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SHORT COMMUNICATION

Comparison of a Newly Developed HPV Genotyping Assay (Mojin HPV Kit) with Cobas 4800 HPV Test for Detection of High-Risk HPV in Specimens Collected in SurePath Solution

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SUMMARY

Background: **Human papillomavirus (HPV) detection based on cervical cytology specimens is useful for cervical cancer screening. The aim of this study was to compare Mojin HPV kit (a newly developed HPV genotyping assay) with the Cobas 4800 HPV test in detecting high-risk (HR) HPV.**
Methods: **A total of 347 cervical exfoliated cell specimens were tested using the Mojin HPV kit and Cobas 4800 HPV test. When the results from the two tests were inconsistent, gene sequencing was performed for correction.**
Results: **For HR-HPV, the results of the two assays agreed by 96.3% [Kappa = 0.911; 95% confidence interval (CI): 0.863 - 0.958)]. The positive and negative coincidence rates between the two tests were 96.0% (95% CI: 92.7% - 98.0%) and 97.0% (95% CI: 91.5% - 99.4%), respectively. Of the 13 samples with discordant results, 3 samples were false positive and 10 samples were true negative for Mojin HPV test, according to the identification by sequencing. For HPV16 genotyping, the total coincidence rate between the 2 tests was 100% (Kappa = 1.000), and 99.7% (Kappa = 0.973; 95% CI: 0.905 - 1.000) for HPV18.**
Conclusions: **Mojin HPV kit may be as effective as Cobas 4800 HPV assay in detecting the total HR-HPV, especially HPV16 or HPV18.**
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INTRODUCTION

For cancer related death among women all over the world, cervical cancer is the fourth leading cause, which culminates in cervical intraepithelial neoplasia 1 (CIN1), CIN2, CIN3, and invasive cancer [1]. Human papillomavirus (HPV) infection, the main cause of cervical cancer, kills more than 260,000 women worldwide every year [2]. According to the homology of DNA, HPV is divided into more than 100 subtypes, and 35 types associated with genital tract infection are categorized as high, intermediate, and low-risk genotypes de-

pending on their oncogenic potential [3]. At least 14 high-risk (HR) HPV genotypes have been identified in humans, with common genotypes being HPV 16 and HPV 18 [4]. Although more than one half of women show cervical HPV infection soon after their sexual debut, 90% of infection is spontaneously cleared within 3 years, while 10% persists and < 1% develops into cervical cancer [5]. Therefore, persistent infection by HR-HPV genotypes can lead to cervical carcinomas.

Papanicolau (Pap) screening test can markedly reduce incidence of cervical cancer. However, some limitations such as low sensitivity exist [6]. Patients infected by HR-HPV had a higher risk of developing CIN2 or worse, while their Pap screening test results were negative. Moreover, the estimated absolute risk for CIN2 or worse was 13.6% and 7%, respectively, in women who were cytologically negative but positive for HPV 16 or HPV 18 genotyping [7]. Hence, the HR-HPV DNA test, especially for HPV 16 or HPV 18 typing detection, plays a pivotal role in cervical cancer screening.

The Roche Cobas 4800 HPV test, approved by the U.S. Food and Drug Administration (FDA) in 2011, is a reliable automated nucleic acid preparation and real-time polymerase chain reaction (PCR) system. It can rapidly distinguish HPV 16 or HPV 18 genotyping from other HR-HPV types [8]. However, due to its high cost, many developing countries, where 80% of HPV infected women live, prohibit its use. In order to promote HPV detection technology in developing countries, especially in rural areas, a new HPV detection and genotyping assay named Mojin HPV kit was developed by Mojin Biological Engineering Technology Co., Ltd (Nanjing, Jiangsu, China). The test is a powerful and precise HPV-DNA detection method based on multiplex fluorescent quantitative PCR, and can detect 12 types of HR-HPV in addition to distinguishing HPV 16 and HPV 18 (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The aim of this study was to evaluate the accuracy of the Mojin HPV kit compared with the Cobas 4800 HPV test in detecting HR-HPV using cervical swab specimens obtained from Chinese women.

MATERIALS AND METHODS

Samples

Between June and November of 2016, exfoliated cell specimens were collected from 347 patients (mean age, 37.8 years; median, 35 years; range, 20 - 83 years) who visited gynecologists for the treatment of cervical cancer or follow-up care at the Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine. Patients were included in the study if they were ≥ 20 years old and not pregnant. All specimens were placed in SurePath Preservative Fluid (BD, Franklin Lakes, NJ, USA) and were stored at 4°C. Each specimen was coded randomly by the sample receivers of Clinical laboratory so that Cobas4800 HPV test and Mojin HPV test could be carried out blindly and independently. Cobas4800 HPV (Roche, Meylan, France) and Mojin HPV (Mojin, Nanjing, China) tests were performed by different operators, according to the manufacturers' instructions.

