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Research Article

Reactivation of Human Papillomavirus in the Glans Penis Persists for a Long time in Japanese Male Cases

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Abstract

Background and Objective: HPV is known to cause uterine cervical cancer. HPV in the glans penis may be the causative agent for female cervical cancer. Probably, men infect women with HPV by sexual intercourse. However, it is said that mean HPV elimination time was 6 to 8 months. Supposedly, it is too short to infect women. In order to clarify long term persistence of HPV, 31 patients were examined repeatedly 2 to 5 times for detecting HPV infection in the glans during 1 to 9 years. **Materials and Methods:** Cells were collected with a saline-wet cotton swab which was used to rub the entire surface of the glans. DNA was extracted from the cells of the glans. HPV-DNA and genotyping were evaluated using the GENOSEARCH-HPV 31 kit in 31 cases (94 samples in total) adopting PCR method. **Results:** About 2/3 (67.7%) showed continuous shedding of almost the same HPV type(s), while HPV(s) was intermittently detected in about 1/3 (32.3%). These data indicate that reactivation of HPV is occurring every moment in about 2/3 of cases. However, 1/3 of the cases may reactivate intermittently. Additionally, HPV was demonstrated to persist at least 9 years in the glans. **Conclusion:** HPV in the glans is supposed to persist for a long time and to be almost always shed to the outside from more than 2/3 of the patients once it is infected with HPV regardless of their age. However, in the rest of the cases (1/3 of the patients), HPV is shed intermittently.

Key words: Human papillomavirus, long time persistence, continuous shedding, cervical cancer, genotyping

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Human papillomavirus (HPV) is known to be the causative agent of uterine cervical cancer¹. HPV has more than 300 types², of which high-risk HPV types³ like as types 16 and 18 cause uterine cervical cancer. Other types of HPV (e.g., types 6, 11 and 42) cause condyloma acuminatum but any malignant tumors could not occur from them⁴. Genital HPV prevalence in females has been reported from 20 to 38%⁵⁻⁹, while that in males has been recognized from 21 to 53%¹⁰⁻¹³. This percentage is rather comparable in both sexes. With regard to genital HPV persistence in females, there are so many papers. Percent of any HPV persistence is 35% in 12 months⁶, 20% in 19 months⁷, 4.2% in 2 years⁸ and 7% in 5 years¹⁴. On the other hand, there is a report of Capra *et al.*¹⁵ that any HPV persistence in men's genitalia showed 49% in 6 months, 36% in 12 months, 26% in 18 months and 11% in 2 years. These values are also comparable in both sexes. However, clinical data for longer-term of HPV persistence are very limited from the literature. It is feasible that a longer follow-up time of patients could elucidate the real image of HPV persistence.

In 2018, a paper was reported by us in the International Journal of STD and AIDS¹⁰. It was entitled "Long-term persistence of human papillomavirus in the skin of the glans penis of elderly men above 80 years of age". In that report, HPV persistence of more than 10 years was estimated in 6 cases. Ho *et al.*¹⁶ said that most genital HPV infections are transient and disappear within 2 years in approximate 90% of women. However, considering our recent report, HPV in the glans penis is conceivable to persist throughout life. In the current research, the effects of long-term persistence of HPV in the glans penis were investigated.

MATERIALS AND METHODS

Patients: Thirty-one patients (from 29 to 86 years, mean age 65.6 ± 16.1 years) were selected who had positive HPV in the glans penis at the first test from the population (739 cases) of latest report¹⁰ of Int. J. STD and AIDS. They were examined continuously in the about 1-year interval for mean 4.8 ± 2.5 years (6 months-8 year 9 months). All patients were recruited in out-patient clinics of Asoka Hospital and Nagareyama Central Hospital from May 2011 to January 2020. These patients visit every 3 months continuously because of urological diseases mainly as patients with benign prostate hypertrophy. Prior to starting the study, written informed consent was obtained from all 31 patients. The participants

then completed the questionnaires, which included questions regarding their age, number of lifetime sex partners, age at the initiation of sexual activity and sexual intercourse during the previous year of their visit. To focus on the persistence and reactivation of HPV infection, these patients were followed up for at most about 9 years and DNA samples were taken about 1 to 2 year-interval for 2 to 5 times.

