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ABOUT COVER

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AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases* (*WJCC*, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

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Retrospective Study

Follow-up study on ThinPrep cytology test-positive patients in tropical regions

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As shown in the statistics from the World Health Organization, it is estimated that approximately 75000 new cases of cervical cancer occur every year in China. In 2008, 33000 people died of cervical cancer in China. It is proven that most women are at risk of cervical cancer. The progression from human papillomavirus (HPV) infection to cervical cancer can be several years or decades, which offers a unique opportunity to prevent cancer.

AIM

To observe the changes in ThinPrep cytology tests (TCT) and HPV infection in patients who were detected to be positive *via* TCT screening of cervical cancer and further explore the biopsy results.

METHODS

This paper performed a follow-up study on 206 cervical cancer screening-positive patients of 12231 total cases from our previous research. We conducted an observational study on the TCT results based on the interpretation of The Bethesda

System.

RESULTS

Over a 5-year period, 10 cases received consistent follow-up. The proportions of cases in which glandular epithelial lesions were detected increased over the follow-up period. The differences between the years were statistically significant ($P < 0.01$). Over the 5 years, the proportion of patients whose squamous epithelial lesions transformed into glandular epithelial lesions increased yearly. Annual positive rates of HPV infection were: year 1, 73% (24/33); year 2, 43% (6/14); year 3, 36% (9/25); year 4, 50% (9/18); and year 5, 25% (6/24). The positive detection rate after biopsy over a 9-year period was 29%.

CONCLUSION

The follow-up study for 5 years to 9 years revealed a tendency to change from squamous epithelial lesions to glandular epithelial lesions and an improvement of the disease (which had not been reported previously). The HPV test indicated a high negative conversion ratio of the viral infection. However, the follow-up cases were not found to have persistent infection of high-risk HPV. Therefore, early intervention of cervical cancer screening is necessary. Low re-examination compliance, patient education, and preventive measures should be enhanced.

Key Words: Cervical cancer; ThinPrep cytology test screening; Human papillomavirus; Follow-up study; Screening; Tropical regions

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Core Tip: In China, 75000 new cases of cervical cancer occur every year, and 33000 people died of cervical cancer in 2008. The progression from human papillomavirus infection to cervical cancer can take years, offering a unique opportunity to prevent cancer. Our study of 206 cervical cancer screening-positive cases revealed a tendency to transform squamous epithelial lesions to glandular epithelial lesions and an improvement of the disease. The human papillomavirus test indicated a high negative conversion ratio of the viral infection, and persistent infection of high-risk human papillomavirus was not detected. Early intervention of cervical cancer screening is necessary.

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INTRODUCTION

As shown in the statistics of the World Health Organization, it is estimated that approximately 75000 new cases were of cervical cancer occur every year in China. In 2008, 33000 people died of cervical cancer in China[1]. Ermel *et al*[2] proved that most women are at risk of cervical cancer. The progression from human papillomavirus (HPV) infection to cervical cancer can be several years or decades, which offers a unique opportunity to prevent cancer[3].

The prevention and control of cervical cancer is extremely important, and it is divided into three levels of prevention: Primary prevention is the use of the HPV vaccine; secondary prevention is the screening and treatment of cervical precancerous lesions; and tertiary prevention is the treatment and palliative therapy of cervical cancer. From 1920 to the present, screening technology has undergone several significant evolutions, including visual examination, PAP test (PAP smear), acetic acid and compound iodine stain visual observation, ThinPrep cytology test (TCT), and the HPV test. It should be noted that the screening tests are not a diagnostic test. They are preliminary tests that indicate which patients should receive further confirmed.

Currently, the accepted “three steps” of diagnosis are cervical cytology, colposcopy, and histopathology. The core principle of comprehensive prevention and control of cervical cancer is to find the best time for effective intervention at specific age stages in according to the natural history of the disease. At the national level, cervical cancer prevention and control programs will benefit from multidisciplinary collaboration and should begin with community education, public mobilization, vaccination, screening, and palliative therapies. If the HPV vaccine has been approved in China, the screening work still needs to be carried out or strengthened. In response to the above strategies, this

paper carried out a follow-up study for 5-9 years on 206 patients who were positive for cervical cancer screening out of 12231 total cases during our preliminary research[4].

