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Prevalence and genotype distribution of human papillomavirus in cytology specimens containing atypical glandular cells: A case–control study

S.B. Namugenyi[a], M.J. Balsan[b], S.N. Glick[a], J.A. Jordan[a,*]

[a] The George Washington University, School of Public Health and Health Services, 2300 Eye St., NW, 231 Ross Hall, Washington, DC 20037, United States
[b] University of Washington, Department of Rehabilitation Medicine, 325 9th Ave., Seattle, WA 98104, United States

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ABSTRACT

Background: Incidence of glandular cell cancers has risen. While atypical glandular cell (AGC) grade cytology results represent only a small percentage of all Pap test results reported annually in the US, a significant percentage represents a corresponding high-grade lesion on follow-up biopsy. The 2006 ASCCP consensus guidelines for AGC-grade cytology results include colposcopy, endocervical sampling, and high-risk (HR) HPV testing for patient management.
Objective: Determine HPV prevalence and genotype distribution in AGC-grade cytology specimens ($n = 53$) compared to cytology specimens negative for intraepithelial lesion or malignancy ($n = 338$).
Study design: DNA extracted from residual, de-identified liquid-based cytology specimens, using QIAamp MinElute Media Kit was analyzed by PCR using Roche Linear Array HPV Genotyping and Detection Test Kits. Multivariate logistic regression compared HPV prevalence and genotype distribution between cases and controls to generate age-adjusted odds ratios (ORadj) and 95% confidence intervals (CI).
Results: HR-HPV DNA was found in 34.0% of cases and 7.4% of controls (ORadj = 9.11; 95% CI: 4.08–20.33, p-value < 0.001). Limiting analysis to HPV-16 and/or -18 resulted in finding HPV DNA in 20.8% of cases and 1.2% of controls (ORadj = 40.10; 95% CI: 10.73–149.88, p-value < 0.001). In contrast, prevalence of low-risk HPV DNA was similar between groups: 13.2% of cases and 17.2% of controls (ORadj = 0.91; 95% CI: 0.35–2.31, p-value = 0.834).
Conclusions: AGC-grade cases contained a significantly higher rate of HR-HPV compared to controls, supporting earlier recommendations for HPV testing of AGC-grade cytology specimens. Our findings also suggest that follow-up genotyping of HR-HPV containing AGC cases for HPV-16 and/or -18 specifically would be useful in patient management.

1. Background

Human papillomavirus (HPV) is the second leading cause of cancer deaths in women worldwide and is estimated to contribute to more than 99% of all cervical cancer [1] with high-risk (HR) HPV types 16 and 18 being associated with 70% of all cervical cancer cases [2]. Data from 2008 show that there were 529,828 new cervical cancer diagnoses and 275,128 deaths reported globally [2], while the most current estimate for cervical cancer incidence in the US in 2012 was 12,170 new diagnoses and 4220 deaths [3].

While there has been a marked decline in the incidence of squamous cell cervical cancers, the incidence of glandular cell cancers has increased [4,5]. Cytologic diagnosis of glandular lesions is challenging due to the complexity of the lesions, and the variety of other site(s) that may be involved ranging from exocervix, endocervix, endometrium, fallopian tubes, ovary and extra-genital organs [6,7]. In addition, multiple studies have been published demonstrating the poor reproducibility between observers when interpreting atypical glandular cell (AGC) grade Pap smears [8]. This can frequently be attributed to benign changes not associated with HR-HPV. However, although AGC-grade Pap test results represent only a small percentage (0.2–0.7%) of all Pap test diagnoses made annually, 38.0% have a corresponding high-grade lesion on the follow-up biopsy as compared to squamous cell biopsy with a 9.7% risk of a high-grade lesion [9,10].

Clinically, women with AGC-grade Pap test results are managed with cervical conization. This aggressive treatment is due in part to the lack of sensitivity of colposcopy and endocervical curettage for excluding cervical lesions. The most recent guidelines suggest a role for HR-HPV testing for all women regardless of

* Corresponding author. Tel.: +1 202 994 7062; fax: +1 202 994 7113.
E-mail address: jajordan@gwu.edu (J.A. Jordan).

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the AGC-subcategory. In 2006, consensus guidelines for managing AGC released by the American Society for Colposcopy and Cervical Pathology (ASCCP) include the combined use of colposcopy, endocervical sampling along with HR-HPV testing [11] for women with AGC-grade Pap test results.

Several studies have been published describing the prevalence of HR-HPV in liquid-based cytology (LBC) specimens with AGC-grade Pap test results [9,12–25]. However, most of these studies did not perform genotyping but instead used the Hybrid Capture 2 (HC2) assay to determine the HR-HPV result. Additionally, four of the five published studies that did perform genotyping did not include a control group for comparison [19,20,22,25] and the one that did, did not include the ages of the women in the two groups [21]. Given the strong association between younger age and HPV prevalence, adjusting for age will provide a more precise estimation of the association between HPV and AGC-grade diagnoses.

