

RESEARCH ARTICLE

Investigation of Transition Types of HPV DNA Test Results over Time in Korean Women

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Abstract

Background: Understanding the history of human papilloma virus (HPV) infection is important for interpretation of a positive HPV DNA screening test, future work-up and treatment. We investigated the transition of HPV DNA test results in Korean women, and analyzed the association of cytology result with transition type. **Materials and Methods:** We retrospectively reviewed annual HPV DNA test results for 5,274 subjects between January 2005 and December 2012. Each subject had a minimum of five annual tests over the eight-year period. Based on the pattern of results, the transition type for each subject was assigned to one of the following: negative, persistent, latent, transient, and unclassifiable. Associations of cytology results with the HPV DNA transition types, number of positive results, and the durations of positive results were also analyzed. **Results:** The proportion of abnormal cytology findings decreased in the following order of transition patterns: persistent, latent, transient, and negative. Among transient patterns, a duration of three years or more significantly correlated with cytology results of non-high grade squamous intraepithelial lesion (HSIL; $p < 0.001$). In the persistent group, duration of five years or more correlated with both non-HSIL and HSIL ($p < 0.001$). Latent group showed no correlation with duration. Irrespective of patterns, having five or more positive results was significantly associated with HSIL ($p < 0.001$). **Conclusions:** Our findings may contribute to better understanding of HPV infection, interpretation of HPV DNA screening results, and prediction of prognosis according to transition type.

Keywords: HPV - Korean women - pattern - screening test - transition

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Introduction

The human papillomaviruses (HPVs) are small double-stranded DNA viruses that infect squamous epithelia. They are exquisitely tissue tropic, undergoing a complete infectious cycle only in fully differentiating squamous epithelium (Stanley, 2010). They can be divided into those predominantly associated with benign anogenital warts or condylomata; low-risk HPV types 6, 11, and their relatives; and those associated with anogenital cancers and precursor lesions, particularly of the cervix, HPV 16, 18, 31, 33, 35, 45, 52, 58 and minor types (Stanley, 2010; Pandey et al., 2012). Virtually 100% of cervical cancers, the second most common cancer in women worldwide, contain HPV DNA sequences from a high-risk oncogenic genital HPV (Stanley, 2010; Johnson et al., 2014; Kwang et al., 2014). The two most common oncogenic types, HPV 16 and 18, cause approximately 70% of all cervical cancers worldwide (Castellsague, 2008). A growing consensus classifies HPV as a necessary but insufficient cause because a large number of HPV infections resolve spontaneously (Castellsague, 2008; Wang et al., 2013; Khunamornpong et al., 2014).

HPV infection is very common; most women in the

world will be infected with genital HPV at some time in their lives, with a lifetime risk of infection of 50%-80%. The pattern of infection, often including multiple genotypes, is consistent with peak rates in young women (<25 years old), a rapid fall in the 30s and 40s, and a slight rise in postmenopausal age groups (Clifford et al., 2005; Stanley, 2010; Rai et al., 2014). The half-life of HPV infections has been estimated at 8-10 months for high-risk types and approximately half that for low-risk viral types (Castellsague, 2008). HPV 16 infections present the most prolonged longevities, with average persistence durations of 16 months in some studies. In more than 90% of infected individuals, infections become "clear", that is, DNA-negative, within two years (Woodman et al., 2001; Castellsague, 2008). The infections that "persist" have a higher risk of progression to true cervical cancer precursor lesions, and these lesions are likely to progress to cervical cancer over a period of several years if left untreated (Bosch et al., 2008; Gravitt, 2011; Nessa et al., 2014).

Despite some progress, there is still incomplete understanding of viral latency and natural immunity. Solving these mysteries could help guide protocols for cervical cancer screening and vaccine use (Gravitt, 2011). Although it is now widely believed that a persistent

infection with a high-risk HPV type is necessary for development of high-grade cervical intraepithelial neoplasia and invasive disease, the term “persistence” has often been loosely defined when testing this hypothesis. The number of positive results and durations used to define persistent infection in studies have varied; however, many studies have defined a persistent HPV infection as positive for HPV on two or more occasions (Evander et al., 1995; Ho et al., 1998; Clavel et al., 2000; Elfgrén et al., 2000; Giuliano et al., 2003; Cuschieri et al., 2004).

