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Assessment of high risk human papilloma virus test on abnormal cytology of cervix.

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Abstract

Objective: To assess the effect of the combination of abnormal cytology and High Risk Human Papilloma Virus (HR-HPV) test on preventing Cervical Intraepithelial Neoplasia (CIN) II$^+$ and cervical cancer.

Methods: In the study, colposcopy examination and cervical biopsy were obtained and analysed from 206 women with abnormal cytology and positive/negative HR-HPV. The research grouping was according to cytological examination results.

Results: Among 206 cases, HR-HPV was positive in 45.15% (93/206), and CIN II$^+$ 15.53% (32/206). And the HR-HPV positive rate of atypia squamous epithelial cells (ASCUS), Lower-Grade Squamous Intraepithelial Lesions (LSIL), and High-Grade Squamous Intraepithelial Lesions (HSIL)$^+$ was 40.52% (62/153), 54.76% (23/42), 72.73% (8/l), respectively, with a statistical difference (χ^2=6.1800, P<0.05). For the cervical biopsy finding of CIN II$^+$, the overall predictive value of HR-HPV test was 87.50% in sensitivity and 56.90% in specificity, the positive predictive value 27.18%, and the negative 96.12%; for CIN III$^+$, the overall predictive value of HR-HPV test was 100% in sensitivity and 51.50% in specificity, the positive predictive value 5.83%, and the negative 100%.

Conclusion: HR-HPV test can evaluate the risk of the finding of cervical cytology and guide its distributions, with extremely high sensitivity and negatively predictive value for CIN II$^+$ and CIN III$^+$.

Keywords: Cervical cytology, High risk human papilloma virus, Cervical intraepithelial neoplasia.

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Introduction

Cervical cancer caused by the persistent High-Risk Human Papilloma Virus (HR-HPV) infections is the fourth most common cancer among women worldwide [1]. In the year 2012, almost 528,000 women diagnosed would have the cervical cancer, 266,000 women of which would die from this disease [2]. 99.7% of cervical cancer patients were infected by the Human Papillomavirus (HPV), which is considered as the major risk factor for cervical cancer [3]. Cervical cancer is a most common malignant tumor in gynecology, which originates from Cervical Intraepithelial Neoplasia (CIN). Cervical cytological detecting is generally considered as a potent method for screening CIN and early cervical cancer. Performing cytology on HR-HPV positive women has been recommended as a triage strategy [4]. While, it is observed that cervical cytological screening has high specificity but low sensitivity, and many females are diagnosed with obscurely cytological abnormality, like Atypia Squamous Epithelial Cells (ASCUS). However, cytology has several limitations like modest sensitivity [5]. Current research suggested that HR-HPV testing is more effective in reducing the burden of CIN3 and cervical cancer compared to cytology [6]. HR-HPV testing provides more sensitivity compared with cytology for detecting cervical precancer [7-9]. At present, the combination of cervical cytological screening and HR-HPV test is suggested to further the identification on the female at high risk of CIN and cervical cancer and classify ASCUS people [10]. Therefore, colposcopy detection and cervical biopsy screening were performed for the women underwent gynecological examination and HR-HPV test in our gynecological clinic and got abnormal cytology, so as to assess the effect of combining abnormal cytology and HR-HPV test on predicting CIN II$^+$ and cervical cancer. Few previous studies were conducted to evaluate the efficacy of the combination of abnormal cytology and HR-HPV test. The aim of this study was to assess the effect of the combination of abnormal cytology and HR-HPV test on preventing CIN II$^+$ and cervical cancer.

Data and Methods

Objects

206 females who received gynecological examination in our gynecological clinic with abnormal cytology and positive/ negative HR-HPV findings were involved in the study, age ranged from 20-75 y, sexual life over 3 y, without cervical circumcise or conization. Both inclusion criteria and exclusion criteria refer to this literature [11]. This research was approved by the Ethical Committee of Nanjing medical university

affiliated Nanjing Maternity and Child Health Hospital according to the declaration of Helsinki promulgated in 1964 as amended in 1996, the approval number is 2014002.

Methods

Sample collection the cervix was completely exposed using a vaginal speculum, then a cotton swab was applied for wiping overmuch secretion from the cervix. A cervical brush was placed on the cervix and rotated clockwise 8~10 laps to get samples, then the brush head was put into a storage bottle. Before tightening the bottle cap, the brush should be broken along the handle crease. Finally, the bottle was stored at 20°C for cytological examination and HPV-DNA test.

