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Clinical Application of Folate Receptor-Mediated Staining Solution Detection in Cervical Cancer Screening

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Abstract. ***Objective***. Cervical cancer is the fourth most deadly women's cancer worldwide, and regular screening is essential to lower mortality rates. The folate receptor-mediated staining solution detection (FRD) has been suggested to be a rapid and cost-effective screening method. We aim to evaluate the validity of FRD testing in cervical cancer screening. ***Methods.*** A total of 207 participants were enrolled in the study. The validity of screening by FRD, cytology screening, and a HPV test were compared using histopathology as the gold standard. Sensitivity, specificity, positive predictive value, negative predictive value, Kappa value, positive likelihood ratio, negative likelihood ratio, percent agreement, and positive detection rates were compared among the three screening methods. ***Results.*** 83(40.1%) participants were diagnosed as NILM, 50(24.15%) were diagnosed as CIN1, and 74(35.74%) were diagnosed as CIN2+. For CIN2+, the detection rates for the FRD, cytology screening, and HPV were 75.68%, 82.09% and 93.22%, respectively. For CIN2+, the sensitivity of HPV testing (93.22%) was significantly higher than that of cytology screening (82.09%) and FRD (75.68%), while the specificity of FRD (63.91%) was higher than that of cytology screening (35.34%) and HPV test (7.56%). The percent agreement and Kappa value of FRD were significantly higher than those of the cytology screening and HPV test. In HPV-HC2+ and ASCUS patients, FRD was associated with a lower false positive rate compared to other screening methods. ***Conclusion***. Our study indicates that FRD has a good sensitivity and high specificity in cervical cancer screening, and could be a rapid, valid and cost-effective screening test.

Key words: folate receptor-mediated, staining solution detection, cervical cancer screening.

Introduction

Cervical cancer is the fourth most deadliest cancer for women worldwide [1]. In 2012, about 527,600 new cervical cancer cases were reported with approximately 265,700 deaths [2]. Countries are disproportionally affected by cervical cancer, with low- and medium- income countries bearing the greatest burden of this disease [3]. In the United States, about 12,990 new cases of cervical cancer will be diagnosed, with roughly 4,120 women dying from cervical cancer each year [4]. In China, there are 100,000 new cases diagnosed with approximately 30,000 deaths every year [5].

Persistent infections of high-risk HPV virus can lead to cervical cancer precancerous lesions, leading to cervical cancer. Regular screening for cervical cancer was found to be associated with an overall mortality prevention rate of 83% [6]. Cytological examination and high-risk HPV testing are currently the major cervical cancer screening methods worldwide [7]. However, these methods require experienced pathologists for reliable interpretation and advanced technology for HPV DNA testing. For rural primary hospitals with limited medical resources in China, there is a lack of pathologists, especially experienced ones. In addition, there are few resources that provide reliable DNA testing technology. A high rate of missed diagnosis is expected in the screening process. The test results usually take several days to get, and many patients fail to follow-up during this period. Therefore, it is necessary to find a screening solution that is fast, valid, and easy to operate.

Recent studies have found that folate receptors are highly expressed on the surface of tumor cells, while there is little to no expression on normal cell surfaces [8]. The folate receptor-mediated staining

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solution detection (FRD) is a dye solution that is used in staining cells and tissues for detecting neoplasms. FRD is mainly used to detect abnormal cervical lesions (CIN2+) in gynecological speculum examination. Compared to other tests, the results of FRD can be obtained within 30 seconds, requiring minimal training and technical support to interpret findings. Therefore, this makes FRD a potentially convenient and cost-effective screening test. In this study, we aimed to evaluate the validity of FRD testing in cervical cancer screening.

Materials and Methods

Participants. This prospective study conducted in Beijing Hospital was approved by the Hospital Institutional Review Board. All patients signed informed consent before being examined. All the researchers and technicians that participated in the research were trained to standardize protocol.

Patients who were seen in the outpatient clinic from April 2015 to August 2015 and provided consent were enrolled. The inclusion criteria were as follows: 1) women in the outpatient clinic with cytology screening and HPV test for cervical cancer screening within 90 days of pathology examination; and 2) cytology ≥ atypical squamous cells of undetermined significance (ASCUS) and/or positive high-risk HPV. Exclusion criteria were: 1) acute inflammation of the lower genital tract; 2) pregnancy or lactating; 3) those with hysterectomy; 4) those who were already diagnosed with CIN2+ before cytology screening; or 5) those who have done cervical treatment.

