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ORIGINAL ARTICLE

Previous Papanicolaou and Hybrid Capture 2 human papillomavirus testing results of 5699 women with histologically diagnosed cervical intraepithelial neoplasia 2/3

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Introduction Cervical cancer remains an important public health problem in Chinese women owing to the lack of a national screening program. The aim of the present study was to evaluate human papillomavirus (HPV) and Papanicolaou (Pap) test results preceding the histologic diagnosis of cervical intraepithelial neoplasia 2/3 (CIN2/3) in China's largest College of American Pathologists-certified clinical laboratory.

Materials and methods All cases of CIN2/3 histologically diagnosed from January 2011 to August 2016 were retrieved from the pathology department records. The Pap cytology and HPV test results from the 6 months before the CIN2/3 diagnoses were analyzed.

Results A total of 5699 patients with histologically diagnosed CIN2/3 had previous Pap and/or HPV Hybrid Capture 2 testing results within the previous 6 months. The average age was 39.5 years (range, 16-82 years). Of these patients, 4288 had Pap test findings (average, 1.5 months) available. The results were high-grade squamous intraepithelial lesion in 44.1%, low-grade squamous intraepithelial lesion in 20.0%, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, in 16.0%, atypical squamous cells of undetermined significance, in 12.3%, atypical glandular cells in 0.7%, and negative in 6.9%.

Tao Wu and Xiangbai Chen are co-first authors.

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Of the 5699 patients, 2546 had HPV Hybrid Capture 2 test results (average, 1.4 months) available. Of these, 91.7% had positive results and 8.3% had negative results. Of 1135 patients with both previous Pap and HPV results, 7.1% had negative HPV results and 8.0% had negative Pap results ($P = 0.38$). Only 21 patients (1.9%) had double negative results.

Conclusions The present study has reported the previous results of HPV testing and Pap cytology for patients with high-grade cervical squamous precursor lesions in a population of women in China who had not undergone intensive previous screening. Both high-risk HPV and Pap cytology had similar negative testing rates for these women, although double negative results were less common. These results support the value of combined testing in the detection of cervical cancer precursors.

Introduction

Cervical cancer is the second most common cancer in woman in China, where women receive inadequate cervical screening. In contrast, cervical cancer is reduced to the sixth most common cancer in women in countries with established cervical screening programs. Recent reported data have revealed that nearly 100,000 new cervical cancer cases will be diagnosed in China annually and that 30,000 women will die of it each year.[1] These data might have been underestimated owing to the lack of a well-established national cancer registry. Women who have never undergone screening or only rarely will develop invasive cancer at a younger age and the cancer will diagnosed at a later stage compared with women who have undergone screening more frequently.[2] Beginning in 2009, a free government-sponsored National Cervical Screening Program in Rural Areas was begun in >200 counties, including every provincial-level administrative division.

Although ~90% of human papillomavirus (HPV) infections will resolve or become latent within <2 years, persistent HPV infection with high-risk genotypes will result in the development of precancerous lesions and cancer of the cervix in most cases. The development of a cervical intraepithelial neoplasia (CIN) 2/3 lesion after persistent high-risk HPV (hrHPV) infection will usually require a few years.[3] In contrast, the transformation of CIN3 to invasive cervical cancer will generally require >1 decade.[3] Therefore, it would be reasonable to screen for cervical cancer every 3 to 5 years.

At present, 3 common strategies are available for cervical cancer screening: Papanicolaou (Pap) cytology only, HPV testing only, and Pap/HPV combined testing. The Cobas HPV test (Roche Molecular Systems, Inc, Branchburg, NJ) was approved by the Food and Drug Administration for primary cervical cancer screening in women aged ≥25 years in 2014. The American screening guidelines have been well acceptable worldwide; however, different nations should establish their own cervical screening program according to their own prevalence of HPV genotypes, the characteristics of their screened population, and access to physicians. Genotypes 16 and 18 have been the most common hrHPVs in America; however, genotypes 16 and 52 have been the most common in China.[4]

KingMed Diagnostics is the largest nationwide pathology laboratory in China and the first pathology laboratory certified by the College of American Pathologists in China. The objective of the present study was to provide retrospective data to evaluate the hrHPV and Pap test results before the histologic diagnosis of CIN2/3 at the KingMed Diagnostics Guangzhou Laboratory. The Pap cytology and HPV tests were analyzed to determine the efficacy of a cervical screening program in a population receiving inadequate cervical screening.

