A comparison of two strategies that detect human papillomavirus in ThinPrep cytology samples - a preliminary study.

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Abstract

In order to evaluate the clinical use of the polymerase chain reaction (PCR) and Hybrid Capture II (HC-II) for the detection of genital human papillomavirus (HPV) in liquid based cytology (ThinPrep) samples, a sample of 40 patients who attend routine cervical cancer screening were randomly recruited. HPV DNA was detectable among the cohort of 40 patients undergoing cervical cytology screening by the PCR and HC II in 57.5% (23/40) and 40.0% (16/40) respectively. The overall concordance was 77.5% and HC-II sensitivity and specificity versus PCR were 65.2 % and 94.1%. PCR sensitivity and specificity versus HC-II were 93.8% and 66.7%. For sample with normal cytology, the agreement between both assays was 70.8%. Sensitivity, specificity, positive predictive value and negative predictive value of the HC-II test were 41.7%, 100.0%, 100.0% and 63.2% respectively. For samples with abnormal cytology, the level of agreement was 87.5%. Sensitivity, specificity positive predictive value and negative predictive value of the HC-II test were 90.9%, 80.0%, 90.9% and 80.0% respectively. The results of PCR and the HC-II assay indicated good reproducibility. For sample with abnormal cytology, the concordance was good and resulting in substantial reproducibility.

Key words

Cervix Neoplasms, DNA, Viral, Papillomavirus, Human, Polymerase Chain Reaction , Sensitivity and Specificity

Introduction

Genital human papillomavirus (HPV) infection is causally associated with cervical cancer, a leading cause of cancer-related mortality in women worldwide. Infection with high risk type is the single most important risk factor for cervical intraepithelial neoplasia.[1-3] Traditionally, the screening for cervical cancer is based on the manually prepared Pap smear. The sensitivity and specificity depend on the skill of the observer. In order to enhance the sensitivity for detecting cervical cancer, the fluid-based ThinPrep method (Cytyc Corporation, USA) and the nucleic acid based assay have been developed.[4] The ThinPrep method can improve cytologic diagnosis of precancerous lesions compared with the conventional Pap method.[5] The commercially available Hybrid Capture II (HC II) (Digene Corporation, USA) and polymerase chain reaction (PCR) methods using various primer pairs are validated and available for routine use.[6] The PCR assay can distinguish most of the pathogenic types of HPV whereas the HC-II can only differentiate between 13 high/intermediate risk and 5 low risk HPV types.[7] The PCR methods are based on the amplification of HPV DNA. Two amplification methods can be used. The first is the use of type specific DNA primers which can detect the specific types of HPV. Second, the amplification can be carried out by using consensus primers where different HPV types can be detected by a pair of primers. The consensus primers system has been validated extensively. The most common consensus primers systems are GP5+/6+ PCR, SPF1/2 PCR and MY09/11 PCR.[6] The SPF1/2 system has the highest analytical sensitivity among these systems.[8] In this study, GP5+/6+ PCR and SPF1/2 PCR were used.

PCR has been the "gold standard" technique in HPV diagnostic. The sensitivity and specificity is very high. However, it is technically demanding and requires vigorous contamination controls. In order to avoid cross contamination, we strictly followed the uni-directional workflow of PCR procedure which had separate room for DNA master mix preparation, DNA extraction and amplification of DNA. Positive control and negative control were included in each run to safeguard the validity of results.

To evaluate the usefulness of HPV PCR and HC-II as a screening tool from ThinPrep® cytology specimen in Hong Kong, both assays were performed in a clinical study comprising 40 women. Results obtained by the HC-II assay were compared with a PCR amplification strategy which included type specific and consensus PCR primer systems.

Methods

Sample collections

The study population consists of 40 Hong Kong Chinese women aged between 29 to 57 years (mean 42.9 years, median 42 years, 6 patients <35 years, 34 patients = 35 years).

The cervical broom was used to collect specimens for liquid based cervical cytology and HPV DNA testing. Specimens were placed directly into PreservCyt fixative vial (Cytyc Corporation, USA). A ThinPrep (Cytyc Corporation, USA) cytologic preparation was produced from the PreservCyt vial. HPV DNA testing of the remaining aliquot of the fixed specimen was done by using HC-II [9]. DNA extraction of the remaining liquid based cytology specimen followed by PCR were performed after HC II was done. Both high risk HPV probe (16, 18, 31, 33, 35, 45, 51, 52 and 56) and low risk HPV probe (6, 11, 43 and 44) for HC-II were carried out according to the manufacturer's protocol.

Cytological evaluation

All ThinPrep[®] cytological smears were screened and classified according to the Bethesda 2001 nomenclature and reporting of gynecologic cytology results.