Methods: A urologist (T.K.) working at the two hospitals collected all rubbing samples of the glans penis. Briefly, cells were collected with a saline-wet cotton swab which was used to rub the entire surface of the glans with 10 to 12 back-and-forth movements. A picture describing for collecting DNA samples is shown in Fig. 1. Cells were suspended in 2.5 mL of preservative solution.

Samples were then shipped to the LSI Medience Corporation (Tokyo, Japan). The method to detect HPV types has been described previously¹⁰. Briefly, 1 mL aliquots of a preservative solution containing cell samples were centrifuged at 5,000 r/min for 5 min and the supernatant was discarded. The cell pellet was washed twice with 300 μ L of 10 mmol L⁻¹ Tris HCl (pH 8.0). Deoxyribonucleic acid (DNA) was extracted from the cells using a DNA extraction kit (SMI test, G and G Science Co., Fukushima, Japan) according to the manufacturer's instructions. HPV-DNA and genotyping were evaluated using the GENOSEARCH-HPV 31 kit (Medical and Biological Laboratory, Nagoya, Japan). This kit contains a probe for detecting β -globin in the DNA extracted from all samples and samples without β -globin amplification were removed from the analysis. This assay can be used to identify 31 HPV genotypes, consisting of 18 high risk types (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 70, 73 and 82) and 13 other types (types 6b, 11, 42, 44, 54, 55, 61, 62, 66,

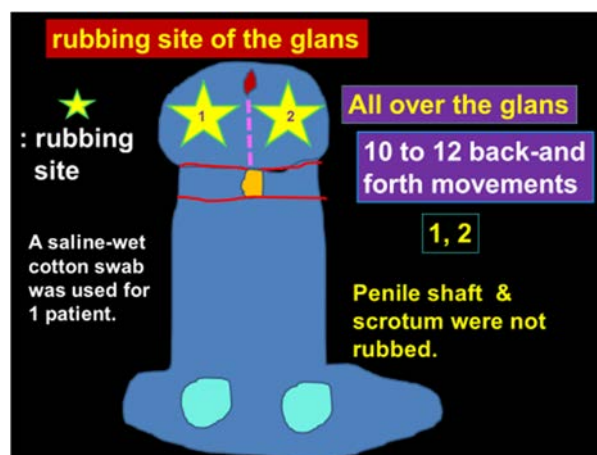


Fig. 1: Rubbing sites on the glans penis

71, 84, 90 and CP6108) by a combination of multiplex polymerase chain reaction and Luminex technology¹⁰. In this study, the high-risk HPV types were defined according to the current International Agency for Research on Cancer classification³.

Classification of complete and incomplete match cases:

In the current report, complete and incomplete match cases were defined as follows. In cases that quite the same HPV(s) is detected in every test or quite the same HPV(s) is found in at least 2 tests along with negative HPV test(s), it was defined as complete match case, while if at least one type of HPV is same in two tests and another type(s) is found in some tests, it was defined as incomplete match case. And as the third group, if a single test was positive and another test(s) was negative, it was defined as an only once positive case.

Ethical approval: This study was approved by the Ethics Committees of Asoka Hospital (approval No. 00008) and Nagareyama Central Hospital (approval No.00001), which are located in the Tokyo Metropolitan District. The study design

and purpose were explained to the candidates during their first visit to our clinics. Patients who did not accept our study protocol were excluded from this study.

Statistical analysis: All the data were presented with Mean ± standard deviation.

