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Human papillomavirus genotyping among women with cervical abnormalities in Ulaanbaatar, Mongolia

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ABSTRACT

Objectives: Few studies on human papillomavirus (HPV) have been conducted in Mongolia. This study was performed to evaluate the prevalent HPV genotypes and their associations with cytology and demographic and behavioral characteristics in Mongolian women with cervical abnormalities.
Methods: Exfoliated cell samples of 100 women who had a previous history of cervical abnormality were collected. Cytological interpretation was conducted microscopically and HPV genotyping was performed using the Roche Linear Array test. Study questionnaires were completed.
Results: Overall, 25 HPV genotypes were detected in 47% of participants, and the most prevalent were HPV 16, 52, 58, and 33. Cytological examination revealed 12% of participants had atypical squamous cells of undetermined significance (ASC-US), 8% had low-grade squamous intraepithelial lesions (LSIL), 7% had high-grade squamous intraepithelial lesions (HSIL), and 14% had squamous cell carcinoma (SCC), while 59% of women had a normal cytology. HPV 16 was the most common type among women with a normal cytology and cervical cancer. However, women with cervical abnormalities including LSIL and HSIL were predominantly infected with HPV 52. Moreover, women aged <35 years had a significantly higher risk of HPV infection than those in the other age groups ($p < 0.05$).
Conclusions: The prevalent trend of HPV genotypes observed in this cohort differs from that reported previously in Mongolia. These data may contribute to developing an effective strategy for cervical cancer prevention in Mongolia.

Introduction

Mongolia is a landlocked country with a total population of three million. About two-thirds of the population live in the capital city Ulaanbaatar (Pezzulo et al., 2017); remote areas are sparsely populated. Mongolia has undergone significant socioeconomic changes accompanied by an unstable government and poor systemic policy. Thus, a large number of the population has migrated abroad over the last two decades (Tsilaajav et al., 2013; Kohrt et al., 2004).

Cervical cancer is the fourth most common cancer in women and the seventh most common cancer overall worldwide. Compared with Japan and other countries, Mongolia has one of the highest age-adjusted cervical cancer rates in Asia (Organization WH, 2014). The current situation in Mongolia indicates that cervical cancer is the fourth most common cancer in the overall population and the second most common cancer in women (Ferlay et al., 2013). According to statistical data for 2015, cervical cancer morbidity and mortality rates were 29.8 per 100 000 women and 13.6 per 100 000 women, respectively; 51.1% of patients were diagnosed in the late stage and the 5-year survival rate was estimated as 44.2% (National Center for Cancer, 2015).

Cervical screening systems are becoming more complex. The conventional Papanicolaou (Pap) test was a major leap forward in cervical cancer screening and continues to be used efficiently in many countries. However, the liquid-based cytology test has increased sensitivity and specificity compared with the Pap test

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(Aziz et al., 2006). Moreover, the introduction of new technology to test for human papillomavirus (HPV), which has a high sensitivity (>90%) and high negative predictive value compared to liquid-based cytology and the Pap test, is now recommended by the World Health Organization (WHO) for cervical cancer screening.

HPV infection is the leading cause of cervical cancer progression and is sexually transmitted (Castellsagué, 2008). HPV infection can occur at the beginning of a woman's sexual life and take several decades to advance into cervical cancer (Franceschi et al., 2006; Woodman et al., 2007). However, most HPV infections are transient and only persistent HPV infections, particularly those with high-risk HPV (HR-HPV) genotypes, progress into high-grade lesions or cancer. Individuals with low-risk HPV (LR-HPV) genotypes are likely to develop genital warts. About 15 HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) have been classified as having high oncogenic potential among >100 HPV genotypes (Muñoz et al., 2003).