HPV detection by PCR

To analyze the cervical scrapes by type-specific and general primer PCR, 3 ml of PreservCyt fixative solution containing cervical scrapes was extracted after the standard Proteinase K-Phenol Chloroform treatment. The purified DNA solution was precipitated with absolute ethanol and dissolved in 100µl distilled water. 10µl of the extracted DNA was used for type specific PCR as described by van den Brule et al.[10] The type specific PCR consisted of two separate tubes which could detect HPV 6, 16, 33 and HPV 11, 18, 31 respectively. The general primer GP5+/6+ which span a region of 140 to 150 bp from the L1 open reading frame of a broad spectrum of HPV genotypes were used.[11] The second general primer PCR SPF1/2 with higher sensitivity which amplified 65bp product was performed for negative GP5+/6+ result.[12] To assess the quality of extracted DNA, human beta-actin PCR was performed for all PCR with negative results as an internal quality control measure to make sure that the negative reaction was not due to sample DNA degradation or the presence of PCR inhibitors.

Statistical analyses

Agreement was measured by both Cohen's kappa statistic and absolute agreement.[13] Approximate 95% confidence intervals (CIs) were computed.

Results

In primary screening, HPV DNA was detectable among the cohort of 40 women by the PCR and HC II in 57.5% (23/40) and 40.0% (16/40) respectively. The concordance between the two tests is shown in Table 1. The two tests gave concordant results for 15 positive samples and 16 negative samples. The overall level of agreement was 77.5% (Kappa (k) = 0.563, 95% CI, 0.31-0.81), and HC-II sensitivity and specificity vs. PCR were 65.2 % and 94.1%. The positive predictive value and the negative predictive value were 93.8% and 66.7% respectively. PCR sensitivity and specificity vs HC-II were 93.8% and 66.7%. Among the samples with discrepant results, 8 were positive by PCR but negative by the HC II assay, whereas 1 sample were HC II positive but PCR negative.

Table 2 summarizes the separate evaluations for women with normal and abnormal cytology. The values were slightly different. For sample with normal cytology, the agreement between both assays was 70.8% (Kappa (k) = 0.417, 95% CI, 0.05-0.78). Sensitivity, specificity, positive predictive value and negative predictive value of the HC-II test were 41.7%, 100.0%, 100.0% and 63.2% respectively. For samples with abnormal cytology, the level of agreement was 87.5%. Sensitivity, specificity positive predictive value and negative predictive value of the HC-II test were 90.9%, 80.0%, 90.9% and 80.0% respectively.

Table 3 summarizes the results of HPV DNA detection in relation to Pap smear abnormalities. Of the patients classified as negative for intraepithelial lesions, HPV was detected in 50.0% of the samples by either or both of the tests. In general, the rate of detection increased in parallel with the increasing severity of the cytological abnormality, with the rate increasing up to 100 % in LGSILs. However, the rate of detection decreased to 50% in HGSIL. It was due to the HPV DNA in one HGSIL sample (50%) was missed by PCR and only HC II could detect it.

ThinPrep Pap tests showed 60.0% normal smears (24/40), 20.0% ASCUS (8/40), 15.0% LGSIL (6/40), 5.0% (2/40) HGSIL and no squamous cell carcinoma was detected.

Discussions

In recent years, HPV DNA testing is becoming part of an established triage strategy after cytological screening with liquid based cytology material. In general, the result of HC-II is similar to the well-established PCR system, although the sensitivity of PCR was slightly better. It can detect more HPV in normal and ASCUS cytology samples. The overall agreement with PCR results of 77.5% was obtained.(Table 1) The result was slightly lower than the previous studies (91.7%) which utilized both type specific PCR primer system and consensus primer system (MY9/11).[14] It may be due to the small sample size in the current study that limited the statistical power to the test. Besides, the use of SPF1/2 primer system may lower the concordance as the primer system has higher analytical sensitivity than that of HC-II, which was supported by our results in the current study (Table 3). Several research groups had reported that the agreement of HC-II and PCR was good, with the level of agreement being greater than 80%.[1, 14-16] When comparing HPV test result in normal and abnormal cytology, different levels of agreement as well as the sensitivity were observed (Table 2). The concordance (70.8%) was lower among women with normal cytology than those of abnormal cytology (87.5%). This is in agreement with the findings of Venturoli et al.,[16] who observed PCR and HC-II

assays showed good agreement for the detection of high-grade lesion (73%) but substantial disagreement for the detection of low-grade lesion (44%) and normal women (50%). In view of the lower detection sensitivity of HC-II for HPV as compared with PCR in women with normal or low grade lesion, a negative HC-II result should not be construed as a true negative HPV detection in the ThinPrep cytology samples.