The study was based on the statement of ethical principles of the Declaration of Helsinki and conformed with Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. The purpose and significance of the study was explained to the voluntary patients before they signed informed consent forms. The study received ethical permission from the Hospital Ethics Committee (No. 2016LW005).

Cobas 4800 HPV test

The Cobas 4800 HPV test features fully automated nucleic acid preparation and real-time PCR combined with a software analytical system. The test is designed to extract, amplify, and detect a broad spectrum of HR HPV genotypes as well as human cellular β-globin genes serving as inner quality control. In this study, magnetic beads are used for DNA extraction, and 4 different reporter dyes track different targets in the multiplex reactive PCR system. Dye1 tracks HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; dyes 2 and 3 track HPV 16 and 18; and dye 4 targets β-globin.

Mojin HPV kit

The Mojin HPV kit is designed for the detection of HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and the distinguishing of type 16 or 18. The DNA templates were extracted from exfoliated cells, precipitated by adding 5% Chelex-100 and boiling. The PCR reaction system was composed of primers, probes, dNTP, Taq polymerase, uracil DNA glucosylase, and Tris-Hcl buffer solution. Amplification was undertaken as follows: after a reverse transcription at 25°C for 10 minutes and a pre-heating at 95°C for 10 minutes, 40 amplification cycles were carried out in the thermal cycler: denaturation at 95°C for 15 seconds annealing/extension at 60°C for 30 seconds. Amplification was completed with a final extension at 40°C for 1 minute. Four different reporter dyes tracked different targets in the multiplex reactive PCR system. FAM tracked HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; Cy5 and JOE tracked HPV-16 and 18, respectively; ROX targeted β-globin. PCR products were detected by monitoring the fluorescence increase of the reporter dyes at each PCR cycle. Software plotted the normalized reporter signal against the number of amplification cycles and also determined the cycle threshold (Ct) value, i.e., the PCR cycle number at which fluorescence increased above a defined threshold level. Ct values of > 40 were taken as no PCR amplification; Ct values of < 40 were taken as positive for PCR amplification.

Gene sequencing

When the results from the Mojin HPV kit and Cobas 4800 HPV test were inconsistent, gene sequencing was

Table 1. The concordance rates between the Mojin HPV kit and Cobas 4800 HPV test for detecting HR-HPV was excellent.

Mojin HPV kit	Cobas 4800 HPV test		Concordance rate (%)	Kappa coefficient	95% CI
	Positive	Negative			
Positive	237	3	96.3	0.911	0.863 - 0.958
Negative	10	97			

Table 2. Discrepant results between the Mojin HPV kit and the Cobas 4800 HPV test.

Mojin HPV kit	Cobas 4800 HPV test	The results of DNA sequencing	Test result analysis	n (%) (total = 347)
Negative	Positive	Negative	TN	10 (2.88)
Positive	Negative	Negative	FP	3 (0.86)

Abbreviations: TN - true negative, FP - false positive.

Table 3. Correlation between the Mojin HPV kit and Cobas 4800 HPV test for detecting HPV 16 or HPV 18 genotyp.

Results of HPV test			Cobas 4800 HPV test		PCR	NCR	TCR	KC	95% CI
			Positive	Negative					
Mojin HPV kit	HPV 16	Positive	31	0	100% (95%CI: 0.908 - 1.000)	100% (95%CI: 0.991 - 1.000)	100% (95%CI: 0.991 - 1.000)	1.000	-
		Negative	0	316					
	HPV 18	Positive	19	0	95.0% (95%CI: 0.751 - 0.999)	100% (95%CI: 0.989 - 1.000)	99.7% (95%CI: 0.984 - 1.000)	0.973	0.905 - 1.000
		Negative	1	327					

Abbreviations: RCR - positive coincidence rate, NCR - negative coincidence rate, TCR - total coincidence rate, KC - Kappa coefficient.

performed, using Big-Dye terminator sequencing and an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing primers were designed on different regions of the HPV genome for detecting gene products. The detection limit for sequencing was about 5 x 10^3 copies per reaction. The sequencing test included 3 positive quality controls and 1 negative quality control. The positive quality controls were plasmids containing the sequencing product fragments of HPV 16, HPV 18, or HPV 56. The negative quality control was human DNA. When the DNA sequence could not be detected, the DNA sequencing result was defined as negative. Primer sequences and amplification conditions are available on request.