Cytology screening. A cervical cytology screening was performed using a liquid-based cytology method by Thinprep (Hologic, Inc., Massachusetts, USA). A specified cytobrush was placed into the cervical canal about 1 cm, and rotated 5 cycles clockwise. The cytobrush was then put into a container. The liquid was sent for examination after the brush was washed 10 times. A ThinPrep automatic pelleter was used to make smears. A diagnosis was made by 2 independently experienced pathologists.

HPV test. HPV DNA testing was performed by the Digene Hybrid Capture 2 (HC2, Qiagen, Crawley, UK) test, which was blind to TCT results. The Digene Microplate Luminometer 2000 (DML 2000; Qiagen, Crawley, UK) was used for reading and calculating the results of HPV testing.

FDR staining. FRD cervical staining was carried out according to the kit instructions (Shaanxi Gaoyuan Medical Equipment Service Co., Ltd, Xi'an, China). The FRD staining solution was applied to the ecto-cervix with a cotton swab by rotating the attached handle clockwise for five times and then pressing it for 10 sec. The FRD staining solution was similarly applied to the cervical canal. After removing the cotton swab, color changes were inspected within 30 seconds. The color change was compared to the reference color. Blue, dark blue, or black, which were shown on either cotton swabs in an area of no less than 2*2 mm indicated a positive result. Brown or green colors were classified as negative results. The results of each screening were hidden from gynecologists who performed the test.

Pathological examination. Colposcopies with biopsy and/or endocervical curettage (ECC) were performed by experienced gynecologists in all patients. The suspected abnormal site was biopsied under colposcopy. If there was no obvious abnormality, the four-quadrant biopsy was performed at the same time as the ECC. The ECC was performed by placing a long, thin instrument into the cervical canal and scraping a sample from that area. Biopsy samples were processed using paraffin-embedding. These samples were then sectioned and stained. The sample was then sent for 2 independent pathologists to review. The pathology results were treated as the gold standard. CIN nomenclature was used to categorize the CIN: CIN negative, CIN1, CIN2, CIN3 and cervical cancer.

Analyses. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for FRD, cytology screening, and HPV testing through the use of histopathology as the gold standard. The kappa value, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and percent agreement were used to assess the agreement between histopathology and FRD, cytology screening, and HPV testing. The positive detection rate (PDR) was compared among the three screening methods using a Chi-square test. All analyses were done with SAS 9.2. A two-sided $P<0.05$ was considered statically significant.

Results

A total of 207 participants were enrolled in the study. The mean age was 43.6±10.9 years old (range, 20-73). Among these patients, 8.7% of the participants were 20-29 years old, 31.4% were 30-39 years old, 25.6% were 40-49 years old, 31.9%

Table 1. *The detection rate of FRD for cervical lesions.*

Histo-pathology diagnosis	n(%)	FRD		TCT		HPV	
		Negative n(%)	Positive n(%)	NILM n(%)	≥ASCUS n(%)	Negative n(%)	Positive n(%)
NILM	83(40.10)	53(63.86)	30(36.14)	32(38.55)	51(61.45)	16(19.28)	67(80.72)
CIN1	50(24.15)	32(64.00)	18(36.00)	14(28.00)	36(72.00)	6(12.00)	44(88.00)
CIN2	27(13.04)	11(40.74)	16(59.26)	5(18.52)	22(81.48)	4(14.81)	23(85.19)
CIN3	31(14.98)	7(22.58)	24(77.42)	5(16.13)	26(83.87)	3(9.68)	28(90.32)
SCC	16(7.73)	0(0)	16(100.00)	2(12.50)	14(87.50)	0(0)	16(100.00)

Abbreviations: CIN, cervical intraepithelial neoplasia; FRD, folate receptor-mediated staining solution detection; HPV, Human papillomavirus; NILM, intraepithelial lesion or malignancy; SCC, squamous cell carcinoma. TCT, Thinprep cytologic test.