We performed a retrospective, long-term, annual tests-based study to investigate the transition of HPV DNA screening test results in Korean women, and to analyze their association with cytology. We aimed to increase understanding of the interpretation of HPV DNA screening results and the basis of the definition of persistent infection.

Materials and Methods

HPV DNA test results from 5274 subjects, who visited Kangbuk Samsung Hospital Total Healthcare Center between January 2005 and December 2012, were retrospectively reviewed (Table 1). Subjects who were 19 years or older and had at least five annual HPV DNA test results were included in the study. Those who had history of cervical cancer (or cervical cancer identified on initial HPV testing) were excluded. HPV DNA testing was done using Hybrid Capture II (Digene, Gaithersburg, MD, USA) with only the high-risk group probes, which are designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Demographic data, such as history of childbirth, marital status, education level and smoking status were also obtained for analyses. Data for cervical cancer development was obtained from the National Cancer Center (Il-san, South Korea) for subjects who had agreed to the release of information. This study was approved by the institutional review board of Kangbuk Samsung Hospital (KBC13169).

We classified the serial annual HPV DNA results into five patterns: negative, persistent, latent, transient, and unclassifiable. Negative pattern was defined as all negative HPV testing results (e.g., -, -, -, -, -). Persistent

Table 1. Demographic Characteristics of Subjects (n=5,274)

Characteristics	Number (%)
Age (years, median±SD)	42.2±5.4 (range: 36-68)
History of childbirth	
Yes	4949 (93.8)
No	102 (1.9)
No data	223 (4.2)
Marital status	
Married	5204 (98.7)
Unmarried	38 (0.7)
No data	32 (0.6)
Education level	
Middle school or lower	121 (2.3)
High school	1547 (29.3)
Community college	803 (15.2)
University	2739 (51.9)
No data	64 (1.2)
Smoking status	
Never/former smoker	5146 (97.6)
Current smoker	126 (2.4)
No data	2 (<0.1)

pattern was defined as at least two consecutive positive results, including a positive result at the point of analysis (e.g., -, -, -, +, +). Latent pattern was defined as a case that was not persistent, but had at least a one-time clearance between positive results (e.g., -, +, -, -, -). Transient pattern was defined as a case that was neither persistent nor latent, but had clearance after positive results (e.g., -, +, -, -, -). Unclassifiable pattern was defined as a case that could not be classified as one of the other four patterns (e.g., -, -, -, -, +). The duration of positive results was measured for persistent, latent, and transient patterns. For latent patterns, both negative and positive results were included in the duration because low levels of HPV infection (below detection level) might exist even during the period of negative results.

Cytology data were obtained and compared according to HPV patterns and duration of positive results. Cytology results were classified as normal; non-high grade squamous intraepithelial lesion (HSIL); atypical squamous cells of undetermined significance (ASC-US); low grade squamous intraepithelial lesion (LSIL); atypical squamous cells-cannot exclude HSIL (ASC-H); and HSIL. We also analyzed association of demographic data such as history of childbirth, marriage status, education degree, and smoking status with transition patterns and cytology results. The IBM SPSS program version 18.0 (IBM, Armonk, New York, USA) was used for statistical analyses. Pearson correlation analyses and multivariate analyses were used and a P value less than 0.05 was considered to be significant.

Results

Classification of HPV DNA patterns

The mean age of 5274 subjects enrolled in this study was 42.2 years (range, 36-68; SD, 5.4). Among them, 68.4%, 21.9%, 4.0%, 3.6%, and 2.1% were classified as negative, transient, latent, persistent, and unclassifiable patterns, respectively. The unclassifiable pattern (n=113) was excluded from further evaluation due to the insignificance.

Cytology results according to HPV DNA patterns

Cytology results were classified into non-HSIL (n=261) and HSIL (n=14). When comparing cytology results by pattern, the proportion of abnormal cytology significantly decreased in the following order of patterns: persistent, latent, transient, and negative (p<0.001) (Table 2).