Since 2012, HPV DNA testing has played an important role in the investigation of abnormal cytology findings to clarify risk types. Indeed, highly developed countries have included HPV DNA testing as a part of their triage system in guidelines for the early detection of cervical cancer (Saslow et al., 2002). In 2011, the Mongolian Ministry of Health approved clinical guidelines for a uterine cervical cancer prevention program for women with the target age of 30–60 years. Since 2012, a uterine cervical cancer screening program has been implemented in Mongolia for women in the target age group, which generally consists of a Pap test every 3 years (Chimeddamba et al., 2015; Ministry of Health Mongolia, 2007). Unfortunately, liquid-based cytology and HPV tests are not accessible because of their high costs. Moreover, data on HPV prevalence and the type distribution in Mongolia are outdated and scarce. According to a 2008 study, HPV 16 was the most common type (54%) among Mongolian women, followed by HPV 58, 18, and 33 (Dondog et al., 2008). Recently, many studies on the implementation of vaccination for HPV infections with the aim of preventing cervical lesions related to HPV infection or repeated infection have been started. The Mongolian Ministry of Health and the Millennium Challenge Corporation (Washington DC, USA) organized a pilot HPV vaccine introduction program for schoolgirls aged 11–15 years in 2012. However, concerns about the side effects of the HPV vaccines led to the interruption of the pilot program in the same year (Toh et al., 2017).

The primary aim of this study was to determine the most prevalent HPV genotypes and their associations with abnormal cervical cytology in Mongolian women. It was also sought to analyze the relationships between HPV infection and cervical abnormalities, demographic data, and behavioral changes. The results of this pilot study may contribute to future approaches for cervical cancer screening and HPV prevention and vaccination in Mongolia.

Methods

Study population and sample collection

This was a hospital-based, cross-sectional study involving 100 women who visited the gynecological outpatient clinic of the National Center for Cancer in Mongolia during March to May 2017. Women aged 18–65 years (mean ± standard deviation 45 ± 10 years) who had a previous history of cervical abnormalities and were attending a gynecological examination in order to confirm whether there was an abnormality or not were eligible for this study. However, pregnant women, women who had received radiation therapy or a hysterectomy, and women with any mental illness/disease history who could not make decisions for themselves were excluded from this study. All participants signed an informed consent form after being provided with an explanation of the study purpose. They also answered a socioeconomic background questionnaire. Women with an abnormal cytology classified as high-grade squamous intraepithelial lesion (HSIL) or more were advised to undergo colposcopy and/or biopsy; however, women with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL) were advised to undergo a repeat cervical smear test after 6 months; all patients were treated in accordance with the guidelines for management standardized by the Ministry of Health in Mongolia. Cervical samples were collected by a gynecologist using Cervex-Brush Combi (Rovers Medical Devices B.V., Oss, The Netherlands). The brush head was directly suspended in a TACAS GYN Vial (Medical & Biological Laboratories Co., Ltd, Tokyo, Japan) and kept in a refrigerator at 4 °C until transported to the laboratory at Gunma University.

Ethical considerations

This study was conducted under cooperation between the Mongolian National University of Medical Sciences, Mongolia and Gunma University, Japan. The study was approved by the ethics and research committees of the Graduate School of Health Sciences, Gunma University (number 2016/020) and the Mongolian National University of Medical Sciences (number B/191). Study approval was also obtained from the Ethics Review Board of the Ministry of Health, Mongolia.

HPV DNA genotyping and cytological examination of samples

HPV DNA testing was performed using exfoliated cervical cells from the TACAS GYN Vial before any procedure to minimize contamination. The Linear Array HPV Genotyping Test (Roche Molecular Systems, Branchburg, NJ, USA) was used to detect the 37 HPV genotypes in this study. Three specific kits (the Amplicor Linear Array Extraction Kit, Linear Array HPV Genotyping Kit, and Linear Array Detection Kit) were used for the following processes: specimen preparation, DNA extraction, PCR amplification of target DNA using HPV primers, and hybridization of the amplified products to oligonucleotide probes and detection of the probe-bound amplified products by colorimetric determination according to the manufacturer's instructions (Jamison et al., 2009). Final color precipitation of testing strips allowed for manual reading of genotype detection by comparison with a reference card. Residual cell samples in the vial were manually processed according to the protocol of the TACAS system. Processed cytological smears were dyed with Papanicolaou stain and interpreted by senior and junior cytopathologists according to the Pap test and Bethesda System 2014 (Nayar and Wilbur, 2015).

