RESEARCH COMMUNICATION

Efficacy of Pap Test in Combination with ThinPrep Cytological Test in Screening for Cervical Cancer

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Abstract

Background: Our aim was to investigate the efficacy of the Pap test in combination with the ThinPrep cytological test (TCT) in screening for cervical cancer in China. Design: From March 2006 to October 2008, 988 women with the mean age 46.4 ± 10.5 years (range, 23-80 years) were recruited to receive cervical cancer screening. Pap test results ≥ grade III and TCT findings ≥ ASCUS/AGUS were considered abnormal. Subjects with a Pap test result ≥ grade IIb received TCT. Colposcopy and biopsies were performed in all participants, and final diagnosis was based on pathological findings. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden index for predicting CIN I or above were determined. Results: The sensitivity, specificity, PPV, NPV and Youden index of the Pap test were 43.1%, 97.2%, 70.0%, 91.9%, and 40.3%, respectively. The same values for TCT in predicting CIN were 80.0%, 63.2%, 16.0%, 97.3%, and 43.2%, respectively. The two tests in combination gave values for predicting CIN of 64.8%, 87.6%, 43.6%, 94.4%, and 53.5%, respectively. Combined testing exhibited the highest Youden index (53.4%). Conclusion: The Pap test with a reduced threshold in combination with the TCT has high sensitivity and high specificity in screening for cervical cancer.

Keywords: Cervical cancer - screening method - pap test - ThinPrep cytological test - Youden index - China

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Introduction

Although cervical cancer is a preventable malignancy, the incidence of cervical cancer is increasing, especially in developing countries. China is in a rapid development stage, and the economic development and medical resources are unevenly distributed. Currently, China has the largest population with cervical cancer. (Yang et al., 2000; Parkin et al., 2005; Li and Kang, 2011; Shi et al., 2012). Thus, developing an applicable method and screening program in China is of great importance. The Pap test has been used for cervical cancer screening for more than 50 years, and it has been confirmed as an economic, simple and effective method. However, it has some disadvantages including low sensitivity and high false negative rate (Fahey et al., 1995; Abulafia et al., 2003; Carns and Fadare, 2008; Li et al., 2009).

To overcome the disadvantages of the Pap test, liquid-based cytological methods and DNA-based methods of screening have become available (Li et al., 2009; Shi et al., 2011). The ThinPrep cytological test (TCT), a liquid-based method has been shown to have an increased sensitivity over that of the Pap test in screening for cervical cancer; however, it is more costly and requires specific equipment and specially trained personnel (Abulafa et al., 2003; Carns ey al., 2008). However, screening of very large numbers of individuals in a largely rural setting is associated with significant costs and logistical difficulties (Belinson et al., 1999; Belinson et al., 2001; Belinson, 2002; Pan et al., 2003; Li et al., 2009). Currently, the TCT has not been widely applied in China.

The purpose of this study was to develop a screening method for cervical using the Pap test with a reduced threshold as the initial method and then the TCT as follow-up to abnormal Pap tests. We hypothesize that this method may increase the quality of screening for cervical cancer while reducing the costs.

Materials and Methods

Subjects

From March 2006 to October 2008, a total of 988 women with the mean age 46.4 ± 10.5 years (range, 23-80 years) from 9 town of Shanghai Pudong New Area (Caolu, Gaohang, New Chuansha, Sanlin, Shiwan, Huamu, Puxing, Jinyang and Yangjing) were recruited to receive cervical cancer screening. Of the 988 participants, 362 (41.2%) were > 50 years of age, 34 (3.4%) were > 65 years of age. All women were local residents, married, non-pregnant, and having their menstrual period.

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Methods

All women received a routine gynecological examination performed by a physician gynecologist and cervical cells were collected for cytological examination.

A Pap test was performed for all subjects and sample preparation, fixation, and hematoxylin and eosin (H&E) staining were done by an experienced pathologist. The results of the Pap test were classified into 5 grades: grade I, normal cells; grade II, presence of dysmorphic cells which might be attributed to inflammation; IIb, presence of dyskaryosis in several cells but malignancy was excluded; grade III, suspected malignancy but evidence was insufficient; grade IV, malignancy highly suspected malignancy; grade V, definite malignancy.