Thinprep cytologic test (TCT)

The sample was processed with Thin-Prep2000 system to crank out a thin layer cellular smear with a diameter of 2 cm, and the cells were fixed in 95% alcohol, with papanicolaou staining. Adopting TBS ranking system for cytological diagnosis.

HPV-DNA typing test

Flow fluorescence hybridization was used for HPV-DNA typing test under the introduction of high risk HPV nucleic acid detection kit (Jiangsu food and drug administration devices (Permission) number: 2013 No. 26660680), obtaining 19 high risk sub-types (HPV16, 18, 26, 31, 3335, 3945, 515253, 55, 56, 585955, and 688283) and 7 low risk sub-types (HPV6, 11, 40, 42, 44, 61, and 73). Luminex200 multi-functional flow lattice analyzer was used for the offspring of PCR, to read microsphere code and its corresponding fluorescence signal, whose findings were interpreted by matching software of Lumi-nex200IS2.3. The trial set up a positive internal control (the signal value of globin gene coated with microsphere was >150, and ≥ 2.5 times than the background signal value) and a negative internal control (a section of nucleic acid sequence coated with microsphere unrelated to HPV and globin gene reflected the background signal). At the same time, trial designed an external positive quality control (the positive type were HPV 16 and HPV 18, as confirmed by gene sequencing, provided by Tellgen Corporation) and blank (deionized water). If the probe signal value of any HPV sub-type was >150, and ≥ 2.5 times than background signal, it was interpreted that the HPV sub-type corresponding to the probe was positive.

Colposcopy examination and pathology detection

The female would receive colposcopy examination 3 to 7 d later after menstrual period. The cervix was daubed 5-acetic acid and 5Lug0ls iodine solution, of which results were recorded. The female, who had abnormal adenocyte that was found through cytological test, or who had a doubtful area that was discovered by colposcopy examination, or who was unsatisfied with the findings of colposcopy, were performed endo-cervical scraping and multiple punch biopsy. The specimens were stored in 10% formalin and sent to our pathology department for examination.

Statistical analysis

The research used software SAS8.0. The enumeration data used test and $P<0.05$ was considered statistically significant.

Results

Compared the finding of cytology, HR-HPV and cervical biopsy of 206 patients, ASCUS was 74.27% (153/206), LSII 20.39% (42/206), HSIL+ 5.34% (11/206).

And HR-HPV was positive in 45.15% (93/206); moreover, there was statistical difference in HR-HPV positive rate of ASCUS, LSIL, and HSIL+ ($\chi^2=6.1800$, $P<0.05$). Abnormal findings in cervical biopsy accounted for 47.57% (98/206), CIN1 II+ 5.53% (32/206). The abnormality of three groups accounted for 42.48 (65/153), 54.76% (23/42), and 90.91% (10/11) respectively, with a significant difference ($\chi^2=15.0380$, $P<0.05$); CIN II accounted for 8.50% (13/153), 30.95% (13/42) and 54.55% (6/11) separately, with a significant difference ($\chi^2=1.1240$, $P<0.05$) (Table 1).

Table 1. *Results of TCT, HR-HPV test, and cervical biopsy screening in 206 patients (cases (%)).*

TCT	n	HR-HPV		Pathology				
		+	-	Normal	CIN I	CIN II	CIN III	Cervical cancer
ASCUS	153	62 (40.52)	59 (59.48)	88 (57.52)	52 (33.99)	12 (7.84)	1 (0.65)	0 (0.00)
LSIL	42	23 (54.76)	19 (59.48)	19 (45.24)	10 (23.81)	12 (28.57)	1 (2.38)	0 (0.00)
HSIL	11	8 (72.73)	3 (27.27)	1 (9.09)	4 (36.36)	2 (18.18)	2 (18.18)	2 (18.18)
Sum	206	93 (45.15)	113 (54.85)	108 (52.43)	66 (32.04)	26 (12.62)	4 (1.94)	2 (0.97)

Comparison on the results of HR-HPV test, abnormal cytological examination, and pathological examination

The positive rate of HR-HPV in cervical biopsy accounted for 29.63% (32/108) normally, CIN Iwas 50.00% (33/66), CIN II 84.62% (22/26), CIN III+ 100% (6/6), with a significant difference (χ^2=34.7728, P<0.05). In the women with HR-HPV (+), those with a normal cervical biopsy finding accounted for 34.41% (32/93), CIN I 35.48% (33/93), CIN II$^+$ 30.11% (28/93); while In women with HR-HPV (-), those with a normal cervical biopsy finding accounted for 67.26% (76/113), CIN I 29.20% (33/113), CIN II$^+$ 3.54% (4/113) (Table 2).