Our study is in accordance with the previous finding that high risk HPV positivity is correlated with the degree of abnormal cytological results.[16, 17] In our study, HPV can be detected in women with normal cytology. The HPV prevalence in women with normal cytology by the use of PCR and HC-II are 50.0% and 20.8% It is noteworthy that the subjects in our study were not young, with a mean age of 42.9 years and only 15.0% aged less than 35 years. The chance of getting transient HPV infection in young women is low. The prevalence calculated from HC-II was in accordance with Venturoli et al.[16] who reported 21.3% prevalence in women with normal cytology. However, the prevalence calculated from PCR was higher than expected. It suggests that PCR detects more HPV positive smears than the HC-II assay in smears in which no cytological abnormality can be detected. It can be attributed to the high analytical sensitivity in PCR assay that can detect latent HPV infection as well as a broader spectrum of pathogenic HPV types. The PCR assay used in this study may disclose HPV infection at a very early phase. It is noteworthy that the discrepant result of cytological diagnosis and HPV testing is not uncommon. One should carefully consider the target populations and the age groups tested. Previous study had shown that the HPV detection rates are different between sexually transmitted disease clinic and clinic for routine gynecological screening.[18, 19] Furthermore, difference in age group also affects the period of HPV infection. It is because transient anogenital HPV infections are common in young, sexually active women.[20] The majority of transient anogenital HPV infection will not develop into persistent infection, and only the persistent infection of high risk HPV may cause intraepithelial lesions.[21] Thus, the detection of HPV DNA does not increase the risk for cervical cancer.

In the present study, only samples with high risk HPV have abnormal cytology smears. In screening for high grade lesions, the estimated sensitivities for the detection of high risk HPV types have been 50% by both PCR and HC-II assay (high risk probe). Both assays fail to detect HSILs in 1 of 2 samples by HC-II high risk probe or type specific PCR. However, it showed positive result by HC-II low risk probe. The failure of PCR amplification may due to insufficient DNA template. Previous study also indicated that HPV typing of HC-II is influenced strongly by the cellularity of the specimens.[22] Samples with scanty cells may lead to false negative results. Kulmala et al. reported that the false negative results were due to low copy number of HPV DNA and deficient sample quality,[15] which was supported by other similar studies.[9, 23, 24]

Although the combination of SPF1/2 PCR and GP5+/6+ PCR is able to detect wide range of HPV infection, HPV type 57, MM4, MM7, MM8, PAP155 and PAP 291 can only be detected by using MY09/11 PCR. The HPV PCR can be further improved by incorporating a separate MY09/11 PCR.

In conclusion, the results of PCR and the HC-II assay were concordant for 77.5% of samples which indicated good reproducibility. For sample with abnormal cytology, the concordance was 87.5%, resulting in substantial reproducibility. The sensitivity of PCR is better than HC-II. On the other hand, the specificity of HC-II is better than PCR. The HC-II is technical well designed and easy to perform. Although the sensitivity of PCR is excellent, it requires carefully controlled laboratory environment and skillful technologist to perform the test.

Table 1. Comparison between PCR and HC-II on 40 samples

HPV-DNA test*	PCR+	PCR -	Total
HC-II +	15	1	16
HC-II -	8	16	24
Total	23	17	40

* +, positive result; -, negative result

Table 2. PCR and HC-II results for women with normal and abnormal cytology.

	PCR+	PCR -	Total
Cytology normal			
HC-II +	5	0	5
HC-II -	7	12	19
Total	12	12	24

	PCR+	PCR -	Total
Cytology abnormal			
HC-II +	10	1	11
HC-II -	1	4	5
Total	11	5	16

* +, positive result; -, negative result

Table 3. Correlation of HPV detection with cytological abnormalities in ThinPap® smears

HPV profile	No. (%) of samples with the following cytological grade†:					
	Normal	ASCUS	LGSIL	HGSIL	Cancer	Total
PCR+, HC2+	5 (20.8)	3 (37.5)	6 (100%)	1 (50%)	0 (0)	15
PCR+, HC2-	7 (29.2)	1 (12.5)	0 (0)	0 (0)	0 (0)	8
PCR-, HC2+	0 (0.0)	0 (0.0)	0 (0)	1 (50%)	0 (0)	1
PCR-, HC2-	12 (50.0)	4 (50.0)	0 (0)	0 (0)	0 (0)	16
Total	24	8	6	2	0	40

* +, positive result; -, negative result

†ASCUS: atypical squamous cells of undetermined significance; LGSIL: low grade squamous intraepithelial lesion; HGSIL: high grade squamous intraepithelial lesion.

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