Cytology results according to duration of patterns

Among 1154 subjects with the transient pattern, 79.8%, 12.6%, and 7.6% showed duration of one year, two years, and three years or more, respectively. There was a significant decrease in the proportion of normal cytology, and an increase of the proportion of non-HSIL, in subjects with duration of three years or more (p<0.001).

Among 210 subjects with the latent pattern, 37.1%, 34.3%, and 28.6% showed duration of three years, four years, and five years or more, respectively. Cytology results did not differ significantly by duration (p=0.659).

Table 2. Cytology Results According to the HPV DNA Patterns

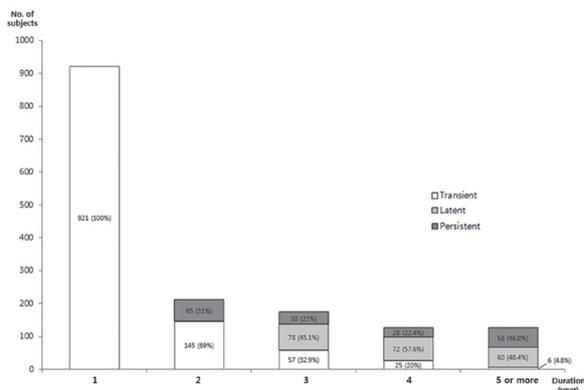
Patterns	No. (%) of cytology results				
	Normal	Non-HSIL			HSIL
		ASC-US	LSIL	ASC-H	
Negative (n = 3608)	3552 (98.4%)	43 (1.2%)	55 (1.5%)	0 (0%)	
Transient (n = 1154)	1030 (89.3%)	67 (5.8%)	119 (10.3%)	5 (0.4%)	
Latent (n = 210)	177 (84.3%)	15 (7.1%)	32 (15.2%)	1 (0.5%)	
Persistent (n = 189)	127 (67.2%)	33 (17.5%)	54 (28.6%)	8 (4.2%)	
			15 (7.9%)	6 (3.2%)	

*Total 5161 subjects were evaluated

Table 3. Cytology Results According to the Total Number of HPV DNA Positive Results

Total No. of HPV DNA positive results	No. (%) of cytology results				
	Normal	Non-HSIL			HSIL
		ASC-US	LSIL	ASC-H	
One (n=921)	864 (93.8%)	38 (4.1%)	56 (6.1%)	1 (0.1%)	
Two (n=339)	279 (82.3%)	27 (8.0%)	56 (16.5%)	4 (1.2%)	
Three (n=142)	142 (70.4%)	21 (14.8%)	42 (29.6%)	0 (0%)	
Four (n=82)	54 (65.9%)	18 (22.0%)	27 (32.9%)	1 (1.2%)	
Five or more (n=69)	37 (53.6%)	11 (15.9%)	24 (34.8%)	8 (11.6%)	
			8 (11.6%)	5 (7.2%)	

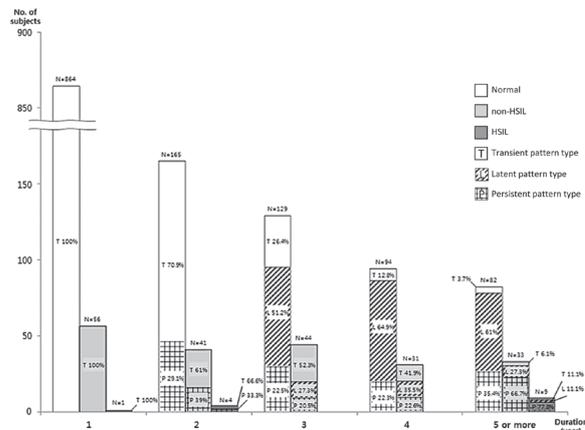
*Total 1553 subjects were evaluated

**Figure 1. Distribution of Pattern Types According to the Duration of Positive Results**

Among 189 subjects with the persistent pattern, 34.4%, 20.1%, 14.8%, and 30.6% showed duration of two years, three years, four years, and five years or more, respectively. While there was no significant difference in cytology results among subjects with duration of two to four years, there was a significant decrease in the proportion of normal cytology and an increase in both non-HSIL and HSIL in subjects with duration of five years or more ($p < 0.001$). Thirty-four (18%) of 189 subjects with the persistent pattern had the transient pattern up until the point of analysis. Of these 34, 29 showed transient duration of one year, and five showed transient duration of two years (Figures. 1 and 2).