Statistical analysis

The chi-square test was performed to compare the proportions of positive samples (HPV 16, HPV 18, or total HPV) between the specimens tested by the Mojin HPV kit and Cobas 4800 system. Concordance rates and kappa coefficient (κ) with 95% confidence intervals (CIs) were calculated to estimate the concordance between the results from the two tests. Significant differences were defined as those with p-values less than 0.05. All statistical analyses were carried out using SPSS 19.0.

RESULTS

In all 347 samples, the percentage of patients testing positive for HPV with Mojin HPV kit and Cobas 4800 HPV test was 69.16% (240/347) and 71.18% (247/347), respectively. Table 1 shows the concordance rates between the Mojin HPV kit and the Cobas 4800 HPV test. Regardless of HPV genotypes, the results of the 2 HPV detection tests agreed in 96.3 (95% CI: 93.7% - 98.0%) of all samples. The positive and negative coincidence rate in two kinds of detection are 96.0% (95% CI: 92.7% - 98.0%) and 97.0% (95% CI: 91.5% - 99.4%), respectively.

Discrepant results between the two tests are shown in Table 2. Of all samples, 3 (0.86%) were positive in the

Mojin HPV detection but negative in the Cobas 4800 HPV test, which were false positives after further confirmation by DNA sequencing. There were 10 (2.88%) cases negative only in the Mojin HPV detection and these results were true negatives according to gene sequencing.

Analyses were also conducted for the detection of HPV 16 and HPV 18. The percentage of patients positive for HPV 16 with both Mojin HPV kit and Cobas 4800 HPV test was 8.93% (31/347), while 5.48 (19/347) for HPV 18 in Mojin HPV kit and 5.76 (20/347) in Cobas 4800 HPV test. Table 3 shows the concordance rates between the two tests for HPV 16 and HPV 18 genotyping.

DISCUSSION

More and more studies demonstrate that precancerous lesions are related to HR HPV genotypes, HPV 16 and HPV 18 being most contributive [9,10]. Based on WHO worldwide data, the five most frequent HR HPV types in patients with cervical cancer were HPV 16, 18, 33, 45, and 31 [11]. HPV 16 in cervical cytology was twice as frequent as any other high-risk type in all regions and the next most common high-risk types were HPV 33 and HPV 56 in Asia, HPV 58 in South America, and HPV 31 in Europe in general population [12]. In China, HPV 16 and HPV 18 were the predominant types, followed by HPV 58, 31, and 52 in histological samples of cancer [13].

Stanczuk et al. reported that the sensitivity of the Cobas 4800 HPV test for CIN2+ detection was 92 - 97.7% (95% CI: 82 - 97%) for the clinician-collected liquid-based cytology samples [14]. Cobas 4800 HPV test had been licensed by the China Food and Drug Administration (CFDA) [15]. Therefore, this testing can be used as a standard to judge the feasibility of other HPV detection kits. This research shows that the Mojin HPV kit is as effective as the Cobas 4800 HPV test in detecting the total HR HPV, especially HPV 16 or HPV 18.

As HPVs cannot be cultured *in vitro*, almost all HPV detection assays rely on the assessment of viral nucleic acids. Currently, HPV detection methods can be subdivided into target amplification methods and signal amplification methods. Target amplification methods utilize nucleic acid polymerases, target-specific oligonucleotides, and a mixture of four (deoxy) ribonucleotides to amplify a specific nucleic acid sequence up to a level high enough to be easily detected [16]. As a target signal amplification method, the Mojin HPV kit is designed to use consensus primers directed against highly conserved sequences of the HPV genome within E1, E6, E7, L1, and L2 open reading frames. However, primers from the Cobas 4800 system target in the viral L1 regions. Thus, the discrepancies in the results of the two assays can be explained by the differences in the primer design. Another factor accounting for the differences in the results may be HPV DNA extraction. The extraction efficiency of the Cobas 4800 fully automated DNA extraction system may be higher than that of the Mojin HPV kit.

Although some discrepancies exist, the Mojin HPV kit could be used to detect HPV precisely. The low cost of the kit could make it widely available in underdeveloped areas for early detection of cervical cancer. In addition, Mojin HPV detection can prevent contamination by using uracil-DNA-glycosylase before amplification, while the PCR product of some low-cost kits can easily pollute the laboratory.

There are several limitations in this study, such as the relatively small sample size. Additionally, we could not assess the sensitivity of CIN2+ detection by just two tests, due to insufficient biopsy data. Further studies with a larger sample size are needed to determine the accuracy of CIN2+ prediction in cervical cancer screening using the Mojin HPV detection assay.

CONCLUSION

In summary, our analytical data for the Mojin HPV kit and Cobas 4800 HPV test are in good agreement. Because of the low price, the Mojin HPV kit is suitable for screening women with CIN in developing regions.

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Declaration of Interest:
The authors declared that they have no conflict of interest.

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