Table 2. Comparison on the findings of HR-HPV test, abnormal cytological examination, and cervical biopsy.

Examination items	n	Normal	CIN I	CIN II	CIN III	Cervical cancer
HR-HPV (+)	93	32	33	22	4	2
ASCUS		22	29	10	1	0
LSIL		10	2	10	1	0
HS1L+		0	2	2	2	2
HR-HPV (-)	113	76	33	4	0	0
ASCUS		66	23	2	0	0
LSIL		9	8	2	0	0
HSIL+		1	2	0	0	0

Prediction of HR-HPV test for the cervical biopsy finding in the women with abnormal cytology The overall predictive value of HR-HPV test for CIN II$^+$ was 87.50% in sensitivity and 56.90% in specificity, the Positive Predictive Value (PPV) 27.18%, and the Negative Predictive Value (NPV) 96.12%; for CIN II+, the overall predictive value of HR-HPV test was 100% in sensitivity and 51.50%% in specificity, PPV 5.83%, and NPV 100% (Table 3).

Table 3. Prediction of HR-HPV for cervical biopsy outcomes in women with abnormal cytology (%).

TCT	CIN II+				CIN III+			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
ASCUS	84.62	63.57	17.46	97.78	100.00	59.87	1.61	100.00
LSIL	84.62	58.63	47.83	89.47	100.00	46.34	4.35	100.00
HSIL	100.00	60.00	75.00	100.00	100.00	42.86	50.00	100.00
Overall	87.50	56.90	27.18	96.12	100.00	51.50	5.83	100.00

Discussion

Cervical cytological examination has been the essential method for screening cervical cancer for many years. Even though the cervical cytological screening decreases the incidence and mortality of cervical cancer, many women are diagnosed with ambiguous cytological abnormity, such as Atypia Squamous Epithelial Cells (ASCUS). The management of abnormal cytology finding is in dispute, and in clinical practice, the medial models include performing colposcopy detection and repeated cytological screening at specified intervals. A majority of abnormal cytology represent a small change of cervical cells which tends to self-recovery. However, since it is the presentation of the lower-grade cytology, up to 28% ASCUS females suffer CIN II or CIN III, who are at high risk of developing higher-grade cervical injury [12]. On the other hand, if the females receive colposcopy examination at once, they may face poor pregnancy in potential because of over-treatment [13]. A randomized controlled trial shows that an immediate colposcopy examination at baseline may cause a higher detection rate for CIN II and above in women. The infection of HPV is nearly associated with all cervical cancer.

And long-term infection of HPV may lead to cervical mishap and cancers [14]. International Agency for Research on Cancer (IARC) recommends HR-HPV to be the major screening method for cervical cancer. An analysis reveals that, HR-HPV test is able to distribute ASCUS, but it's still controversial [15]. The study is to assess its prediction for the risk of abnormal cytology.

In 206 cases of abnormal cytology, ASCUS takes up the majority (74.27). The positive rate of HR-HPV increases along the development of abnormal cytological grade and the rise of grade of the cervical biopsy findings. Moreover, the ratio of abnormal results in cervical biopsy and CIN II$^+$ enlarges with the rise of abnormal cytological grade. According to the

analysis on positive/negative HR-HPV typing, it is known that the proportion of abnormal results of cervical biopsy and CIN II^+ in the women with positive HR-HPV are higher than that in the women with negative HR-HPV; on the basis of further analysis, we know that the CIN II^+ proportion of ASCUS, LSIL, and HSIL in the women with positive HR-HPV raises successively, while that in the women with negative HR-HPV declines in turn. Evidently, HR-HPV examination can be an important method to screen cervical cancer, and distribute the findings of abnormal cytology. What's more, it can pick out the CIN from ASCUS, the majority of abnormal cytology. But the females who just have positive HR-HPV should accept colposcopy examination and biopsy; the women with negative HR-HPV should be followed up carefully, so as to reduce the usage frequency of colposcopy, lest over-diagnosis and treatment give rise to burdens on patients and doctors. A study finds that HR-HPV test is an ultimately precise screening trial, and the combination of cytology and HR-HPV test may get an extremely high sensitivity and nearly 100% negative predictive value [16,17]. The present study suggests that, HR-HPV test has higher sensitivity (87.50%) and negative predictive value (96.12%) for CIN II^+; it gets 100% sensitivity and negative predictive value for CIN III^+, which is consistent with other literature reports [18]. For the women with abnormal cytology, negative HR-HPV predicts that they don't have high grade cervical disorders. In addition, for the patients with cytology HSIL, positive HR-HPV suggests the existence of CIN II^+ [19].