Women were randomly selected for the TCT. In addition, women with a grade IIb Pap test also received TCT. Materials and reagents for the TCT were purchased from Beijing TCT Medical Technology Co., Ltd., China. Sample collection, section preparation, and staining were performed according to the manufacturer's instructions. Pathological examination was done by an experienced pathologist. Results were classified into 6 grades according to The Bethesda System (TBS): within normal limit (WNL); atypical squamous cells of undetermined significance (ASCUS); atypical glandular cells of undetemined significance (AGUS); low grade squamous intraepithelial lesion (LSIL); high grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma (SCC) (Thrall et al., 2001).

Colposcopy and biopsy for pathological examination

Colposcopy was performed by an experienced gynecologist with a SKLL Colposcope (Shenzhen Certainn Technology Co., Ltd., China). The subjects who received colposcopy had: 1) ≥ IIb Pap test results; 2) ≥ ASCUS/AGUS TCT results; or 3) were volunteers with a negative Pap test. The lesions were carefully identified and biopsies of tissues suspicious for cervical cancer were collected for pathological examination. In patients in whom no abnormal areas could be identified, biopsies were performed at the 3, 6, 9, and 12 o'clock positions. Biopsy tissues were fixed in 10% formaldehyde followed by paraffin-embedding, sectioning, and staining. Results were expressed as chronic cervicitis, cervical intraepithelial neoplasia I (CIN I), CIN II, CIN III, or squamous cell carcinoma (SCC). A result of ≥ CIN I (CIN I+) was considered to represent an abnormality.

Grouping

Subjects were grouped based on the pathological examination of cervical cells: Pap test group; TCT group; Pap test + TCT group. A ≥ grade III Pap test was considered abnormal, and for the TCT, ≥ ASCUS/AGUS was considered abnormal. In the Pap test + TCT group, when the Pap test result was grade IIb, the TCT test was performed and abnormal was considered a Pap test result ≥ grade III or a TCT result ≥ ASCUS/AGUS.

Observations

Definitive diagnosis was based on the pathological findings of colposcopy biopsy specimens. The sensitivity (true positive/[true positive + false negative]), specificity (true negative/[true negative + false positive]), positive predictive value (PPV; true positive/[true positive + false positive]), negative predictive value (NPV; true negative/[true negative + false negative]), and Youden index (sensitivity + specificity -1) were calculated. True positive was defined as 1) grade III-V pap smear and ≥ CIN on colposcopy biopsy specimens or 2) ≥ ASCUS/AGUS on TCT and ≥ CIN on colposcopy biopsy specimens. True negative was defined as 1) grade I-II pap smear and negative colposcopic biopsy pathological examination or 2) negative TCT and negative colposcopic biopsy pathological examination. False positive was defined as 1) grade III-V pap smear and negative colposcopic biopsy pathological examination or 2) ≥ ASCUS/AGUS on TCT and negative colposcopic biopsy pathological examination. False negative was defined as 1) grade I-II pap smear and ≥ CIN on colposcopy biopsy specimens or 2) negative TCT and on colposcopy biopsy specimens.

Statistical analysis

Results were summarized by the frequency (count), sensitivity, specificity, PPV, NPV, and Youden Index (sensitivity + specificity - 1).

Results

Pap test screening

A total of 988 women received Pap tests and colposcopy. Of these women, the results were grade I-IIa in 742 (abnormal rate 5.66%), grade IIb in 166 (abnormal rate 19.28%), grade III in 66 (abnormal rate 68.18%), and grade IV in 14 (abnormal rate 78.57%). None had grade V. Results are shown in Table 1.

In Pap testing, grade III was used as a threshold to detect CIN I+. Results showed true positive in 56 women, true negative in 834, false positive in 24, and false negative 74. The sensitivity, specificity, PPV, NPV and Youden index were 43.08%, 97.20%, 70.00%, 91.85% and 40.28%, respectively.

TCT screening

TCT and colposcopy were performed in 310 women, and results are shown in Table 2. Of these women, 185 were negative (abnormal rate 2.7%), ASCUS was found in 51 (abnormal rate 11.76%), AGUS in 38 (abnormal rate 5.26%), LISL in 23 (abnormal rate 21.74%), and HISL in 13 (53.85%). True positive was noted in 20 women, true

Table 1. Comparison of Colposcopy Findings and Pap Test Results

Pap smear results	No.	Colposcopy Findings				Abnormal (%)
		Negative	CIN I	CIN II-III	Cancer	
I-II a	742	700	36	5	1	5.66
II b	166	134	14	15	3	19.28
III	66	21	20	22	3	68.18
IV	14	3	2	7	2	78.57
Total	988	858	72	49	9	13.16

A total of 988 participants received both Pap smear and colposcopy. Abnormal indicates colposcopy findings were CIN I, II, or III or malignancy