Table 2. *Clinical performance of FRD, cytology screening and HPV test*

	FRD% (95%CI)	TCT% (95%CI)	HPV% (95%CI)
Sensitivity	75.68(65.90-85.45)	82.09(72.91-91.27)	93.22(83.54-98.12)
Specificity	63.91 (55.75-72.07)	35.34(27.21-43.46)	7.56(2.81-12.31)
PPV	53.85 (44.26-63.43)	39.01 (30.96-47.06)	33.33(26.14-40.53)
NPV	82.52 (75.19-89.86)	79.66 (69.39-89.93)	69.23(38.57-90.91)
PLR	2.10(1.62-2.72)	1.27(1.07-1.50)	1.01(0.93-1.10)
NLR	0.38(0.25-0.58)	0.51(0.29-0.89)	0.90(0.29-2.79)
Percent agreement	68.12 (61.77-74.46)	51.00 (44.07-57.93)	35.96(28.91-43.00)
Kappa	36.32 (24.18-48.46)	13.68 (3.76-23.60)	0.54(-4.96-6.03)

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

were 50-65 years old, and 2.4% were older than 65. Histopathologically, 83 (40.1%) participants were diagnosed as NILM, 50 (24.15%) were diagnosed as CIN1, 27 (13.04%) were diagnosed as CIN2, 31 (14.98%) were diagnosed as CIN3, and 16 (7.73%) were diagnosed as SCC.

Detection rate by histopathology diagnosis. Among 207 patients, 74 (35.7%) cases were diagnosed with CIN2+ pathologically. The overall positivity rates of FRD, cytology screening, and HPV testing were 50.24%, 70.50%, and 92.70%, respectively **(Table 1)**. For CIN2+, the detection rates of FRD, cytology screening, and HPV were 75.68%, 82.09%, and 93.22%, respectively. The false positive rate for CIN2+ in FRD, cytology screening, and HPV testing were 36.09%, 64.66% and 92.44%. The lowest false positive rate was in FRD. As the abnormality of cervical lesions increases, the detection rate of FRD gradually increases.

Performance of FRD, cytology screening and HPV test. Among the three screening methods, for CIN2+, the specificity of FRD (63.91%) was higher than that of cytology screening (35.34%) and HPV testing (7.56%) **(Table 2)**. The positive predictive value (53.85%) of FRD was also higher than cytology (39.01%) and HPV (30.58%), respectively. The percent agreement (68.12%) and Kappa value (36.32%) of FRD testing were the highest compared to those of cytology and HPV testing. The difference of each index was statistically significant (p<0.05 for all). The sensitivity of HPV testing (93.22%) was significantly higher

Table 3. *Detection rates in patients with smooth cervical surface.*

Histo-pathology diagnosis	FRD Negative	FRD Positive	TCT Negative	TCT Positive	HPV Negative	HPV Positive	Total
Normal	47(67.14)	23(32.86)	27(38.57)	43(61.43)	6(8.57)	64(91.43)	70
CIN1	20(66.67)	10(33.33)	8(26.67)	22(73.33)	0(0)	30(100)	30
CIN2	5(38.46)	8(61.54)	4(30.77)	9(69.23)	0(0)	13(100)	13
CIN3	5(45.45)	6(54.55)	1(9.09)	10(90.91)	0(0)	11(100)	11
Total	77(62.10)	47(37.90)	40(32.26)	84(67.74)	6(4.84)	118(95.16)	124

than that of cytology screening and FRD. However, there was no significant difference in sensitivity between cytology screening and FRD.

Detection rates in patients with smooth cervical surface. There were 124 patients with smooth cervical surface under colposcopy. Among them, 54 (43.55%) participants were diagnosed as CIN1+ **(Table 3)**. To detect CNI1+, the sensitivity of HPV testing was 100%. The HPV sensitivity was statistically higher than that of FRD (44.44%, $p<0.05$) and TCT (75.93%, $p<0.05$). The specificity of FRD (67.14%) was statistically higher than that of cytology screening (38.57%) and HPV testing (8.57%). The percent agreement of FRD (57.26%) was statistically higher than that of cytology screening (54.84%) and HPV testing (48.39%). If colposcopy referral is based on positive results of the test, the colposcopy referral rate based on FRD was 37.9%, which was 57% lower compared to HPV testing (95.16%), and 30% lower compared to cytology screening (67.74%).

Performance of cytology screening and FRD in HPV-HC2+ patients. A total of 167 patients were positive for HPV-HC2. Among them, 100 cases (59.88%) were pathologically diagnosed with CIN1+ **(Table 4)**. For the diagnosis of CIN1+, the sensitivity of cytology screening (≥ASCUS) and FRD for CIN1+ were: 76.00% and 54.00%, respectively. The difference was statistically significant for each of these screenings. The specificity of FRD for the diagnosis of CIN1+ was significantly higher than that of cytology screening (70.15% and 47.76%, respectively). The percent agreement was similar in the two screening methods (64.67% in cytology screening versus 60.48% in FDR). On the basis of this test's positive results, the colposcopy referral rate based on FRD was 44.31%, which was 22% lower compared to cytology screening (66.46%).

