Application of TCT and Human Papillomavirus (HPV) in Screening for Cervical Lesions in the South of Sichuan Province, China

Xiaoqin Tang[1], Dingyao Jiang[1], Yuhan Wang[1], Sibei Ruan[2], Mingxi Tang[2]

[1] Department of Pathology, the People's Hospital in Longmatan District of Luzhou, Luzhou 646000, Sichuan, China

[2] Department of Pathology, Affiliated Hospital of Southwest Medical University, Luzhou 646000, Sichuan, China

Abstract: Objective: To explore the role of thin prep liquid-based cytology test (TCT) and human papillomavirus (HPV) genotyping detection in early diagnosis of cervical lesions, and assess the distribution of HPV subtypes among women in southern Sichuan. Methods: The results of TCT and HPV detection last 5 years were analyzed, and compared with the clinical pathological examination of 1126 cases. Results: The TCT-positive rate was 3.22% (2435/75512), including atypical squamous cells of undetermined significance (ASC-US) 1410 cases, atypical squamous cells cannot exclude HSIL (ASC-H) 388 cases, low-grade squamous intraepithelial lesions (LSIL) 301 cases, high-grade squamous intraepithelial lesion (HSIL) 300 cases, squamous cell carcinomas (SCC) 14 cases, atypical glandular cell (AGC) 19 cases, and adenocarcinoma 3 cases. With increase of pathological diagnosis grade, the grade of cervical cytology increased ($P<0.001$). 12.74% (508/3989) of women were tested HPV positive, the most frequently detected high-risk types (HR-HPV) and low-risk types (LR-HPV) were HPV 52, 16, 58, 33 and HPV 6, 11, 43, respectively. Overall HPV infection rate was high among 50 ~ 59 and 20 ~ 29 age group. With the grade of pathological diagnosis increasing, the positive rate of HPV infection also rose ($P<0.001$). Conclusion: TCT with HPV genotyping technology can significantly increase the detection rate of high grade cervical lesions. The most common HR-HPV types were HPV 52, 16, 58, 33, and LR-HPV types were HPV 6, 11, 43. HPV prevalence showed a bi-modal age in southern Sichuan.

***Keywords*:** Cervical lesions; Thin prep liquid-based cytology test; Human papilloma virus; Histopathology; Southern Sichuan.

1. Introduction

Cervical cancer is the fourth most generally-encountered malignant tumor in women around the world, with 18.1 million new cases in 2018 and 350,000 deaths worldwide each year, and about 85% occurring in developing countries[1]. These data indicate that cervical cancer has been a serious threat to women's health and caused a heavy economic burden on developing countries. The Thin Prep cytological test (TCT), a liquid-based method to detect cervical exfoliated cells, is widely applied to diagnose cervical lesions. Cervical TCT combined with biopsy under colposcope and human papillomavirus (HPV) detection, which greatly increases the detection rate of early cervical epithelial lesions, has become the most advanced technique in screening of cervical cancer and precancerous lesions among women[2,3].

2. Materials and methods

2.1 Study population

Cervical lesion screening was performed on 75512 women from the Affiliated Hospital of Southwest Medical University and the People's Hospital in Longmatan District of Luzhou in recent 5 years. They were 15-94 years old, and the mean age was (40.92±9.93) years old. Among them, 3989 cases attended the HPV detection and 1126 cases demonstrated the positive by TCT and had valid histological diagnosis.

2.2 TCT and histological diagnosis

The patients were required to sign an informed consent and the samples were taken to avoid the menstrual period. They had no sexual life 24 hours before examination and no application of vagina examination, douching and placing vaginal suppository 3 days before examination. The methods of collecting TCT specimens and biopsy under colposcope were described previously[2]. Cytological findings were diagnosed according to the 2001 Bethesda System[4] and classified as follows: (1) negative. (2) squamous cell abnormalities: a) atypical squamous cells (ASC), including ASC of undetermined significance (ASC-US) and ASC cannot exclude HSIL (ASC-H) ; b) low-grade squamous intraepithelial lesion (LSIL); c) high-grade squamous intraepithelial lesion (HSIL); d) squamous cell carcinoma (SCC). (3) glandular cell abnormalities: a)

atypical glandular cells (AGC); b) endocervical adenocarcinoma in situ (AIS); c) adenocarcinoma. Histological examination was accomplished to determine cervical intraepithelial neoplasia (CIN) grade. CIN grades were classified as CIN1, CIN2 and CIN3 according to the Richart's classification[5], and CIN1 is defined as LSIL ,CIN2 and CIN3 are defined as HSIL.