Table 2. Comparison of Colposcopy Findings and TCT Result

Pap smear results	No.	Colposcopy Findings				Abnormal (%)
		Negative	CIN I	CIN II-III	Cancer	
Negative	185	180	3	2	0	2.7
ASCUS	51	45	4	2	0	11.76
AGUS	38	36	2	0	0	5.26
LSIL	23	18	3	2	0	21.74
HSIL	13	6	2	5	0	53.85
Total	310	285	14	11	0	8.06

A total of 310 participants received both TCT and colposcopy. Abnormal indicates colposcopy findings were CIN I, II, or III or malignancy

Table 3. Colposcopy Findings in Women Who Received the Pap Test and the TCT

Test results	Number of Women	Colposcopy Finding				Abnormal (%)
		Negative	CIN I	CIN II-III	Cancer	
Grade I-IIa	742	700	36	5	1	5.66
Grade IIb						
TCT: negative	44	42	2	0	0	4.55
TCT: ASCUS	59	51	5	3	0	13.56
TCT: AGUS	24	24	0	0	0	0
TCT: LSIL	9	4	2	3	0	55.56
TCT: HSIL	14	2	3	8	1	85.71
Grade III	66	21	20	22	3	68.18
Grade IV	14	3	2	7	2	78.57
Total	972	847	70	48	7	12.86

A total of 972 participants received Pap smear, TCT, and colposcopy. Abnormal indicates colposcopy findings were CIN I, II, or III or malignancy

Table 4. Comparisons of Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, and Youden Index of Different Tests

Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Youden Index (%)
Pap test (grade III)	43.08	97.2	70	91.85	40.28
TCT	80	63.16	16	97.3	43.16
Pap test + TCT	65.85	87.6	43.55	94.64	53.35

negative in 180, false positive in 105, and false negative in 5. The sensitivity, specificity, PPV, NPV, and Youden index of ASCUS in predicting CIN were 80.00%, 63.16%, 16%, 97.3% and 43.16%, respectively.

Pap test combined with TCT screening

A total of 742 women with grade I-IIa and 44 women with grade IIb Pap test results and negative TCT underwent colposcopy. Results showed CIN I+ in 44 women (Table 3). Screening with the Pap test and TCT revealed true positive in 81 women, true negative in 742, false positive in 105, and false negative in 44. The sensitivity, specificity, PPV, NPV, and Youden index of the Pap test combined with TCT in predicting CIN I+ were 64.80%, 87.60%, 43.55%, 94.40%, and 53.45%, respectively.

Comparisons among different tests

Results following screening with different tests are shown in Table 4. The Pap test exhibited the lowest sensitivity (43.08%), followed by combined testing (65.85%), and TCT exhibited the highest sensitivity (80%). The Youden index of the TCT and Pap test was 43.16% and 40.28%, respectively. Combined testing exhibited the highest Youden index (53.35%).

Discussion

American Cancer Association statistics indicate that the mortality of cervical cancer patients was reduced by 74% during the period of 1955-1992 after the Pat test was introduced as a screening test (American Cancer Society, 2008). In 1970s, the mortality of cervical cancer patients was approximately 9.98/100000 in China, while a survey reported that during the period from 1990-1992 the mortality was reduced to 3.25/100000 (Cao 2002). Despite this reduction, data has shown that the incidence of cervical cancer increased 14% during the period from 2000-20005 (Yang et al., 2000; Shi et al., 2012). There are about 493,100 new cases of cervical cancer annually worldwide, and 273,000 women die of cervical cancer (Parkin et al., 2005). Moreover, 80% of cervical cancer patients are found in underdeveloped countries (Goldie et al., 2003; American Cancer Society, 2008), which may be attributed to the low economic level and less use of screening.

China is a country with large population with a relatively low standard of living. In addition, the distribution of medical resource is markedly uneven, and the use of cervical cancer screening varies widely in the different regions of China. For these reasons, the incidence of cervical cancer is relatively high in China. Although the Pap test has been widely used for cervical cancer screening and has reduced the incidence and mortality from cervical cancer, it has poor sensitivity and is gradually being replaced by newer methods. TCT has been used for a number of years, and the detection rate for cervical cancer (especially poorly differentiated cervical cancer) (Guo et al., 2005) is markedly greater as compared to the Pap test (Abulafia et al., 2003). However, the TCT is costly as compared to the Pap test and requires specialized equipment and skilled personal, thus is application in clinical practice has been slow. Screening a large, rural population must take into consideration the sensitivity and specificity of the testing, ease of administration, and costs involved (Belinson et al., 1999). Although there have been a number of studies evaluating different methods and screening programs for cervical cancer in China, no ideal one has been identified (Belinson et al., 1999; Belinson et al., 2001; Belinson, 2002; Pan et al., 2003; Li et l., 2009).