RESULTS

Table 1 shows the number of tests performed, duration of follow-up and mean age in 31 cases. Out of 31 patients, 4 patients received 5 times of test, likewise, 8 received 4 times, 3 received 3 times and 16 received 2 times. Their duration of follow-up years and mean age are 4.1±2.1 years and 72.3±10.9 year-old in Group 1, 5.3±2.7 years and 64.4±16.4-year-old in Group 2 and 4.6±2.3 years and 52.6±19.6-year-old in Group 3, respectively. The mean follow-up period in 31 cases was 4.8±2.5 years. The mean age in 31 cases was 65.6±16.1 years.

Table 2 demonstrates the percentage of a complete match, incomplete match and only once positive in 31 cases.

Table 1: Number of tests, duration of follow-up and mean age in 31 cases

	Number of cases	Number of tests	Duration of follow-up (year)	Age (Mean ±SD)
Group-1	12	2 to 5	4.1±2.1	72.3±10.9
Group-2	14	2 to 5	5.3±2.7	64.4±16.4
Group-3	5	2 to 5	4.6±2.3	52.6±19.6
Mean±SD in 3 Groups			4.8±2.5	65.6±16.1

Table 2: Percentage of complete match, incomplete match and others in 31 cases

Category	Percentage
*Complete match in 2 to 5 tests	38.7 % (12/31 patients)
#Incomplete match in 2 to 5 tests	45.2% (14/31 patients)
Only once positive in 2 to 5 tests	16.1% (5/31 patients)

*: Complete match case was defined as follows. Quite the same HPV type(s) is detected in every test or quite the same HPV type(s) is found in at least 2 tests along with negative HPV test(s), #: Incomplete match case was defined as follows, One type of HPV is quite same in at least two tests in each case and other type(s) is found in some tests

Table 3: HPV types detected in Group 1 (12 complete match cases)

Period	Age [#]	HPV genotypes detected by each examination					Test period	HPV persistent period
		1st	2nd	3rd	4th	5th		
Group-1-1	68	71	Negative	71	Negative	71	6 year	6 year
Group-1-2	80	90	Negative	90	Negative		4 year 5 months	3 year 3 months
Group-1-3 [§]	64	51	51	51	51		7 year 3 months	7 year 3 months
Group-1-4	81	56	56	56	56		4 year 4 months	4 year 4 months
Group-1-5 [§]	63	52	52	52			7 year 4 months	7 year 4 months
Group-1-6	86	62	62	Negative			2 year 11 months	1 year 8 months
Group-1-7	84	55	55	Negative			3 year 7 months	9 months
Group-1-8	64	62	62				4 year 3 months	4 year 3 months
Group-1-9	61	62	62				4 year 3 months	4 year 3 months
Group-1-10 [§]	55	90	90				2 year 11 months	2 year 11 months
Group-1-11 [§]	85	52	52				1 year 3 months	1 year 3 months
Group-1-12 [§]	77	52	52				6 months	6 months
Mean±SD							4.1±2.1 year	3.6±2.4 year

*: Bold digits indicate high-risk HPV types, while other digits indicate other HPV types, #: Age at the first visit, §: Patient who is still sexually active within the recent year

Table 4: HPV types detected in Group 2 (14 incomplete match cases)