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). Statistical means, *p*-values, and odds ratios (OR) were determined. Associations between demographic/behavioral characteristics and HPV positivity and cytological abnormalities were analyzed by logistic regression analysis. All tests were considered statistically significant at the level of $p < 0.05$.

Results

Cytological diagnosis of all participants

Among the 100 women who participated in this study, the Pap test revealed that 12% had ASC-US, 8% had LSIL, 7% had HSIL, and

14% had squamous cell carcinoma (SCC). The remaining 59% of participants had normal cytological results (negative for intraepithelial lesion or malignancy, NILM) (Table 1).

Overall HPV prevalence and type distribution

Overall, HPV DNA was detected in 47% (47/100) of participants, of whom 32% were infected with single HPV infections and 68% were infected with multiple HPV infections. Twenty-five genotypes were detected including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 53, 70, 73, 82, 42, 54, 55, 61, 62, 81, 83, and CP6108 (Figure 1). The most prevalent HR-HPV types were 16 (21%, 21/100) and 52 (14%, 14/100), followed by 58 (6%, 6/100), 33 (6%, 6/100), and 31 (5%, 5/100). HPV 55 (6%, 6/100) and 54 (5%, 5/100) were dominant among LR-HPV types. The other genotypes had frequencies of 4% or less.

HPV prevalence according to cytological abnormalities

The prevalence of HPV-positive women among those with normal cervical smears was 32% (15/47), whereas the prevalence among women with ASC-US, LSIL, HSIL, and SCC were 13% (6/47), 17% (8/47), 13% (6/47), and 26% (12/47), respectively (Table 2). Single LR-HPV types were detected in 13.3% (2/15) of women with a normal cytology and 16.6% (1/6) of women with ASC-US, while there was no detection in women with LSIL, HSIL, or SCC. Single HR-HPV types were detected in 20% (3/15) of women with a normal cytology, 16.6% (1/6) of women with ASC-US, 33.3% (2/6) of women with HSIL, and 50% (6/12) of women with SCC. The proportions of multiple HPV infections detected in women with cytological NILM, ASC-US, LSIL, HSIL, and SCC were 66.7% (10/15), 66.7% (4/6), 100% (8/8), 66.7% (4/6), and 50% (6/12), respectively (Table 2).

Relevant HPV genotypes within different cytological abnormalities

The prevalence and distribution of the most frequent HPV genotypes among cytological abnormalities are shown in Figure 2. HPV 16 was the most prevalent genotype detected in women with SCC, followed by HPV 52, 33, 54, and 31. HPV 52 was the most common genotype in women with LSIL and HSIL, followed by HPV 58 and 59 in LSIL and HPV 33, 58, and 82 in HSIL. HPV 16, 52, and 55 were the most common genotypes in women with ASC-US, while women with a normal cytology were frequently infected with HPV 16 and 45.

Demographic and behavioral risk factors of women with HPV infection and cytological abnormalities

Multivariable analyses of demographic and behavioral risk factors for HPV DNA positivity and cytological abnormalities are summarized in Table 3. Women aged between 25 and 34 years were 4.1 times (95% confidence interval 1.0–16.8) as likely to be HPV DNA-positive as women aged ≥55 years ($p < 0.05$). Other variables and risk factors within cytological abnormalities showed no statistically significant differences.

Table 1
Representation of cytological findings among all participants ($n = 100$).