Materials and methods

Patient selection

Guangzhou KingMed Diagnostics is the largest independent pathology laboratory that has been fully certified by the College of American Pathologists in China. Patients with a histological diagnosis of CIN2/3 were retrospectively identified in the pathology database at Guangzhou KingMed Diagnostics for a 68-month period from January 2011 to August 2016. Previous HC2 hrHPV and PAP test results at Guangzhou KingMed Diagnostics were recorded and only those cases with previous hrHPV and/or PAP cytology test results within 6 months before the histological CIN2/3 diagnosis were included.

Of these samples, >90% were from Guangdong Province. Surgical specimens were collected from ~1000 hospitals throughout Guangdong Province and referred to Guangzhou KingMed Diagnostics. Most cases originated from local community hospitals, which serve populations primarily from suburban and rural areas. The Pap and HPV test specimens were collected by clinicians from >1000 local community hospitals, women's healthcare centers, clinics, and physical examination centers. Because China has no well-established national cancer registry or national cervical cancer screening program, clinicians request Pap and/or HPV testing for a variety of reasons. Pap tests and HPV testing specimens are received at Guangzhou KingMed Diagnostics separately and analyzed by different departments (ie, the Cytopathology Department and Molecular Microbiology Department, respectively). hrHPV testing is performed at KingMed Diagnostics for all studied

cases; however, some patients might have had Pap cytology test results reported by another laboratory and, therefore, were not included in the present study.

Pap testing

Pap tests were performed at Guangzhou KingMed Diagnostics cytology laboratory using a variety of methods, including conventional Pap smears and 4 liquid-based cytology preparations (ie, ThinPrep [Hologic, Bedford, MA]; SurePath [BD Diagnostics, Franklin Lakes, NJ]; Liqui-PREP [LGM International, Melbourne, FL]; and LITUO [Lituo Biotechnology Co, Ltd, Hunan, China]), and conventional Pap testing. Detailed methods, The Bethesda System report rates, and introduction to the KingMed Diagnostics cytology laboratory have been previously described in our recent reports.[1,5]

hrHPV testing

hrHPV Tests were performed at Guangzhou KingMed Diagnostics molecular laboratory using the Hybrid Capture 2 (HC2) assay (Qiagen, Hilden, Germany), which tests for hrHPV and intermediate-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. These samples were collected and stored in digene Standard Transport Medium (Qiagen) in containers that differed from those used for the Pap samples. The results of HC2 HPV testing were reported as either positive or negative. The cases with equivocal HC2 results were excluded from the present study.

Histopathologic diagnosis

All diagnoses of CIN2/3 in the present study were from histopathologic interpretations of surgical specimens, including cervical biopsy, endocervical curettage, and excisional procedures using loop electrosurgical excision or cold knife cervical conization. Histologic diagnoses were rendered by surgical pathologists at the surgical pathology department at Guangzhou KingMed Diagnostics. Immunohistochemical staining with p16 and Ki-67 was liberally used to increase the reliability of the CIN2/3 diagnoses.

Statistical analysis

Statistical analysis using the Pearson χ^2 test was conducted using SAS, version 9.1, software (SAS Institute, Gary, NC). P values <0.05 were considered statically significant.

Results

A total of 15,415 patients with a histologic diagnosis of CIN2/3 were identified during the study period. Of these patients, 5699 with a histologic diagnosis of CIN2/3 had previous Pap and/or HC2 HPV testing results available from the previous 6 months. The average age of these patients was 39.5 years (range, 16-82 years).

Previous cytologic results

Of the 5699 patients, 4288 (75.2%) had Pap test results within 6 months before the CIN2/3 diagnostic surgical procedures available in the Guangzhou KingMed cytology laboratory. The average interval between the histologic diagnosis and previous Pap test result was 1.5 months (range, 0.2-6 months). Details of the Pap test results before the histologic diagnosis of CIN2/3 are listed in Table 1. A high-grade squamous intraepithelial lesion (HSIL) was the most common abnormal cytologic result (44.1%). In contrast, a finding of a low-grade squamous intraepithelial lesion and a finding of atypical squamous cells, cannot exclude HSIL, preceded 20% and 16% of the CIN2/3 diagnoses, respectively. Atypical squamous cells of undetermined significance preceded 12.3% of the CIN2/3 diagnoses, and atypical glandular cells preceded <0.1% of the CIN2/3 diagnoses. A previous negative Pap result was found in 6.9% of cases. The negative Pap reporting rate with conventional Pap testing was lower than that with liquid-based cytology (4.1% versus 7.2% [275 of 3803]; $P = 0.0106$). The negative Pap testing rate was the highest with Liqui-PREP (LGM International); however, the total case number was too small to obtain a significant conclusion. The negative rate with ThinPrep (Hologic) was not significantly different statistically compared with the rate with SurePath (BD Diagnostics; $P = 0.2796$) but was significantly different from that with LITUO (Lituo Biotechnology Co, Ltd; $P < 0.001$).