2. Objective

The aim of this study was to determine the prevalence and genotype distribution of HPV from LBC specimens among women with an AGC-grade diagnosis compared to women whose Pap smears were negative for intraepithelial lesion or malignancy (NILM), with ages known for both groups of women.

3. Study design

3.1. Selection of cases and controls

We conducted a case–control study. Quest Diagnostics Laboratories provided remnant, de-identified LBC specimens containing more than 1 ml of volume for both cases and controls. These specimens were collected from women seeking routine clinical care who resided in the Midwest, Northeast and the mid-Atlantic states of the US. Cases included all women with an AGC-grade Pap test result between 2007 and 2012. Because the AGC sub-classifications of "not otherwise specified" (AGC-NOS) or "favor neoplasia" (AGC-FN) were not available for all cases we received, these sub-classifications were not included here. Controls included a random sample of women with NILM results for a Pap smear between 2011 and 2012. Following our initial request for control specimens, an additional specific request was made for a random sample of NILM-grade LBC specimens from women 50 years and older to ensure an adequate sample ratio of controls to cases. Other than age and cytology results, we had no additional demographic or clinical information about these women. As only the birth year and cytology result were available to the investigators from these unlinked, de-identified remnant LBC specimens, this study was determined to fall outside the category of human subjects research according to the federal regulations and therefore subject consent was not required.

3.2. LBC processing and HPV DNA extraction

The LBC specimens were stored at room temperature from the time of collection until the time of extraction, with DNA extracted close to the time when the sample arrived in our research laboratory. The QIAamp MinElute Media Kit (Qiagen Inc., Valencia, CA) and QIAvac vacuum manifold (Qiagen Inc.) were used according to the manufacturer's instruction to extract DNA from 0.25 ml of LBC samples. The concentration of DNA for each eluate was measured immediately after extraction using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and the DNA extract stored at −20 °C for several months until tested for HPV and the internal control (beta globin) DNA targets by PCR.

3.3. HPV DNA PCR amplification

PCR amplification was performed using the Linear Array HPV Genotyping Test (Roche Molecular Systems Inc., Branchbury, NJ). To 58 μl of HPV master mix, 125 μl of HPV Mg^{2+} and 10 μl of 1 M Tris HCl pH 7.5 were added. 50 μl DNA extract from each specimen was added to 50 μl of the 2× working HPV PCR master mix. The positive and negative controls included in the kit were run with each batch of samples tested. The PCR amplification was performed using the gold-plated 96-well GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. After amplification, 100 μl of denaturation solution was added to each sample. Denatured samples were stored according to the manufacturer's instructions before HPV genotyping was performed, with most samples being genotyped within 1–2 days post-amplification.

3.4. HPV DNA genotyping test

HPV genotyping was performed using the Linear Array Detection Kit (Roche Molecular Systems Inc.) according to the manufacturer's instructions. The completed linear array strips were allowed to air dry on a clean, dry surface at room temperature for a minimum of 1 h or up to 72 h prior to reading and interpreting the test results using a reference guide template provided in each genotyping kit. Two people independently read the results and the two sets of results and their interpretations were compared. Any discrepancies between the readers were reviewed again by the two independent persons and resolved.

Test results were considered valid if the internal controls (beta globin DNA high and low) bands were present, or in some instances, if the internal control (beta globin DNA high) and HPV DNA band(s) were present. A cross-reactive probe 52/33/35/58 for 4 HPV genotypes is found on the genotyping strip and was interpreted according to the manufacturer's instructions. HPV-52 co-infection with one or more of the other 3 HR-HPV cross-reacting genotypes cannot be distinguished using the Roche genotyping kit. Thirty-seven HPV genotypes are detectable using the HPV genotyping test of which 13 are classified as HR-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) [26,27] and 24 LR-HPV (types 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108).

3.5. Statistical analysis

We compared the prevalence of individual HPV genotypes, HPV-16 and/or -18, any HR-HPV, and any LR-HPV between cases and controls. To generate age-adjusted odds ratios (ORadj) and 95% confidence intervals (CI), we used multivariate logistic regression. All analyses were conducted using Intercooled Stata 12.1 (StataCorp, College Station, TX, USA).

4. Results

4.1. Study population

Cases ($n = 53$) had a mean age of 57 years (range, 18–95), while controls ($n = 338$) had a mean age of 45 years (range, 20–91).

4.2. Prevalence of HPV DNA in AGC-grade cases and NILM-grade controls

One or more HR-HPV types were detected in 34.0% of the AGC-grade cases compared to 7.4% of the NILM-grade controls (Table 1); the prevalence of HPV-16 and/or -18 was 20.8% for AGC-grade cases and 1.2% for the NILM-grade controls (Table 3). In contrast, the