Period	Age [#]	HPV genotypes detected by each examination					Test period	HPV persistent period
		1st	2nd	3rd	4th	5th		
Group-2-1 [§]	62	56 , 71, 81	42, 62, 71	42, 62, 71	42, 62, 71	42, 62, 71	8 year 9 months	8 year 9 months
Group-2-2 [§]	65	51 , 53, 58 , 61	51 , 61	51 , 58 , 61	61	61, 90	6 year 7 months	6 year 7 months
Group-2-3	79	35 , 66, 71	35 , 71, 90	35 , 52 , 62, 71, 90	35 , 90		8 year 8 months	8 year 8 months
Group-2-4	75	61	Negative	52 , 61	Negative		7 year 3 months	2 year 10 months
Group-2-5	79	67	11	56	56		8 year 5 months	8 year 5 months
Group-2-6	75	90	62	16 , 53 , 62, 90	16 , 52 , 62, 90		3 year 11 months	3 year 11 months
Group-2-7	73	16	16 , 59	16 , 70	16 , 70		8 year 7 months	8 year 7 months
Group-2-8	80	52 , 62	52				5 year 7 months	5 year 7 months
Group-2-9 [§]	29	16 , 52 , 53	52 , 58 , 62, 70				4 year 6 months	4 year 6 months
Group-2-10 [§]	39	52 , 56	18 , 52 , 56 , 73				3 year 11 months	3 year 11 months
Group-2-11 [§]	65	68, 70	70				2 year 10 months	2 year 10 months
Group-2-12	67	16 , 35 , 62	16 , 52 , 62				2 year 9 months	2 year 9 months
Group-2-13 [§]	41	16 , 39 , CP6108	16 , 39				1 year 11 months	1 year 11 months
Group-2-14	73	52 , 56	52				10 months	10 months
Mean±SD							5.3±2.7 year	5.0±2.8 year

*: Bold digits indicate high-risk HPV types, while other black digits indicate other HPV types, #: Age at the first visit, §: Patient who is still sexually active within the recent year

Table 5: HPV types detected in Group 3 (5 cases of only once positive)

Period	Age [#]	HPV genotypes detected by each examination					Test period
		1st	2nd	3rd	4th	5th	
Group-3-1	68	90	Negative	Negative	Negative	Negative	8 year 1 months
Group-3-2 [§]	56	39	Negative				4 year 11 months
Group-3-3	74	71	Negative				4 year 10 months
Group-3-4 [§]	29	90	Negative				2 year 7 months
Group-3-5	36	90	Negative				2 year 6 months
Mean±SD							4.6±2.3 year

*: Bold digit indicates high-risk HPV types, while other black digits indicate other HPV types, #: Age at the first visit, §: Patient who is still sexually active within the recent year

Twelve cases were classified as a complete match (38.7% in 31 cases), 14 cases as an incomplete match (45.2% in 31 cases) and 5 cases as only once positive (16.1% in 31 cases).

Table 3 describes HPV types detected in Group 1 (12 complete match cases). In 8 cases of case number 3, 4, 5, 8-12 in Group 1, quite the same HPV type was detected every time in 2 to 4 tests (these 8 cases were designated as genuine type). In 4 cases of the rest of the 12 cases, a single quite the same HPV type was found in 2 to 3 tests with 1 to 2 negative tests. All tests identified only one type of any HPV or negative HPV. The mean follow-up period was 4.1±2.1 years. The mean persistent period was 3.6±2.4 years.

Table 4 illustrates HPV types detected in Group 2 (14 incomplete match cases). In 13 incomplete match cases, quite the same HPV type(s) was detected in at least 2 tests, but in some other test(s) different HPV(s) was found. Some other different HPV type(s) were found often when tested in 13 out of 14 cases (these 13 cases were designated as mixed type). Case 4 in Group 2 showed the same type in 2 tests along with 2 times of negative test. The mean follow-up period was 5.3±2.7 years. The mean persistent period was 5.0±2.8 years.

Table 5 depicts HPV types detected in Group 3 (5 cases of only once positive). In 2 to 5 tests, a single HPV type was positive only once in each case (these 5 cases were designated as only once positive type). The mean follow-up period was 4.6±2.3 years. The persistent period is not able to be calculated because one HPV type was obtained once in each case.

DISCUSSION

The major finding in this study is that HPV in the skin of glans penis persists at least for 9 years. In addition, HPV is continuously shed in about 2/3 of patients (67.7%), while, about 1/3 of patients (32.3%) excrete HPV intermittently. HPV is almost always reactivated in the skin of glans penis where HPV infected once.

Table 1 illustrates that the duration of follow-up is longest in Group 2 (mean 5.3±2.7 year) and is shortest in Group 1 (mean 4.1±2.1 year). This observation period is not so long but yields valuable data for explaining HPV persistence. Capra *et al.*¹⁵ followed their cases for only 2 years which was much shorter than 5 years of our cases.

