biopsy from patients presenting with oropharyngeal abnormalities. In the second (PAP2), a nested case-control study, bilateral tonsillar cytology samples were collected at 12-month intervals from HIV-infected individuals. The presence of cytopathological abnormality in HPV16-positive tonsil brush biopsies (cases) was compared to HPV16-negative samples (controls) matched on age and gender. HPV16 was detected in samples by consensus primer PCR and/or type-specific PCR. Univariate logistic regression was used to evaluate associations. In PAP1, HPV16 alone (OR 6.1, 95%CI 1.6-22.7) or in combination with abnormal cytology (OR 20, 95%CI 4.2-95.4) was associated with OSCC. In PAP2, 4.7% (72 of 1524) of tonsillar cytology specimens from HIV-infected individuals without oropharyngeal abnormalities were HPV16-positive. Tonsillar HPV16 infection was not associated with atypical squamous cells of unknown significance (ASCUS), the only cytological abnormality identified.

HPV16 was associated with OSCC among individuals with accessible oropharyngeal lesions, but not with cytological evidence of dysplasia among high-risk individuals without such lesions. An oropharyngeal Pap-test equivalent may not be feasible, likely due to limitations in sampling the relevant tonsillar cryptepithelium. Despite limitation of this study, this study represents the first attempt to evaluate an oropharyngeal Pap-test equivalent for HPV-positive cancers at this anatomic site. This study indicated that any screening modality for OSCC will have to allow both visualization and sampling of lesions deep within the tonsillar crypts. We envision using such a screening modality in a population at increased risk for development of OSCC such as individuals with persistent oral HPV16 infection.

Zuna RE, Moore WE, Shanesmith RP, Dunn ST, Wang SS, Schiffman M, Blakey GL, Teel T. 2009. Association of HPV16 E6 variants with diagnostic severity in cervical cytology samples of 354 women in a US population. *Int J Cancer.* **125**:2609-13.

Persistent infections with high risk human papillomavirus type(HPV), especially type 16 are associated with the majority of invasive squamous cell carcinomas of the cervix globally, as well as in American women. Being the most prevalent type, HPV16 genome has been extensively sequenced to characterize its variants. Sequencing of the E6 region of the HVP16 genome alone is sufficient for categorizing the infected virus to the following six major groups: European (EP), European variant (EV), African (Af), Asian (As), Asian -American (AA) and North American (NA). Several epidemiological studies have shown that specific HPV16 variants are preferentially associated with high grade and/or progressive cervical lesions, suggesting intratype genetic variations are implicated in determining the clinical outcome of HPV infections. The product of two early genes, E6 and E7, are mainly responsible for the oncogenicity of the virus. The functional significance of E6 polymorphisms and carcinogenic potential of E6 has also been proven by in vitro and in vivo studies. This study analyzed the association of HPV16 variants with diagnostic severity in 354 HPV16-positive Oklahoman women by PCR amplification and DNA sequencing of the E6 open reading frame. European prototype (EP) sequences were identified in 43% of samples, 43% European variant (EV) and 14% were non-European(NE). The 14% NE or 51 samples consists of 61% AA, 23% Af and 16% NA variants. In general, the proportion of EV harboring the 350 T → G nucleotide pattern and NE variants increased with diagnostic severity while the EP decreased. When adjusted for age and race, the increased risk for carcinoma/severe dysplasia for NE variants were statistically significant with an odds ration (OR) of 3.8 (1.3 - 10.7) while that for EV did not (OR= 1.6(95% CI 0.7 - 3.6)). The results of this study further supported the strong evidence demonstrated by Xi et al. in 2007 which also examined a US population: there is increased association of non-European (NE) variants, particularly AA, with CIN3. This current study added a population of invasive cervical cancers to the spectrum of cervical lesions; thus the authors were able to show the association of NE variants, particularly AA with increasingly severe cervical neoplasia in the US involves not only progression to CIN3 to extends to invasive cancer as well. In addition, it is interesting to point out that the eight cases of NA-1 variant identified here were invasive cancers, HSIL-S or AIS. NA-1 is very closely related to the AA variant as it shares 5 out of 6 major nucleotide changes of AA. The results of this study has further demonstrated a strong association between HPV16 E6 variant, especially NE, and the development of invasive cancer in a US population.

Richard C, Lanner C, Naryzhny SN, Sherman L, Lee H, Lambert PF, Zehbe I. 2010. The immortalizing and transforming ability of two common human papillomavirus 16 E6 variants with different prevalences in cervical cancer. *Oncogene.* **29**: 3435-45.

Several epidemiological studies reported HPV16 E6 AA variant to be up to 20-fold more prevalent than the E6 prototype in cervical cancer. This study provided new insights that the increased oncogenic potential of the AA variant is due to its increased pathogenicity. The authors for the first time using longitudinal cell culture studies to investigate the immortalizing and transforming abilities of naturally occuring E6 AA variant in primary host cells of HPV - primary human foreskin keratinocytes (PHFKs). Having a limited lifespan, PHFKs normally undergo senescence (crisis) and eventually die around passage 9. In this study, the negative control vector-transduced PHFKs died at passage 9 while both prototype E6/E7 PHFKs and E6 AA/ E7 PHFKs survived to passage 65 (and beyond). Eventhough they both survived to passage 65, E6 AA/E7 PHFKs had overall significantly faster doubling times at the time of crisis and post-crisis; thus this population of cells reached the passage 65 sooner that PHFKs transduced with prototype E56/E7. Two hallmarks of activities implicated in immortalization and transformation, such as telomerase activation and p53 degradation were similar between E6/E7 and E6 AA/E7 PHFKs. Resistance to detachment-induced cell death (anoikis) is also similar in both cell types but only E6 AA/E7 cells showed ability to form colonlies, a sign of cell transformation. Proteomic analyses revealed differentially expressed host cellular proteins associated with metabolic enzymes in E6/E7- and E6 AA/E7-transduced keratinocytes. In summary, these results provide evidence to show that HPV16 AA/E7 PHFKs possessing a phenotype more reminiscent of transformed cells than HPV16 E6/E7 PHFKs. This is the first biochemical experiment providing new insights into the reasons underlying the greater prevalence of the AA variant in cervical cancer as evidenced by characteristics associated with higher oncogenic potential.

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➤ ***Research & Development***
Cervical Cancer & HPV Infection
Continued ..... pg 2

➤ ***Journal Watch***
Summaries of recent topical publications in the medical literature
Full Article ..... pg 5

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