Cytology results according to the total number of HPV DNA positive results

Among the 1553 subjects with at least one positive

**Figure 2. Cytology Results of Each Pattern Type According to the Duration of Positive Results**

result, the proportion of subjects with a single positive result was the highest (59.3%), which included the transient pattern with duration of one year. The total number of positive results was significantly correlated with the proportion of abnormal cytology. There was a significant increase in the proportion of HSIL when the number of positive results was five or more ($p < 0.001$) (Table 3).

HPV DNA patterns and cytology results according to history of childbirth, marital status, education level, smoking status and cancer development

Women without history of childbirth had significantly higher frequencies of transient pattern (odds ratio 1.845, $p < 0.05$) and cytology of non-HSIL (odds ratio 2.860,

$p < 0.05$). For women with history of childbirth, four or less live births was significantly associated with transient pattern (1-4 births: odds ratio 0.554, $p < 0.05$; ≥ 5 births: odds ratio 0.830, $p = 0.641$) and non-HSIL (1-4 births: odds ratio 0.349, $p < 0.05$; ≥ 5 births: odds ratio 0.446, $p = 0.226$). Current smokers also showed a significant association with persistent pattern (odds ratio 2.746, $p < 0.05$), compared to never/former smokers. Marital status and education level showed no significant association with HPV DNA patterns and cytology results.

We received cancer development data for 2531 (48%) subjects. Of these, only one subject had been diagnosed with cervical cancer. This subject had a transient pattern based on review of her six consecutive HPV DNA results from 2007 to 2012: +, -, -, -, -, -. The cytology was negative in 2007, but cervical cancer was detected two months after the initial HPV DNA screening test in 2007. The data of this subject was excluded from evaluation.

Discussion

In our study, we were able to classify the serial HPV DNA results of each subject into five patterns. As the patterns become worse (negative \rightarrow transient \rightarrow latent \rightarrow persistent), the proportion of normal cytology decreased, and the proportion of both non-HSIL and HSIL increased. Studies regarding natural history of HPV infection suggest that 90% of women will transit from "DNA-positive" to "DNA-negative" within two years from initial HPV DNA detection (Woodman et al., 2001; Castellsague, 2008). This transition is classically interpreted as HPV infection clearance. In our study, 92.4% of cases with transient pattern also showed duration of less than two years. Yet, many other studies have demonstrated transition from cleared HPV infection state back to "DNA-positive" state (Brown et al., 2005; Insinga et al., 2010; Theiler et al., 2010). In our study, 244 (4.6%) subjects (210 latent patterns and 34 persistent patterns) showed such transition of state. Typically, a woman in cleared HPV infection state is considered to have cleared the previously detected HPV infection, and is usually presumed to be uninfected. However, due to the uncertainty of natural immunity against HPV reinfection and latency, "DNA-negative" state can represent at least three different states: (a) a woman with prior infection who has completely cleared HPV, and is now HPV uninfected and immune to reinfection; (b) a similarly uninfected woman following prior infection, but one who remains susceptible to reinfection; and (c) an HPV-infected woman with negative HPV DNA test results who becomes reactivated (Gravitt, 2011). Currently, there is no way to conclusively differentiate individuals in "DNA-negative" state into these three subgroups, and the transition probabilities from "DNA-negative" to "DNA-positive" state are likely to depend on the subgroup. The inability to differentiate among "DNA-negative" states inevitably restricts our ability to make conclusive inferences concerning risk of HPV infection (Ho et al., 1998; Brown et al., 2005; Insinga et al., 2010; Theiler et al., 2010; Gravitt, 2011).