Table 2 shows that 2 to 5 tests were performed in cases of the complete match (12 patients), incomplete match (14 patients) and only once positive (5 patients), which were carried out 1 to 2 years apart. This will indicate that quite the same HPV type(s) were detected almost in every case in Groups 1 and 2. This is almost in line with Lai *et al.*¹⁷. They demonstrated that quite the same HPV type(s) were always found in 3 to 5 tests as a longitudinal examination for 3 years. As Table 3 describes 12 cases in Group 1, quite the same HPV type was detected every time in 8 cases (genuine type). Although the rest (cases 1, 2, 6 and 7) of the 12 cases showed sometimes negative results, quite the same type of HPV was excreted in every case of Group 1. The mean observation time was 4.1 ± 2.1 years. Lai *et al.*¹⁷ clarified that quite the same HPV type(s) were detected in every test during 3 to 5 tests for about 3 years with some exception of negative tests. Their median time of HPV persistence was 16.9 months which is much shorter than our cases in Group 1 (3.6 ± 2.4 years).

About Group 2 in Table 4, quite the same HPV type(s) was always found in at least 2 tests and some different HPV type(s) were found whenever tested in 13 out of 14 cases (mixed type). And the last case (No. 4) excreted at least one same type of HPV in 2 tests with negative 2 tests. This No.4 case also enters the group of HPV reproducibility. Resultantly, all 14 cases demonstrated HPV reproducibility. The mean persistent period was 5.0 ± 2.8 years. On the contrary, Molano *et al.*¹⁴ reported HPV persistence of only 7% in 5 years follow-up. Seven percent in 5 years is too low compared with our result. Lai *et al.*¹⁷ support our result that almost same HPV type(s) were detected 3 to 5 times in each of 15 cases who were followed for 3 years with frequent HPV examination.

Table 5 proved that at least 1 out of 2 to 5 tests showed the negative result in all 5 cases, which is indicative that HPV is not always excreted continuously. Giuliano *et al.*¹¹ demonstrated that male genital HPV is eliminated within 1 year. As the mean observation period in Group 3 is 4.6 ± 2.3 years, elimination time may be roughly comparable with their data.

It will be summarized from the above data that if the reproducibility of HPV is defined when at least one same type of HPV is found in more than 2 tests in each case, the reproducibility of HPV was as high as 83.9% (26 out of 31 cases). Moreover, HPV detectability in all 94 tests collected was as high as 81.9% (77/94 tests). In addition, quite the same HPV type was found every time in 8 cases (genuine in Group 1) and quite the same type(s) in at least 2 tests and some other type(s) in the other test(s) were detected every time in 13 cases (mixed in Group 2). This means that HPV(s) was found whenever tested in 21 (8 genuine and 13 mixed) out of 31

cases (67.7%). Considering these results, reactivation of certain HPV type(s) may be occurring every moment in about 2/3 (67.7%) of patients.

On the other hand, like as in cases of 1, 2, 6 and 7 in Group 1, case 4 in Group 2 and 5 cases in Group 3, these 10 out of 31 cases (about 1/3) showed negative HPV result(s) at least once. This will indicate that reactivation occurs sometimes but not so often. As described above, HPV reactivation may be very active in most patients for a long time. The longest duration of almost same HPV types being shed in case 1 of Group 2 is 8 year and 9 months. These data suggest that HPV persists for a long time probably at least 9 years.

Moreover, HPV reinfection in our cases may be very rare because only 13 out of 31 patients experienced sexual intercourse in the previous year and only 2 of the 13 patients were more than 70-year-old. These facts will prove that reinfection of HPV in recent years may have seldom occurred in the current cases.