Cytological diagnosis	*n* (%)
NILM	59 (59)
ASC-US	12 (12)
LSIL	8 (8)
HSIL	7 (7)
SCC	14 (14)

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

Discussion

Considering the current wide range of international communication due to travel or immigration, changes in HPV genotype distribution in certain populations are highly probable. The current study appears to be the first to investigate the most prevalent HPV genotypes and their relationships with cervical abnormalities among Mongolian women.

HPV DNA was detected in 47% of study participants, and more women had multiple infections (68%) than single infections (32%). A previous study conducted in 2008 showed that overall HPV DNA prevalence was 35% among 800 women in Ulaanbaatar city (Dondog et al., 2008). The increased HPV DNA prevalence in this study may be attributed to the study cohort, as women who had a previous history of abnormal cytology were analyzed in this study.

The present study also found HPV 16, 52, 58, 33, and 31 to be the dominant HR-HPV types, and HPV 55 and 54 to be the dominant LR-HPV types distributed among Mongolian women. These results are similar to those reported from Asian countries such as Japan (HPV 16, 18, 52, and 58) and South Korea (HPV 16, 58, 33, and 52) (Onuki et al., 2009; Takehara et al., 2011; So et al., 2016). Previous studies indicated that HPV 16 was the dominant genotype, followed by HPV 58, 18, 33, and 45 (Dondog et al., 2008; Chimeddorj et al., 2008). However, the current study revealed a high prevalence of HPV 52 and low prevalence of HPV 18 among Mongolian women. This minor change in HPV infections suggests that the small sample size of this study could have led to a possible bias, or that study participants with cervical abnormalities may have affected this distribution in the present study. Furthermore, the previous HPV studies in this country, which has a small population, were performed almost 10 years ago. Increased migration of Mongolians throughout the world, especially to Asian countries, and the poor socioeconomic conditions over the last two decades, along with the development of tests, could explain this distribution difference in HPV infections (Rousseau et al., 2001). Thus, we consider it crucial that new research be performed with increased numbers of study participants and with increased reporting of demographic characteristics and risk factors related to HPV types.

The distribution of single and multiple infections was evaluated in participants with a normal cytology and ASC-US (Table 2). The overall multiple infection rate for HPV DNA positivity in LSIL was high (100%) in this study, and was similar to a study in a Latino cohort (Zhou et al., 2014). HPV 52, 58, 59, and 16 were the most common types detected in LSIL. The multiple infection rate (66.7%) for HPV DNA positivity was also higher in HSIL than the single infection rate (33.3%). HPV genotypes commonly detected in HSIL were 52, 16, and 33. However, there was no difference in single and multiple infection rates in participants with SCC. The most common HPV genotypes detected in SCC were HPV 16, 52, 33, and 31, which differs from the previous study in Mongolia (HPV 16, 35, 31, and 58) (Dondog et al., 2008). These findings suggest that only persistent HPV genotypes lead to cervical dysplasia, regardless of single or multiple infections. Since the study identified probable high risk HPV types circulating in this country, it is important to formulate an appropriate, targeted strategy to prevent HPV infection in each region.

According to the demographic and behavioral risk factors questionnaire, Mongolian women aged <35 years were more likely to have HPV DNA positivity than women in the other age groups. This finding supports results from a previous study that showed HPV infection to be highest in women aged <25 years in Mongolia (Dondog et al., 2008). HPV infections generally peak at a young age,

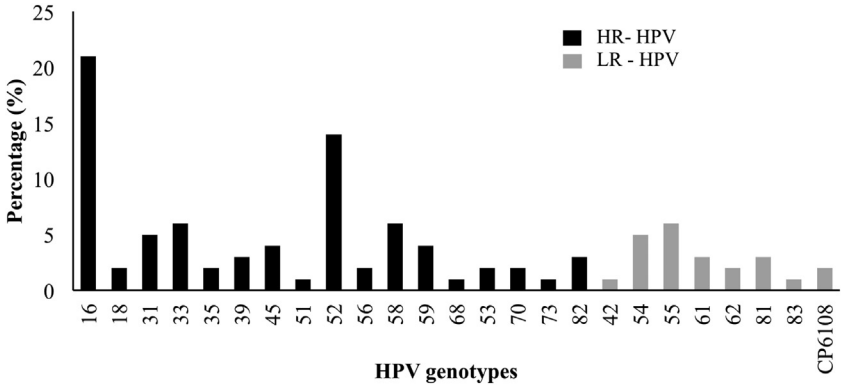

Figure 1. Distribution of HPV genotypes detected in overall participants. HPV, human papillomavirus; HR-HPV, high-risk HPV; LR-HPV, low-risk HPV.

