DOI: 10.5472/MMJ.2011.02168.1

The Persistence and Clearance Rate of Human Papilloma Virus Genotypes in Urban Turkish Women after One Year

Bir Yıl Sonunda Şehirde Yaşayan Türk Kadınındaki Human Papilloma Virus Persistans ve Klereansı

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Abstract

Objective: To evaluate the persistence of the different human papillomavirus (HPV) genotypes in women detected positive for HPV at Marmara University Hospital gynecologic outpatient clinics.

Patients and Methods: Forty out of 79 women who had been tested positive for HPV DNA in our initial prevalance study were re-assessed after one year. HPV types were identified by polymerase chain reaction (PCR) and hybridization using a microarray.

Results: One year after the initial assessment, 52.5% of the women had their initial HPV infection resolved and 35% of the women had acquired another HPV infection. The HPV DNA persistence was detected in 17.5% of the 40 women. Nine women had acquisition of HPV genotype by the same phylogenetic clade. 43.33% of high risk (HR) HPV type and 80% of the low risk (LR) HPV type infection had resolved.

Conclusions: The persistence rate was increased in women with HR HPV types. Multiple and mixed HPV infections have an important impact on the persistence of HPV genotype. *(Marmara Medical Journal 2012;25:10-5)*

Key Words: Cervical cytology, HPV genotype, HPV persistence, HPV clearance

Özet

Amaç: Marmara Üniversitesi Hastanesi kadın hastalıkları polikliniğinde human papilloma virüs (HPV) pozitif saptanan kadınlarda değişik HPV genotiplerinin persistansını değerlendirmek.

Hastalar ve Yöntem: İlk prevalans çalışmasında HPV DNA testi pozitif saptanan 79 kadından 40'ı bir yıl sonra tekrar değerlendirildi. HPV tipleri polymerase chain reaction (PCR) ve mikroarray hibridizasyon teknikleri ile tanımlandı.

Bulgular: İlk değerlendirmenin bir yıl sonrasında kadınların %52,5'nin HPV enfeksiyonunun ortadan kalktığı; %35 'inin yeni bir HPV enfeksiyonu edindiği saptandı. 40 kadının %17,5'inde HPV DNA persistansı saptandı. Dokuz kadın aynı filogenetik ağaçtan HPV genotipi edinmişti. Yüksek riskli (YR) HPV tipi enfeksiyonun %43,33'ü ve düşük riskli (DR) HPV tipi enfeksiyonun %80'i ortadan kalkmıştı.

Sonuç: Yüksek riskli HPV tipleri saptanan kadınlarda persistans oranı artmıştı. Çok sayıda ve karışık tipte HPV enfeksiyonları HPV genotipinin persistansında önemli etkiye sahiptir. *(Marmara Üniversitesi Tıp Fakültesi Dergisi 2012;25:10-5)*

Anahtar Kelimeler: Servikal sitoloji, HPV genotipi, HPV persistansı, HPV kliransı

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States[1]. Nearly 76% of new infections occur in individuals aged 15 to 25 years[2]. By age 50, an estimated 80% of unvaccinated women are infected. The virus causes cervical and other cancers and diseases, as well as genital warts. A cross sectional evaluation of the HPV DNA prevalence in 13 countries estimated that 6.6% of women in the age range 15–74 years with normal cytology are carriers of HPV DNA, with marked variation within and between world regions (range 1.4-25.6%)[3]. The prevalance of HPV DNA in our population was recently found as 13.68%[4].

Most HPV infections are transient: approximately 70% are no longer evident within a year, and up to 91% are cleared within 2 years[5,6]. Low-risk (LR) HPV infections are cleared equally well and probably faster than high-risk (HR) types[5,7]. Studies in 22 countries identified HPV DNA in almost all (99.7%) cases of cervical cancer[8]. Approximately 40 distinct HPV types are known to infect the genital

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tract and epidemiological studies to date suggest that at least 14 of these, called oncogenic or HR types, are significantly associated with progression to invasive cervical cancer[9].