2.3 HPV detection

HPV DNA testing was performed by using polymerase chain reaction (PCR) and HPV genotyping was detected by a commercial Human Papillomavirus Genotyping Kit for 23 Types (Yaneng Bioscience, Shenzhen, China), including 17 high-risk types (HR-HPV) (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82)and 6 low-risk types (LR-HPV) (6, 11, 42, 43, 81 and 83). An internal control was included in each experiment.

2.4 Statistical analysis

The data were analyzed by using SPSS software (IBM, Armonk, New York, USA) (version 22.0) and divided into age groups. Correlation analysis of categorical variables was measured by the Goodman-Kruskal statistics; The comparison of rate was examined by Pearson χ^2 test; Rate trend test was examined by Cochran Armitage trend test. $P<0.05$ was regarded as statistically significant.

3. Results

3.1 TCT results and cervical biopsy results

2435 (3.22%) out of 75512 cases were abnormal by TCT, in which 1410 (57.91%) cases pertained to ASC-US, 388 (15.93%) cases to ASC-H, 301 (12.36%) cases to LSIL, 300 (12.32%) cases to HSIL, 14 (0.57%) cases to SCC, 19 (0.78%) cases to AGC and 3 (0.12%) cases to adenocarcinoma. Among 2435 patients, 1126 ones were given cervical biopsy under colposcope and the pathological results were as follows: chronic cervicitis 400 cases, LSIL 287 cases, HSIL 370 cases, SCC 67 cases and adenocarcinoma 2 cases. The coincidence rates of TCT results and pathological diagnosis in LSIL, HSIL, SCC, adenocarcinoma were 64.60%, 71.65%, 83.33% and 100%, respectively. With increase of pathological diagnosis grade, the positive rate of abnormal TCT was on the rise ($G=0.894$, $P<0.001$) (Table 1).

Table 1. Cervical biopsy results of patients with TCT.

TBS Classification	Total	Cervical Biopsy Results[n(%)]				
		Chronic Cervicitis	LSIL	HSIL	SCC	Adenocarcinoma
ASC-US	526	318(60.46)	115(21.86)	77(14.64)	16(3.04)	0(0.00)
ASC-H	237	54(22.78)	47(19.83)	116(48.95)	20(8.44)	0(0.00)
LSIL	161	18(11.18)	104(64.60)	37(22.98)	2(1.24)	0(0.00)
HSIL	194	10(5.15)	21(10.83)	139(71.65)	24(12.37)	0(0.00)
SCC	6	0(0.00)	0(0.00)	1(16.67)	5(83.33)	0(0.00)
Adenocarcinoma	2	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(1.00)
Total	1126	400(35.52)	287(25.49)	370(32.86)	67(5.95)	2(0.18)

3.2 HPV detection results

3989 out of 75512 patients were given HPV detection. Among them, 508 patients were detected positive HPV infection, accounting for 12.74%, which including 382 (9.58%) single infection and 126 (3.16%) multiple infections. Because of multiple HPV infections, 589 instances of HPV were detected, including 496 (12.43%) HR-HPV genotypes and 93 (2.33%) LR-HPV genotypes. Analysis of the distribution of different HPV genotypes, the most common HR-HPV genotypes were HPV 52, 16, 58,and 33 with infection rate 21.65%, 19.29%, 17.13%, and 5.71%, respectively. Among LR-HPV types, HPV 6 was the most prevalent type with a positive infection rate of 6.30%, followed by HPV 11 (5.51%) and HPV 43 (3.94%) (Table 2).

Table 2. Distribution of HPV types.