Performance of FRD and HPV testing in ASCUS patients. Thirty-eight patients were diagnosed as ASCUS based on cytology screening. Among them, 16 cases (42.11%) were diagnosed as CIN1+ **(Table 5)**. For the diagnosis of CIN1+, the sensitivity of HPV testing was statistically higher than that of FRD (100% versus 20.0%, $p<0.05$), while the specificity was statistically lower than that of FRD (9.1% versus 81.8%, $p<0.05$). The percent agreement of FRD was statistically higher than that of HPV testing (60.53% versus 47.37%, $p<0.05$). On the basis of the FRD diagnosis, the colposcopy referral rate was 23.68% and 94.7% on the basis of HPV testing.

Discussion

In our study, we compared the cytology screen results and HPV test findings. FRD has a higher specificity, higher positive predictive value, and higher percent agreement. Overall, the results suggest that FRD is a valid screening method for cervical cancer.

The folate receptor type alpha is known to be frequently overexpressed in ovarian cancer. In addition, the folate receptor type apha is a target for several novel experimental cancer therapies [9]. Studies have shown that folate receptors are overexpressed on the surface of cancerous cells with an

Table 4. *Performance of cytology screening and FRD in HPV-HC2+ patients.*

Histo-pathology diagnosis	TCT		FRD		
	NILM n(%)	≥ASCUS n(%)	Negative n(%)	Positive n(%)	Total
Normal/inflammation	32(47.76)	35(52.24)	47(70.15)	20(29.85)	67
CIN1	13(29.55)	31(70.45)	28(63.64)	16(36.36)	44
CIN2	5(21.74)	18(78.26)	11(47.83)	12(52.17)	23
CIN3	5(20.83)	19(79.17)	7(29.17)	17(70.83)	24
SCC	1(11.11)	8(88.89)	0(0.00)	9(100.00)	9
Total	56(33.53)	111(66.47)	93(55.69)	74(44.31)	167

Table 5. *Clinical performance of HPV testing and FRD in ASCUS patients.*

Histo-pathology diagnosis	HPV		FRD		
	Negative n(%)	Positive n(%)	Negative n(%)	Positive n(%)	Total
Normal/inflammation	2(9.09)	20(90.91)	18(81.82)	4(18.18)	22
CIN1	0(0)	7(100)	5(71.43)	2(28.57)	7
CIN2	0(0)	6(100)	5(83.33)	1(16.67)	6
CIN3	0(0)	1(100)	1(100)	0(0)	1
SCC	0(0)	2(100)	0(0)	2(100)	2
Total	2(5.26)	36(94.74)	29(76.32)	9(23.68)	38

epithelial origin from gynecological malignancy [10,11]. Compared to cytology screening, FRD enables clinicians to obtain test results within 30 seconds. This speed might increase patient compliance of follow-up and facilitate early intervention. Furthermore, compared to cytology screening and HPV testing, minimal training and technical support are required for FRD, making cervical screening accessible to places with limited medical resources. From our study, the specificity and PPV of FRD were higher than those of cytology screening and HPV testing. Therefore, FRD is a potentially valuable screening method, especially in low risk populations. In the lower risk population with a low prevalence of abnormality, the high specificity minimizes false positive cases. Clinically, this approach might lower unnecessary colposcopy referral, future screening, and prevent unnecessary patient anxiety. Reducing the false positive rate may have extra benefits in allocating limited medical resources appropriately to certain disadvantageous communities.

Our results were consistent with previous studies. Li et al showed that compared to cytology screening (≥ASC-US), FDR was associated with lower sensitivity and higher specificity in the general population [12]. Similar results were shown in Dai et al's work within the general population [13]. Together with our work, FDR was shown to be a rapid, valid, and cost-effective method for cervical cancer screening.

Among strengths of our study are relatively large sample size and standard use of colposcopy. However, our study also has several limitations. First, we could not rule out the misclassification of disease due to subjective interpretation in colposcopy and histopathology. Second, even with a relatively large baseline screening population, the number of participants diagnosed with CIN2+ was small, limiting our ability to examine the performance of FDR in different histopathologic stages.

In conclusion, our work indicates that FRD has good sensitivity and high specificity in cervical cancer screening, and can be a rapid, valid, and cost-effective screening test.

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