HC2 results

Of the 5699 patients, 2546 (44.7%) had HC2 testing results available within 6 months before the histologic diagnosis of CIN2/3. Their average age was 38.5 years (range, 18-77 years). The average interval between HC2 testing and the histologic diagnosis of CIN2/3 was 1.4 months (range, 0.2-6 months). Of these 2546 patients, 2334 (91.7%) had positive HC2 hrHPV results and 212 (8.3%) had negative HC2 test results.

Comparison of Pap and HPV test results

Of the 2546 patients with HPV test results, 1135 (44.6%) also had Pap test results within 6 months before the histologic diagnosis of CIN2/3. Of the 1135 patient with both previous Pap and HPV test results available, 7.1% of the patents had negative HPV test results and 8.0% had negative Pap test results ($P = 0.38$). Details of the Pap cytology results and related HPV test results are listed in Table 2. Of the 1135 patients, 985 (86.8%) had had abnormal Pap and positive HPV test results. Only 21 patients (1.9%) had had

Table 1 Papanicolaou test result before histologic diagnosis of cervical intraepithelial neoplasia 2/3.

Method	Total (n)	HSIL	LSIL	ASC-H	ASC-US	AGC	Negative
ThinPrep	1964	804[a] (40.9)	372 (18.9)	326 (16.6)	271 (13.8)	18 (0.9)	173 (8.8)
SurePath	405	169 (41.7)	130 (32.1)	37 (9.1)	37 (9.1)	3 (0.7)	29 (7.2)
Liqui-PREP	85	31 (36.5)	16 (18.8)	14 (16.5)	11 (12.9)	0 (0.0)	13 (15.2)
LITUO	1348	676 (50.1)	250 (18.6)	226 (16.8)	129 (9.6)	7 (0.5)	60 (4.5)
CPS	486	209[b] (43.0)	89 (18.3)	85 (17.5)	80 (16.5)	3 (0.6)	20 (4.1)
Total	4288	1889 (44.1)	857 (20.0)	688 (16.0)	528 (12.3)	31 (0.7)	295 (6.9)

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cell, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN2/3, cervical intraepithelial neoplasia 2/3; CPS, conventional Papanicolaou smear; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
Data presented as n (%).
[a]Included 9 malignant Papanicolaou test results.
[b]Included 1 malignant Papanicolaou test result.

double negative results. When examining the results of Pap testing in these patients within the context of the HPV test results, the abnormal Pap test rate was 93.4% (985 of 1055 patients) among the patients with positive HPV test results, which was significantly greater than the abnormal Pap test rate of 73.7% (59 of 80 patients) for the patients with negative HPV test results ($P = 0.0001$). The very low double-negative rate (1.9%) and high rate of negative HPV test and positive Pap test results (73.7%) strongly argue against using the HPV test as the primary test for cervical cancer screening in clinical practice.

Discussion

The present study analyzed 5699 patients with a histologic diagnosis of CIN2+ and previous Pap and/or HPV testing results. To the best of our knowledge, the present study is one of the largest studies of similar research retrieved from PubMed. The most common cytologic diagnoses within 6 months before the histologic diagnosis of CIN2+ was HSIL (44.1%), comparable to the data from the University of Pittsburgh, which reported that 41.6% of 2827 cases with a histologic CIN2+ diagnosis was HSIL.[6] Although it has been believed that the ThinPrep test (Hologic) is significantly better to detect high-grade squamous intraepithelial lesions than conventional smear,[7] we found no statistically significant difference in HSIL diagnosis between the ThinPrep and conventional smear groups. The present study of patients with a histologic CIN2+ diagnosis indicated that 92.9% of patients will have positive hrHPV test results, 92.0% of patients will have abnormal Pap smear results, and 1.9% of patients will have double-negative HPV and Pap test results. These data concur with those from the study of 4090 cases with a CIN3+ diagnosis at multiple clinical centers by Blatt et al.[8] They reported that 94% of patients had positive hrHPV test results, 92.3% of patients had an abnormal Pap smear result, and 1.2% patients had double-negative HPV and PAP results.[8]

Table 2 Previous Papanicolaou and high-risk human papillomavirus test results for 1135 patients.