Persistent cervical infection with HR HPV significantly increases the risk of a women developing atypical cervical cytology[6,10]. More importantly persistent infection with the same genotype strongly increases the risk of developing high grade pre-invasive disease[11] and the progression to invasive disease[12]. Specific genotyping data is particularly important because clinical progression only occurs in the presence of a persistent infection with HR HPV[13-15]. A few cohort studies indicate that HPV infection is mostly a transient or intermittent phenomenon; only a small proportion of those positive for a given HPV type tend to harbor the same type in subsequent specimens[16]. In addition, prospective epidemiologic studies show that the risk of subsequent cervical intraepithelial neoplasia seems to be proportional to the number of specimens testing positive for HPV[17], which suggests that only persistent infections may trigger carcinogenic development.

In an attempt to investigate the persistence of HPV genotype after one year, we have re-assessed women who had previously been detected positive for HPV DNA in a prevalance study in our center[4].

Patients and Methods

After approval by the Scientific Ethical Committee of Marmara University Medical School, 79 women seen at the outpatient gynecologic clinics of the Marmara University and Academic Hospitals, an affiliated private institution, were included for the follow-up phase of the cohort study. Women who had high grade cytology or treatment for CIN at the time of the prevalence study were not included in this study. Two patients who had had hysterectomies were excluded. Seventy nine women who had been tested positive for HPV DNA in our initial prevelance study, were called by phone and invited for reassessment of HPV in our center.

All recruited women received detailed information regarding the objective of this follow-up study, consented to participate, and were invited to give their samples which were obtained using a cytobrush between September 1, 2009 and April 30, 2010. The BD SurePath Pap test kit (BD Diagnostics–TriPath, Burlington, NC, USA) was used for liquid-based cytology testing and the Clinical Arrays Papillomavirus kit (Genomica, Madrid, Spain) was used for HPV genotype identification, as previously described[4].

The phylogenetic grouping was based on the L1 sequences of HPV, the region that encodes the major capsid protein and associates with humoral immune responses to HPV infection[18]. Accordingly, types 16, 31, 33, 35, 52, and 58 (all belonging to clade A9); 18, 39, 45, 59, 68, and 70 (clade A7); 26, 51, and 82 (clade A5); and 53, 56, and 66 (clade A6) were phylogenetically classified as HR HPV types. In contrast, types 6, 11, and 44 (clade A10); 34 and 73 (clade A11); 40 and 43 (clade A8); 42 (clade A1); 61, 72, 81, 83, 84, and CP6108 (clade A3); 57 (clade A4) and were classified as LR HPV types.

Women were defined as having: a resolved infection if HPV DNA was detected in the first but not in the second sample(clearence); a persistent infection if the same HPV DNA was detected in both samples; acquired infection if HPV DNA was detected at the first visit (e.g. HPV18) which has resolved and another HPV detected at the second visit (e.g. HPV52); and acquisition if HPV DNA detected at the second visit was a member of the same phylogenetic clade of the HPV DNA detected in the first visit.

The χ^2 and Fischer exact tests were used to compare variables and $P<0.05$ was considered significant.

Results

Since our institute is a tertiary center we receive many patients from different cities of the country. Thirtynine (49%) women unfortunately refused to come for re-assessment mostly because of living far from our center. A total of 40 women (50.63%) agreed to participate. Specific HPV typing was done in 40 women after one year of the initial HPV positivity. Twenty-one women (52.5%) had their initial HPV infection cleared. Twelve women (30%) had lost their initial HPV genotypes and acquired another HPV infection. Seven women (17.5%) had the same HR genotype detected in both the initial and control samples. Among women with HPV genotype persistence, two women had HPV16, one had HPV58, one had HPV51, two had HPV53 and one had HPV66 persistence. HPV16 and HPV58 belong to the same phylogenetic group A9. HPV53 and HPV66 are in the group A6. Nine women out of the 40 women had acquisition of HPV genotype by the same phylogenetic clade in the follow-up tests.