HPV types	Total [n(%)]	Single infection [n(%)]	Multiple infection [n(%)]
HR-HPV			
16	98(19.29)	71(72.45)	27(27.55)
18	20(3.94)	14(70.00)	6(30.00)
31	15(2.95)	9(60.00)	6(40.00)

HPV types	Total [n(%)]	Single infection [n(%)]	Multiple infection [n(%)]
33	29(5.71)	15(51.72)	14(48.28)
35	13(2.56)	6(46.15)	7(53.85)
39	28(5.51)	12(42.86)	16(57.14)
45	5(0.98)	4(80.00)	1(20.00)
51	22(4.33)	12(54.55)	10(45.45)
52	110(21.65)	75(68.18)	35(31.82)
53	2(0.39)	1(50.00)	1(50.00)
56	22(4.33)	13(59.09)	9(40.91)
58	87(17.13)	54(62.07)	33(37.93)
59	10(1.97)	5(50.00)	5(50.00)
66	2(0.39)	1(50.00)	1(50.00)
68	28(5.51)	7(25.00)	21(75.00)
73	1(0.20)	1(100.00)	0(0.00)
82	4(0.79)	1(25.00)	3(75.00)
LR-HPV			
6	32(6.30)	20(62.50)	12(37.50)
11	28(5.51)	18(64.29)	10(35.71)
42	9(1.77)	6(66.67)	3(33.33)
43	20(3.94)	15(75.00)	5(25.00)
81	1(0.20)	1(100.00)	0(0.00)
83	3(0.59)	2(66.67)	1(33.33)

According to the age group, the highest HPV infection rate was found in the 50 ~ 59 year group, significantly higher than 30 ~ 39 and 40 ~ 49 year group (χ^2=15.650, P<0.001; χ^2=33.712, P<0.001). The second age range was between the ages of 20 and 29 years, significantly higher than 40 ~ 49 year group (χ^2=10.962, P=0.001). Besides, there were significant differences between 50 ~ 59 year group and other age groups in terms of the prevalence of HR-HPV (χ^2=13.886, P<0.001; χ^2=36.373, P<0.001; χ^2=45.678, P<0.001; χ^2=8.103, P=0.004). The infection rate of LR-HPV was also mainly distributed in 50 ~ 59 and 20 ~ 29 age group, significantly higher than 30 ~ 39 and 40 ~ 49 year group (χ^2=29.711, P<0.001; χ^2=33.346, P<0.001; χ^2=16.085, P<0.001; χ^2=17.398, P<0.001) (Table 3).

Table 3. Prevalence of HPV infection in each age.

Age groups	Total	HPV-positive [n(%)]	HR-HPV positive [n(%)]	LR-HPV positive [n(%)]
20 ~ 29	760	112(14.74)**	108(14.21)	31(4.08)*****
30 ~ 39	1039	129(12.42)	110(10.59)	12(1.16)
40 ~ 49	1663	168(10.10)	172(10.34)	23(1.38)
50 ~ 59	402	83(20.65)*	92(22.89)***	25(6.22)****
⩾ 60	125	16(12.80)	14(11.20)	2(1.60)
Total	3989	508(12.74)	496(12.43)	93(2.33)

*Compared with 30 ~ 39 and 40 ~ 49 year group(χ^2=15.650, P<0.001; χ^2=33.712, P<0.001).
**Compared with 40 ~ 49 year group(χ^2=10.962, P=0.001).
***Compared with other age groups (χ^2=13.886, P<0.001; χ^2=36.373, P<0.001; χ^2=45.678, P<0.001; χ^2=8.103, P=0.004).
****Compared with 30 ~ 39 and 40 ~ 49 year group(χ^2=29.711, P<0.001; χ^2=33.346, P<0.001).
*****Compared with 30 ~ 39 and 40 ~ 49 year group(χ^2=16.085, P<0.001; χ^2=17.398, P<0.001).

3.3 Relationship between HPV and biopsy

The patients with positive HPV included chronic cervicitis 6 (1.50%) cases, LSIL 168 (58.54%) cases, HSIL 271 (73.24%) cases and cervical cancer 63 (91.30%) cases. There were significant differences between the HPV positive rate of cervical cancer and other groups (χ^2= 378.240, P<0.001; χ^2=26.215, P<0.001; χ^2=10.425, P=0.001). The HPV positive rate of HSIL was significantly higher than patients with chronic cervicitis and LSIL (χ^2=429.524, P<0.001; χ^2=15.767, P<0.001). And the HPV positive rate of LSIL was significantly higher than patients with chronic cervicitis (χ^2=287.435, P<0.001). With increase of pathological grade, HPV positive rate was on the rise (χ^2 for trend=456.437, P<0.001) (Table 4).

Table 4. HPV positivity among biopsy cases.