This study shows that HR-HPV test can evaluate the risk of the finding of cervical cytology and guide its distributions, with extremely high sensitivity and negatively predictive value for CIN II^+ and CIN III^+. Certainly, this study also has its deficiencies, like small sample size and lack of long-term follow-up data, so data should be collected for a more scientific evaluation.

References

1. Guan P, Howell-Jones R, Li N. Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer. Int J Cancer 2012; 131: 2349-2359.
2. Beaslas O, Vihervaara T, Li J. Silencing of OSBPrelated protein 8 (ORP8) modifies the macrophage transcriptome, nucleoporin p62 distribution, and migration capacity. Exp Cell Res 2012; 318: 1933-1945.
3. Jemal A, Bray F. Center MM Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
4. Wentzensen N, Schiffman M, Palmer T. Triage of HPV positive women in cervical cancer screening. J Clin Virol 2016; 76: 49-55.
5. Sahasrabuddhe VV, Luhn P, Wentzensen N. Human papillomavirus and cervical cancer: biomarkers for improved prevention efforts. Future Microbiol 2011; 6: 1083-1098.
6. Arbyn M, Ronco G, Anttila A. Evidence regarding HPV testing in secondary prevention of cervical cancer. Vaccine 2012; 30: 88-99.
7. Rijkaart DC, Berkhof J, Rozendaal L. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol 2012; 13: 78-88.
8. Ronco G, Dillner J, Elfstrom KM. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014; 383: 524-532.
9. Pan Q, Hu S, Zhang X. Pooled analysis of the performance of liquid-based cytology in population-based cervical cancer screening studies in China. Cancer Cytopathol 2013; 121: 473-482.
10. Xie X, Gou WL. Obstetrics and gynecology. Beijing Peoples Med Publ House 2013; 301-304.
11. Koliopoulos AG, Nyaga VN, Santesso N. Cytology versus HPV testing for cervical cancer screening in the general population. Cochrane Datab Sys Rev 2017; 8: 008587.
12. Tshomo U, Franceschi S, Tshokey T. Evaluation of cytology versus human papillomavirus-based cervical cancer screening algorithms in Bhutan. Oncotarget 2017; 8: 72438-72446.
13. Van de Vijver A, Poppe W, Verguts J. Pregnancy outcome after cervical conisation: a retrospective cohort study in the Leuven University Hospital. BJOG 2010; 17: 268-273.
14. Kocsis A, Takacs T, Jeney C. Performance of a new HPV and biomarker assay in management of hrHPV positive women: subanalysis of the ongoing multicenter TRACE clinical trial (n>6,000) to evaluate POU4F3 methylation as a potential biomarker of cervical precancer and cancer. Int J Cancer 2017; 140: 1119-1133.
15. Freitas AC, Oliveira THA, Barros MR. hrHPV E5 oncoprotein: immune evasion and related immunotherapies. J Exp Clin Cancer Res 2017; 36: 71.
16. Flores YN, Bishai DM, Lorincz A. HPV testing for cervical cancer screening appears more cost-effective than Papanico-lau cytology in Mexico. Cancer Causes Control 2011; 22: 261-272.
17. Zhao FH, Lin MJ, Chen F. Performance of high-risk human papillomavirus DNA testing as a primary screen for cervical cancer: a pooled analysis of individual patient data from 17 population-based studies from China. Lancet Oncol 2010; 11: 1160-1171.
18. Gold MA, Thomas MA, Huh WK. High-risk human papillomavirus detection in women with low-grade squamous intraepithelial lesions or higher-grade cytology using the Cer-vista HPV HR test. J Low Genit Tract Dis 2013; 17: 51-57.
19. Li J, Yan Z, Jiarui L. Performance of high-risk human papillomavlrus testing in the triage of abnormal cervical cytology among chinese younger women in Shang-hai, China. Asian Pac J Cancer Prev 2011; 12: 2963-2968.

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