When HPV genotype persistent women (n:7) were compared with those without, except the marital status all demographic parameters were alike (Table I). All HPV persistent women after one year were single. When the drop out women were compared

Table I. Patient demographics of positive HPV tested patients who came for follow-up control

	HPV clearance (n=21)	HPV acquired (n=12)	HPV genotype persistence (n=7)	P value
Age	34.81±9.39	33.25±6.71	30.86±6.06	0.31
Married	12	5	0	0.02
University graduate	10	9	5	0.51
Normal cytology in 2008	19	11	5	0.24
High risk HPV infection 2008	13	9	7	0.14
Mono-infection in 2008	18	8	4	0.23
Normal cytology in 2010	21	11	5	0.07
High risk HPV infection 2010	-	9	7	0.15
Mono-infection in 2010	-	8	5	0.83

Table II. Participant characteristics between study group and drop-out or no show-up group

	Study group (n=40)	Drop-out + No show-up group (n=39)	P value
Age	33.65±8.11	36.51±12.22	0.22
High school graduate	10	7	0.42
University graduate	24	19	0.31
Single	20	16	0.93
Normal cytology in 2008	35	29	0.17
HPV 6 in 2008	4	4	0.97
HPV 16 in 2008	12	8	0.33
HPV 35 in 2008	4	2	0.41
HPV 53 in 2008	4	1	0.18
HPV 56 in 2008	1	2	0.54
HPV 66 in 2008	3	1	0.32
High risk HPV in 2008	30	29	0.85

with the follow-up group in terms of demographic characteristics, all of the parameters were similar between the groups (Table II). Since 40 patients of the initial 79 women attended the follow-up examinations, the drop-out rate was 49%.

Thirty subjects (75%) from the initial analysis were women with HR HPV types. Thirteen out of these HR HPV types had cleared (43.33%). Seven of the initial HR HPV types persisted. Twelve women acquired new infections, nine had HR and three had LR HPV infections. Eight (80%) out of ten of the LR HPV type infection had resolved. One had a new HR and the other a LR HPV infection (Table III). Of the 40 samples that were HPV DNA positive at baseline, 30 (75%) were mono-infections and 10 were multiple infections. Three (30%) of the initially multiple HPV infections had cleared; whereas, eighteen (60%) of the initial mono-HPV infections had cleared in the follow-up tests. Nine of the 12 mono-infections which persisted were all HR HPV types both initially and in the follow-up test. Two had acquired LR HPV infections after being cleared from HR HPV types. One case was initially a LR HPV type and acquired another LR during the follow-up period. Seven of the multiple HPV infected cases were all HR types both in enrollment and at the follow-up tests (Table II).

The cervical cytology was normal in 35 women (87.5%) at the initial assesment. Twentysix of these women (74.28%) had HR HPV genotyes. Thirtyseven women (92.5%) had normal cervical cytology at the follow up visit and 13 (35.13%) of those had HR HPV genotypes. Five of these 12 women had HPV genotype persistence with normal initial and follow-up smears. Seven of these 12 patients had another HPV type from the same phylogenetic clade of the HPV DNA detected in the first visit. Five patients with initial abnormal pap smear result (2 atypical squamus cells of undetermined significance (ASC-US), 1 atypical squamous cells, cannot exclude high-grade intrepithelial lesion (ASC-H), 2 low-grade squamous intrepithelial lesion (LGSIL)) had normal cytologic findings in the follow-up. Of these five women, four had HR HPV type in the first test and two of these persisted in the follow-up period. Three patients with previous normal pap smear results but later had abnormal findings (2 LGSIL and 1 ASC-US) had HR HPV type at the initial assesment. Two of these 3 women had HPV genotype persistence. Thirty-two patients had normal smear results both in the first and the follow-up visits (80%).