Many studies have distinguished persistent infection from transient infection simply by the number of positive

test results. When defined this way, the duration of a persistent infection varies depending on the interval between tests used in each study (Woodman et al., 2007). For example, the same infection might be considered persistent or transient depending on how often the samples were taken, which can be influenced by merely changing the sampling interval. With the definition based on the number of positive tests, an infection is more likely to be considered persistent if a woman is tested more frequently. We defined persistent pattern as a case with at least two consecutive positive results, including a positive result at the point of analysis. Our definition may be also arbitrary, in that it is impossible to know infection status prior to initial tests and can result in inconsistent grouping depending on the period investigated to define the transition pattern. For example, if a case defined as a transient pattern had a history of infection before the period we investigated, this should have been defined as a latent pattern. In addition, if a subject with persistent pattern with duration of two years changes to negative later on, this would actually reflect a transient pattern.

The 2012 Updated Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors recommend women 30 years of age or older with HPV positive but cytology negative results repeat co-testing at one year. At the one year repeat co-test, if the HPV test is positive or cytology is ASC-US or worse, colposcopy is recommended. If the one year repeat co-test result is HPV negative and cytology negative, repeat co-testing in three years is recommended. HPV genotyping is also acceptable. If HPV-16 or HPV-18 tests are positive, colposcopy is recommended. If HPV-16 and HPV-18 tests are negative, repeat co-testing in one year is recommended (Massad et al., 2013). Considering the above guideline and our results (significant decrease in the proportion of normal cytology and increase in the proportion of non-HSIL in subjects with transient pattern and duration of three years or more), subjects with two consecutive, annual, positive results should be treated with colposcopy. When a subject is HPV DNA positive for three consecutive years, it is highly recommended she visit an outpatient clinic for further evaluation. In order to better adhere to the guideline, HPV genotyping should be done when an HPV screening test is positive.

We found association between no history of childbirth and transient pattern/non-HSIL. Considering that the proportion of unmarried subjects in the group with no childbirth history was higher than that in the group with childbirth history (13.7% vs 0.4%), we assumed that higher sexual activity of the unmarried subjects with no history of childbirth might cause significant association with transient pattern/non-HSIL. In the group with history of childbirth, the number of live births (≤ 4) was significantly related with transient pattern/non-HSIL. Previous studies have reported that an increasing number of live births or pregnancies were related with increased risk of HSIL/cervical cancer, which might be resulted by modulated immune response to HPV due to increased levels of estrogen and progesterone, and maintenance of the transformation zone on the exocervix for long time due to high parity. Many studies reported that smoking

increases the risk of developing HSIL/cervical cancer (Castellsague and Munoz, 2003; Jensen et al., 2012). The exposure to tobacco may cause defect of the host's effective local immune response against viral infections, and thus smokers maintain cervical HPV infections significantly longer and have lower clearance compared to women who have never smoked (Giuliano et al., 2002). Our study also showed significant relationship between current smoking and persistent pattern.

The strengths of this study are a relatively long follow-up period, more than five annual HPV DNA test results, and a large number of subjects included in the analyses. Our study design and data collection methods have enabled us to specifically define five different patterns, which is a new approach for investigation of transition of HPV DNA test results, one that closely reflects the natural history of HPV. A limitation of our study is that the positive results are not completely genotype-specific because the detection method we used cannot identify specific genotypes of high-risk HPV. This may have led to a wrong classification of patterns. In addition, we could not obtain data regarding cervical cancer development for all subjects due to the absence of informed consents for provision of cancer development data. Further prospective studies using a genotype-specific HPV DNA detection method are necessary to investigate the relationship between patterns and cancer development.

In conclusion, we classified serial HPV DNA results of each subject into five patterns. There was a significant decrease in the proportion of normal cytology and an increase of the proportion of non-HSIL in subjects with transient pattern and duration of three years or more. Therefore, subjects with two consecutive positive annual results should be recommended to be treated with colposcopy, of which corresponds to the current guideline. This study may contribute to better understanding of the natural history of HPV, interpretation of HPV DNA screening results, and prediction of prognosis according to the transition pattern.

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