Classification	Total	HPV-Positive	HPV-Negative	Infection rate(%)*
Chronic cervicitis	400	6	394	1.50
LSIL	287	168	119	58.54****
HSIL	370	271	99	73.24***
Cervical cancer	69	63	6	91.30**
Total	1126	508	618	45.12

*χ^2 for trend, P<0.001.
**Compared with chronic cervicitis, LSIL and HSIL(χ^2= 378.240, P<0.001; χ^2=26.215, P<0.001; χ^2=10.425, P=0.001).
** *Compared with chronic cervicitis and LSIL (χ^2=429.524, P<0.001; χ^2=15.767, P<0.001).
****Compared with chronic cervicitis(χ^2=287.435, P<0.001).

4. Discussion

Compared with the Pap test, TCT changes the traditional operation method, collecting cervical exfoliated cells with a special cytobrush that was then placed into a fixative solution instead of besmearing on glass directly, thus obtaining almost all of the exfoliated cells, greatly improving the cytological smear quality and consequently increasing the detection rate of cervical intraepithelial lesions[3,8]. In this study, the TCT-positive rate was 3.22%, which was basically consistent with Guangdong (3.17%)[9], but different from Beijing (1.6%)[2], Shanghai (4.4%)[10] and Jiangsu (8.63%)[7]. It may be because this area is border with Yunnan, Guizhou and Chongqing, and the crowd from multi-ethnic live with for a long time. In 2014, the WHO classification of tumours of female reproductive organs put cervical precancerous lesions revision for cervical squamous intraepithelial lesions, which was classified as LSIL and HSIL[11]. LSIL has low risk of developing towards cancer, comprising mild dysplasia, CIN1, condyloma and hollow out cell disease. While HSIL may turn into a direction towards invasive cancer without treatment, comprising moderate and severe dysplasia, CIN2, CIN3 and carcinoma in situ[5,11]. In this way, there is a good correspondence between pathological diagnosis and cytology TBS system for cervical lesions. In our study, the coincidence rates of TCT results and pathological diagnosis in LSIL, HSIL, SCC, adenocarcinoma were 64.60%, 71.65%, 83.33% and 100%, respectively. There was a positive correlation between the grade of cervical TCT and biopsy in LSIL and above lesions. With the pathological grade of biopsy increasing, the cervical cytology grade was on the rise (P<0.001). Thus it can be seen that TCT detection technology is an effective screening method for cervical lesions.

A lot of studies have revealed that HPV infection is closely associated with cervical neoplasms and persistent HR-HPV infection is a high-risk factor of cervical cancer[2,9]. At present, HPV detection has become another effective method to screen cervical cancer. To our knowledge, this is the first study ever conducted on the prevalence and genotype distribution of cervical HPV among women in the south of Sichuan Province, China.

In our study, the infection rate of overall HPV (12.74%) was higher than many cities in China, including Beijing (11.9%) [12], Guangdong (7.3%)[13], and Shandong (6.93%)[14], but lower compared to Shanghai (18.81%)[8], Tianjin (14.71%) [15], Sichuan (23.84%)[16], even the southwest (26.2%)[17] and the pooled data (26%) in China[18]. The most common HPV genotypes are various among different cities in China: in Beijing[12] were HPV 52, 16, 58, 51; in Guangdong[13] were HPV 16, 52, 58, 18; in Shandong[14] were HPV 16, 52, 58, 68; in Shanghai[8] were HPV 52, 16, 58, 53; in Tianjin[15] were HPV 16, 58, 18, 66; in Sichuan[16] were HPV 52, 58, 16; in southwestern China[17] were HPV 16, 52, 58, 18; and the whole country[18] were HPV 52, 16, 58. In our study, HPV 52 and 16 was respectively the first and the second most common type, was in contrast to Guangdong, Shandong and southwestern China, in where HPV 52 was the second common type while HPV 16 was the fist common type[13,14,17]. HPV 58 had been reported as the second[15,16] or the third[8,12-14,17,18] most common type in many different regions, and in our study, HPV 58 was the third most common type. Generally speaking, HR-HPV 52, 16, 58 as the predominant in our study were consistent with many previous surveys[8,12,18]. Moreover, as the first and the second most common type of LR-HPV respectively in our study, HPV 6 and 11 were similar to the whole country (HPV 6, 11, 61) [18].

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Conflicts of interest statement

We declare that we have no conflict of interest.

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