Discussion

In our study 52.5% of the women resolved their HPV infection within one year. This indicated that those HR HPV infections were of a transient nature. The duration of a transient HPV infection was previously stated as 8–13 months[6,19]. Molano M et al. (20) reported the clearance rate as highest in the first 6 months of follow-up. In their study, 23% of HPV infections were still present at 1 year and 7 % at 5 years. Clearance rates were lower for HPV 16 than for low-risk HPV types. HPV types related phylogenetically to HPV 16 (types 31, 33, 35, 52, 58) had intermediate clearance rates and other HR types did not show evidence of slower clearance compared with LR types. They also showed that clearance of HPV infection occurred in the 2 years after HPV was first detected. Franco (21) showed that 12-month clearance was higher for low-risk HPV types (12.2%) than for high-risk HPV types (9.5 %). HR-HPV infections tend to last longer than those of LR-HPV types[19,22]. In the cohort study of Brisson et al. HPV 16, 18, 31/33/35 appeared more persistent than other types[23]. In our study 43.33% of HR HPV types and 80% of LR HPV types cleared after one year.

Thomas et al. found that the risk of acquiring a specific HPV type was not substantially decreased among those with prior infection with a phylogenetically related type (HPV: 16 and 31; 18 and 45; 6 and 11)[24]. An association between persistent HPV infection and the presence of multiple types has been documented[3,25]. Nevertheless Liaw KL et al[26] and Molano M et al.[20] demonstrated that the clearance of a type-specific HPV infection seems to be independent of the presence of a coinfection with other types. Fourteen different HPV genotypes were detected in the follow-up women in our study. Thirty (75%) of the 40 women had mono HPV infections at the first visit; whereas, thirteen (68.4%) of the 19 women had mono HPV infection at the second visit. In our study 60% of the initially mono-infected and 30% of the multiple-infected women had HPV clearance during the follow-up. Ho et al.[6] and Perrons et al.[25]

Table III. Longitudinal detection of HPV by DNA genotyping in a cohort of individuals tested at enrolment and during follow-up

Case no	HPV DNA at enrolment	Cytology at enrolment	HPV DNA at follow-up	Cytology at follow-up	HPV DNA persistence	HPV acquisition
1	70	normal	negative	normal	-	-
2	6,16,18,31,33,83	normal	58	normal	-	+
3	35	normal	6,61	normal	-	-
4	58	normal	58	normal	+	+
5	66	normal	negative	normal	-	-
6	66	normal	51	LGSIL	-	-
7	53	normal	negative	normal	-	-
8	18	normal	negative	normal	-	-
9	53, 66, 85	normal	negative	normal	-	-
10	16	normal	negative	normal	-	-
11	51	ADAS	51	normal	+	+
12	16	normal	negative	normal	-	-
13	16	normal	negative	normal	-	-
14	45, 59	normal	negative	normal	-	-
15	61	normal	negative	normal	-	-
16	70	normal	61	normal	-	-
17	53	normal	53	ASCUS	+	+
18	16	normal	53	normal	-	-
19	16	normal	6	normal	-	-
20	16, 53, 59	normal	51	normal	-	-
21	52	normal	negative	normal	-	-
22	85	normal	53	normal	-	-
23	35,62	ASC-H	negative	normal	-	-
24	59, 84	ASCUS	16,53	normal	-	-
25	62	normal	negative	normal	-	-
26	16	normal	18,53,66	normal	-	-
27	6	normal	negative	normal	-	-
28	33	normal	negative	normal	-	-
29	83	normal	negative	normal	-	-
30	66	LGSIL	66	normal	+	+
31	16	normal	33	normal	-	+
32	83	normal	negative	normal	-	-
33	16,35	normal	16,35,66	normal	+	+
34	16, 56, 59, 66,85	normal	6,16,31,51,58	LGSIL	+	+
35	18, 53	normal	52,58,61,82	normal	-	-
36	6	normal	negative	normal	-	-
37	16	normal	negative	normal	-	-
38	35	normal	negative	normal	-	-
39	6	LGSIL	negative	normal	-	-
40	53,58	normal	53	normal	+	+

found that infection with multiple types of HPV was associated with persistent HPV infection. Rousseau et al. observed that persistence of HPV infection was independent of coinfection with other HPV types[27]. Liaw et al. found that the presence of HPV16 was associated with an excess risk for acquisition of other types without affecting the persistence of the episodes with the additional types[26]. We have detected that three women (30%) with multiple infections and four women (13.3%) with mono-infections at the initial visit had persistent genotype infection at reassesment after one year.