In the present study, the Pap test was used in combination with the TCT as a method for screening for cervical cancer. First, the Pap test was performed for preliminary screening and the Pap test threshold was reduced from grade III to Grade IIb in an attempt to elevate its sensitivity. Patients with a grade IIb Pap test subsequently were tested with the TCT which has a relatively high sensitivity. The combination test screening preserves the advantages of the Pap test (simplicity and low cost) while taking advantage of the higher sensitivity of the TCT.

In studies comparing methods of cervical cancer screening, pathological findings following colposcopy

used as the gold standard. It has been reported that the diagnostic accuracy of biopsies taken during colposcopy is 67% to 97%, and the correlation between colposcopic biopsies and postoperative pathological diagnosis is as high as 97.2% (Carns and Fadare, 2008). In the present study, pathological findings following colposcopy served as the standard, and the predictive value of the Pap test, the TCT, and the combination test were compared. The results showed that the sensitivity of the combination test was 65.85%, which was markedly higher than that of Pap test (43.08%), but comparable to that of the TCT alone (80.00%) which is similar to the sensitivity of the TCT previously reported (Belinson et al., 2001). A study examining the use of only the TCT for screening reported that the TCT with ASCUS as positive had a sensitivity for CIN 2 of 94% and specificity of 78%, a sensitivity for CIN 3 of 98%, and for cancer of 100% (Belinson et al., 2002).

Although the sensitivity of the TCT was higher than that of the Pap test, the specificity of the TCT was lower than that of Pap test. It is known that sensitivity is negatively correlated with specificity (Fahey et al., 1995; Carns and Fadare, 2008). Screening with a test that provides high sensitivity and low specificity will produce a high false positive rate, which is not acceptable when screening a large population with limited resources as in China.

The Youden Index is a receiver operating curve (ROC) summary statistic that optimizes a biomarker's differentiating ability by giving equal weight is given to sensitivity and specificity (Ruopp et al., 2008). In the present study, we compared the Youden Index of different methods of screening, and found that the Youden Index of combined testing was markedly higher than that of the Pap test and the TCT, but the Youden Index of the Pap test was comparable with that of the TCT. This indicates that the increase of sensitivity of the TCT is at the cost of reduced specificity, and the increased specificity of the Pap test is at the cost of reduced sensitivity. The increased sensitivity of the combination test is attributable using a lower threshold for the Pap test, and the increased specificity is related to the TCT with high sensitivity. In this study, a higher sensitivity and specificity was found when a grade IIb Pap smear was used a threshold to perform a secondary screening with TCT.

The results of women who received the TCT alone and those who received it due to a grade IIb Pap test were further analyzed. Results showed that, among women with LSIL or HSIL on the TCT, 73.91% were found to be positive for CIN I in combination testing, which was significantly higher than that of TCT alone (33.3%). This indicates that the TCT exhibits a relatively high false positive rate, which necessitates a second screening.

In the present study, the Pap test threshold for further testing was decreased to grade IIb and 1 woman with grade IIb was diagnosed with cervical cancer and 14 women with grade IIb were diagnosed having CIN II-III. Thus, we postulate that the low sensitivity of the Pap test might be related to the threshold used. Whether the threshold used for the Pap test requires modification may be a subject of further study.

The rate of positive tests and consistency with pathological examination of biopsy specimens for the Pap test alone and the TCT alone were lower than those previously reported in China (Bian et al., 2004; Ling et al., 2004; Carns and Fadare, 2008; Tu et al., 2008). In addition, the proportion of women with grade IIb Pap tests and those with ASCUS on TCT was low in our study. Also, there was a relatively high percentage of elderly women in the present study and cervical atrophy in the elderly can increase the false positive rate of the Pap test (Weintraub et al., 1987). These may be contributing factors for the low rate of positive tests and consistency with biopsy specimens.

The primary limitations of the study are its cross-sectional design, lack of follow-up data, and no cost analysis was performed. In addition, we did not examine HPV-DNA testing.

In conclusions, taken together, our findings demonstrate that using the Pap test with a reduced threshold in combination with the TCT has high sensitivity and high specificity in screening for cervical cancer and is simple to administer.

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