Table 2
Frequency of HPV DNA positivity results according to cytological abnormalities ($n = 47$).

Cytological diagnosis	HPV DNA positivity (%)	Single infection (%)		Multiple infections (%)
		LR-HPV	HR-HPV	
NILM	15 (32)	2 (13.3)	3 (20)	10 (66.7)
ASC-US	6 (13)	1 (16.6)	1 (16.6)	4 (66.7)
LSIL	8 (17)	0 (0)	0 (0)	8 (100)
HSIL	6 (13)	0 (0)	2 (33.3)	4 (66.7)
SCC	12 (26)	0 (0)	6 (50)	6 (50)
Total	47 (100)	3 (6.4)	12 (25.5)	32 (68.1)

HPV, human papillomavirus; LR-HPV, low-risk HPV; HR-HPV, high-risk HPV; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

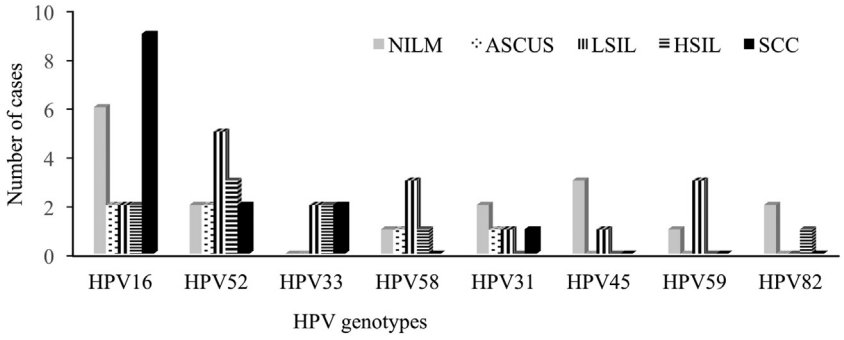

Figure 2. Most frequent HPV genotypes according to cytological abnormality. HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

which corresponds to a woman's first sexual intercourse. However, most infections clear within a few years (Franco and Harper, 2005; Wang et al., 2015). Furthermore, no significant difference in cytological findings according to age group was observed. This result can be explained by the fact that the number of women included in this study was small and HPV DNA testing is more sensitive than cytology testing. Furthermore, this finding suggests that the target age range for the cervical cancer screening program and primary prevention system should be reconsidered for the young generation in Mongolia. Other factors including residency, relationship status, contraceptive use, age at first sexual intercourse, number of lifetime sexual partners, and history of induced abortion were not significantly associated with HPV DNA positivity or abnormal cytology results in this study. Moreover, it was not possible to determine information regarding the exact number of lifetime sexual partners of the participants' partners or husbands, which prevents a complete understanding of the high incidence of multiple infection, but not significant risk factors of sexual behavior in participants. Further investigations are required to clarify these findings by increasing the sample size and including information about partners and husbands.