Zielinski et al.[28] demonstrated the presence of the same HPV type in undisputable normal and subsequent abnormal smears until diagnosis of cervical cancer, and thus showed that high-risk HPV detection precedes the development of abnormal cytology. It was suggested that high HPV DNA copy number was associated with cytologic abnormalities and that HPV-positive women with normal cytology were at minimal risk of subsequent progression to cancer while having very low viral loads[29,30]. Many cross-sectional studies reported an increase in viral load with increasing disease severity, others found either no association, or a higher viral load in women with low-grade squamous intraepithelial lesion (LSIL) than in those with high-grade squamous intraepithelial lesion high-grade squamous intraepithelial lesion (HSIL)[31-34]. Longitudinal studies have also failed to find a consistent association between a baseline measurement of viral load and duration of infection, clearance of disease, and subsequent risk of acquisition or progression of disease[35-37]. In our study, five patients with initial abnormal pap smear results (2 ASC-US, 1 ASC-H, 2 LGSIL) had normal cytologic findings in the follow-up. Four out of these five women had high risk HPV type at enrollment. Two of these women had HPV genotype persistence while two had clearance. Conversely three patients with previous normal pap smear and HR HPV at the initial assessment had abnormal cytologic findings (1 ASCUS, 2 LGSIL) during the follow up visit. Two of these 3 women had HPV genotype persistence. Hence genotypic persistence after one year per se may not predict cytologic abnormality . Longer duration follow-up might be necessary. Nevertheless viral load and HPV persistence may have a complimentary impact on the consequent cytologic changes.

Koshiol J et al[38] stated that the strength of the association between HPV persistence and cervical neoplasia increased with increasing grade of cervical disease. The magnitude of effect for HPV persistence in predicting CIN2-3/HSILs varied widely and was partially dependent on the HPV referent group. Persistent HPV infection resulted in an approximately one extra CIN2-3/HSILs case in every 60 women followed for about 5 years as compared with HPV-negative women. In the present study 35% of the subjects acquired another HPV genotype infection during follow-up. 27.5% of these patients had HR HPV. The acquired HR new infections could be misinterpreted as persistence of HPV when the assessment is dependent only on the presence of HR HPV instead of genotyping.

Kovacic et al.[39] showed that the proportions of mono-infected women exhibiting cytologic abnormalities were analogous to women in the <35 and 35- to 54-year-old age groups but significantly lower in the >54-year-old age group. Viral load was not consistently related to age for all women or stratified by level of cytologic abnormality. However, Molano[20] did not confirm any unfavorable effect of age on clearance. Similarly we did not find any impact of age either on persistency of HPV or cytological abnormalities. Nielssen et al.[40] observed a strong association between marital status and infection with high-risk HPV types among women aged 20 to 29 years. In our study, HPV genotype persistence was significantly less prevalent among married women.

The limitation of our study is the high drop out rate (49%). Despite the invitation of all 79 women with positive HPV at the initial assesment only 40 women were available for reassesment of HPV after one year. Nevertheless when the similar demographic characteristics of the women who drop out and who came for follow up were analysed, we could speculate that our results could be representative of the whole group of 79 women who were HPV positive at the initial assesment.

In conclusion the persistence of HPV genotype was 17.5% after one year in our population. The persistence rate was increased in women with HR HPV types. Multiple and mixed HPV infections have an important impact on persistence of HPV genotype. Future studies with larger groups will shed more light on the influence of viral load and the persistence of HPV infections on developing preinvasive and cervical lesions.

Acknowledgement

The authors declare that they have no conflict of interest

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