Category	HPV positive (n)	HPV negative (n)	Total (n)	HPV positive (%)	HPV negative (%)
HSIL	442[a]	20	462[a]	0.957	0.043
LSIL	216	8	224	0.964	0.036
ASC-H	153	12	165	0.927	0.073
ASC-US	161	19	180	0.894	0.106
AGC	13	0	13	1.000	0.000
Negative	70	21	91	0.769	0.231
Total	1055	80	1135	0.930	0.070

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
[a]Included 2 malignant Papanicolaou test results.

Since HPV testing was approved as the primary cervical screening test in 2014, debates have ensued regarding the best strategy. After assessing the 5-year cumulative risk in 331,818 women, Katki et al[9] concluded that the 5-year cumulative incidence of cervical cancer for HPV-negative women was extremely low (3.8 per 100,000 women annually), only slightly greater than that for women with HPV-negative and Pap-negative test results (3.2 per 100,000 women annually). The results reported by Gage et al[10] support a conclusion that a negative cytology result provides little or no extra reassurance against the presence of cervical cancer beyond that conferred by an HPV-negative test result. However, our study found that 7.8% of patients with CIN2/3 had had a negative HPV test result within 6 months previously, and these high-grade squamous lesions might have been missed using HPV-only screening. Other studies have reported similar findings, with an HPV negative rate of ~8% to 12% for women with a CIN2/3 lesion and ~12% to 15% for women with cervical cancer.[11-14] It has been believed that HPV testing as the primary screening test will miss a substantial number of precancer and cancer cases. In contrast, when the HPV test result is positive, no

definitive guidelines or practical triage are available for intervention. Many studies have shown that HPV testing is more sensitive and less specific than cytology,[15-18] which would lead to overtreatment if every patients with positive HPV results underwent colposcopy. Therefore, the use of the HPV test as the primary screening test has inherent drawbacks.

Our study had 1 promising result for the use of HPV/Pap combined testing in that the negative rate for both tests was 1.9%. Other studies have reported similar results that the double-negative rate for combined HPV and Pap testing was ~0.2% to 0.4% in women with CIN2/3 and 4% to 5% in women with cervical cancer.[19-21] All these studies have shown that HPV/Pap combined testing is more sensitive and specific than HPV testing only and will miss many fewer cases of precancer and cancer. After persistent hrHPV infection, the development of a CIN2/3 lesion will usually require a few years. In contrast, the transformation of CIN3 to invasive cervical cancer will generally require >1 decade. Furthermore, in China, many women are underinsured or uninsured and live in remote rural areas. Data have shown that 5 years would be a reasonable interval for cervical screening in China, which might help women better comply with the screening guidelines. Therefore, the optimal practice might be to perform HPV/Pap combined testing every 5 years for cervical cancer screening in China.

The present study had several strengths. To the best of our knowledge, the present study is one of the largest reviewing data on the efficacy of cervical screening tests. As is well-known, the negativity of HPV testing is affected by the interval between HPV testing and the diagnosis of CIN. Data from the University of Pittsburgh Medical Center study indicated that the HPV test result was 97.4% positive within 4 months of a CIN2/3 diagnosis and only 83% positive with an interval of 4 months to 3 years to the cancer diagnosis.[6] HPV infections will have resolved within 8 months in most cases.[3] Therefore, the interval for HPV testing should be ~8 months, which will increase the sensitivity to detect cervical precancerous lesions and cancer. The interval used in the present study was within that range.

Conclusions

The results from the present study have shown that HPV/Pap combined testing is more sensitive and specific than HPV testing only or Pap testing only. Combined testing could lengthen the interval of cervical screening, which might be a better fit for the Chinese female population, underinsured and uninsured women, and women living in underserved and remote areas. More clinical data are required to establish Chinese national cervical screening guidelines.

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Conflict of interest disclosures

The authors made no disclosures.

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