In 2008¹¹ and 2011¹⁸, Giuliano *et al.* reported that mean HPV elimination time was 5.9 to 7.5 months and 75% of male patients clear HPV within 1 year. Capra *et al.*¹⁵ stated that the persistence of any HPV in male cases is 49% in 6 months, 36% in 12 months, 26% in 18 months and 11% in 24 months. They showed slightly longer persistence but is still shorter than ours (4.4 ± 2.6 years in 26 patients). With regard to female cases, there are so many papers. Percent of any HPV persistence is 35% in 12 months⁶, 20% in 19 months⁷, 4.2 % in 2 years⁸ and 7% in 5 years¹⁴. Although 5 years follow-up is moderately long, HPV persistence of 7% is too low compared with our cases.

In above mentioned all reports, their mean observation time (2 to 5 years) was too short to view the real image of HPV persistence. We think that HPV elimination time may be much longer than they consider. We suppose that HPV elimination is very difficult and rather it persists for a long time in the deep skin and is shed to the outside continuously.

In 2018, Twisk *et al.*¹⁹ reported that reactivation of latent HPV instead of incident infection is common. Ranjeva *et al.*²⁰ estimated that HPV infection arises from autoinoculation, episodic reactivation of the latent virus, or both. As they describe, it seems that HPV reactivates very often instead of reinfection and autoinoculation.

In the current study, we selected participants who had positive HPV at the first visit. For this reason, HPV positivity (81.9%) may be slightly higher than the real value. In spite of the aforementioned drawbacks, it is supposed to be true that HPV persists for a long time and is shed every moment because continuous reactivation of HPV occurs in the glans penis.

More than 2 types of HPV in a case were found in 14 of 31 cases (45.2%). The highest number of HPV type detected was 5 in one case (case number 3 in Group 2). The percentage of high-risk HPV amounted to 61.3% (19/31 patients). In addition, one or more than one type of HPV was found in every test in 21 out of 31 cases (67.7%, 2/3 of cases). The mean HPV follow-up period was 4.8 ± 2.5 years in total of Groups 1, 2 and 3. The mean HPV persistent period was 4.4 ± 2.6 years estimated from 26 cases of Groups 1 and 2.

The results of a questionnaire of 31 patients were summarized as follows. The number of lifetime sex partners was mean 9.4 ± 11.9 females (from 1 to 50). Age at the initiation of sexual activity was mean 20.8 ± 3.1 -year-old (from age 17 to 29). The number of patients having sexual intercourse(s) within the previous year of visit was 13, of whom only 2 patients were more than 70-year-old. 14 patients were more than 70-year-old, of whom only 2 patients (ages 77 and 85) were still sexually active within 1 year. Nine patients experienced venereal diseases in their younger age, 4 of whom had gonorrheal urethritis and 5 of whom were infected with chlamydia trachomatis.

For the future recommendation, male patients are the main reservoir of HPV for developing female cervical cancer even in their older age. Long term examination of HPV in the same individuals for more than 10 years is a prerequisite for achieving the real image of HPV persistence in not only males but also females. In the male case with positive genital HPV, condom use is essential to protect women. Females should receive a gynecological diagnosis if her genital HPV is positive especially in case of high-risk HPV.

CONCLUSION

In conclusion, HPV in the glans penis persists for a long time and reactivation of HPV is always occurring every moment in 67.7% of cases. However, the rest of the cases (32.3%) may reactivate sometimes but not so often. HPV in the glans penis is supposed to be almost always shed to the outside from more than 2/3 of the patients once it is infected with HPV.

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SIGNIFICANCE STATEMENT

This study discovers that HPV in the skin of the glans penis is excreted continuously in 2/3 of patients but is intermittently shed in 1/3 of patients. This discovery can be beneficial for HPV clinicians not only in urology but also in gynecology. Although HPV in the glans is said to disappear within 1 year, it reactivates almost every moment and persists for about 9 years. Moreover, high-risk HPV is shed very often in patients more than 70-year-old. This indicates that older patients can infect women with high-risk HPV. This study will help the researcher to uncover the critical areas of HPV reactivation and persistence that many researchers were not able to explore.

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