This study has some limitations that must be discussed. First, the number of study participants was small, and thus the data do not directly represent the whole population. Second, this was a cross-sectional study and whether the HPV infections were transient or persistent remains uncertain due to the absence of a follow-up system. Although progression to lesion or cancer may occur several decades after infection, it is not possible to detect simultaneous or sequential infections with each HPV genotype and potential combinatorial effects on cytological characteristics. Third, there is a need for new research on demographic characteristics and risk factors associated with HPV types, increasing the number of subjects in the study population. Despite these limitations, the strengths of this study include the use of the

Table 3
Multivariable analyses of demographic and behavioral risk factors for HPV DNA positivity and abnormal cervical cytology ($n = 100$).[a]

Risk factor	Total *n* (%)	HPV DNA positivity[b]			Abnormal cytology[c]		
		Positive *n* (%)	OR (95% CI)	*p*-Value	Positive *n* (%)	OR (95% CI)	*p*-Value
Residency							
Ulaanbaatar city[d]	61 (61)	27 (44)	0.5 (0.1–1.4)	0.2	23 (38)	0.6 (0.2–1.6)	0.35
Country	39 (39)	20 (51)	–		18 (46)	–	
Age group							
25–34 years	19 (19)	12 (63)	4.1 (1.0–17)	0.04	9 (47)	1.8 (0.5–6.7)	0.36
35–44 years	27 (27)	11 (41)	1.2 (0.3–4.4)	0.74	10 (37)	0.9 (0.3–3.1)	0.96
45–54 years	29 (29)	15 (52)	1.6 (0.4–5.8)	0.40	12 (41)	1 (0.3–3.1)	0.94
≥55 years	25 (25)	9 (36)	–		10 (40)	–	
Relationship status							
Single	11 (11)	6 (55)	1.4 (0.2–10.8)	0.69	9 (82)	–	
Married	77 (77)	35 (45)	0.8 (0.2–3.1)	0.75	27 (35)	6.3 (0.9–42.7)	0.06
Divorced/widowed	12 (12)	6 (50)	–		5 (42)	0.7 (0.2–2.6)	0.65
Contraceptive use							
Yes	45 (45)	22 (49)	0.9 (0.3–2.6)	0.95	17 (38)	0.6 (0.2–1.5)	0.34
No	55 (55)	25 (45)	–		24 (44)	–	
Age at first sexual intercourse							
<20 years	33 (33)	15(45)	1.9 (0.3–9.9)	0.40	13 (39)	1.7 (0.3–7.7)	0.45
20–24 years	55 (55)	28 (51)	2.2 (0.5–9.4)	0.28	24 (44)	1.6 (0.4–6.4)	0.45
≥25 years	12 (12)	4 (33)	–		4 (33)	–	
Lifetime number of sexual partners							
1	47 (47)	25 (53)	2.2 (0.8–5.8)	0.08	21 (45)	1.3 (0.5–2.9)	0.53
≥2	53 (53)	22 (42)	–		20 (38)	–	
Induced abortion							
None	26 (26)	12 (46)	0.9 (0.3–2.9)	0.99	13 (50)	2.1 (0.7–5.7)	0.14
1	28 (28)	14 (50)	1.2 (0.4–3.7)	0.70	12 (43)	1.6 (0.6–4.3)	0.33
≥2	44 (44)	19 (43)	–		14 (32)	–	

HPV, human papillomavirus; OR, odds ratio; CI, confidence interval.
[a] Adjustment for age did not substantially change the findings.
[b] HPV DNA positivity includes high- and low-risk HPV infections.
[c] Abnormal cytology includes: ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.
[d] Ulaanbaatar city indicates the capital city of Mongolia.

relatively sensitive TACAS LBC test and the Linear Array HPV genotyping test, which were repeated for inconclusive results. Furthermore, this study described the probable relationship between HPV types and demographic and behavioral risk factors.

In conclusion, this study revealed the prevalence of HPV genotypes among Mongolian women and the frequency in cervical abnormalities. Even with the small population size, these data on the most prevalent HPV genotypes could form the basis for future studies and may contribute to formulating prevention and vaccination programs and a follow-up system for HPV infection in Mongolia.

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Ethical approval

This study was approved by the ethics and research committees of the Graduate School of Health Sciences, Gunma University (number 2016/020) and the Mongolian National University of Medical Sciences (number B/191).

Conflict of interest

All authors declare that they have no conflicts of interest.

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