MATERIALS AND METHODS

Patient recruitment

The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Hainan Medical University, and the study complies with all regulations. Informed consent was obtained from all patients.

We recruited 206 patients who were positive for cervical cancer screening during our previous research[4]. They were followed up for 5-9 years (from January 2011 to December 2019). Of the 206 cases, 101 cases received more than one re-examination. Of those, 8 cases were hospitalized, 18 cases received a physical examination, and 75 cases were outpatients. The patients were between 20-years-old and 72-years-old, with an average age of 39. This study obtained informed consent from these patients.

ThinPrep method

The operation[4] was subject to the operation manual. A special cervical canal brush was used to collect the exfoliated cells at the cervical opening and cervical canal. The collected cells were placed in a vial containing a ThinPrep preservation solution. They were processed *via* the ThinPrep system and filtered with an exact detailed filter. The number of cells was controlled to be less than 70000. A smear with cells evenly distributed within a diameter of 2 cm was prepared. The cells were fixed with 95% ethanol and stained with hematoxylin and eosin. Mx3000P Multiplex Quantitative PCR System (Strata Gene, Germany) and Combi-H2 Hybridization Machine (South Korea) were adopted to analyze the results. The HPV genotyping reagent was produced by Yaneng Bioscience (Shenzhen) Co., Ltd. and used in line with the operation manual. After HPV gene amplification, a reverse dot blot was conducted for HPV genotyping.

Diagnostic criteria of cervical cytology and histopathology

The diagnostic criteria of cervical cytology, based on The Bethesda System, were classified into: (1) Negative for intraepithelial lesion or malignancy (NILM); (2) Low-grade squamous intraepithelial lesions (LSIL); (3) High-grade squamous intraepithelial lesions; (4) Squamous cell cancer; (5) Atypical squamous cells (ASC); (6) ASCs of undetermined significance; (7) ASC highly indicating the existence of high-grade cervical lesions; (8) Atypical glandular cells not otherwise specified (AGC-NOS); (9) AGC-favor neoplasia; and (10) Adenocarcinoma in situ/adenocarcinoma.

The biopsy[4] was completed by the Pathology Department of the Second Affiliated Hospital of Hainan Medical University. Histopathological diagnosis was classified into average or inflammation, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, and cervical cancer (including squamous cell cancer and adenocarcinoma).

Statistical analysis and data processing

SPSS 18.0 statistical software was utilized for statistical analysis. A group design was adopted, and analysis of variance was used for the comparison of the averages of multiple samples. The pairwise comparison was statistically significant. $P < 0.05$ indicated that the differences among the groups were statistically significant.

RESULTS

During the 9-year follow-up study, 105 of the 206 TCT-positive patients were lost to follow-up, with a lost-to-follow-up rate of 50% (105/206). Over 5 consecutive years, 10 patients received consistent follow-up including a TCT. Over the 5 years glandular epithelial lesions were detected in 0% of patients (0/10), 60% of patients (6/10), 70% of patients (7/10), 90% of patients (9/10), and 90% of patients (9/10) ($SS = 231177.9$, $df = 4$, $MS = 57794.474$, $F = 166.252$, $P = 0.000$). The differences among groups were statistically significant ($P < 0.01$) (Table 1).

Of the 206 TCT-positive cases, 101 patients received at least one re-examination over a 5-year period. Over the years, the number of cases that received an examination declined, and the interval between re-examinations was inconsistent. Over the 5 years, the proportion of patients whose squamous epithelial lesions transformed into glandular epithelial lesions increased yearly [year 1: 0% (0/101); year 2: 50% (24/48); year 3: 56% (27/48); year 4: 68% (28/41); and year 5: 70% (17/24)] (Table 2).

The number of patients who received HPV genotyping and the interval between HPV genotyping among the 101 patients during the 5 years were inconsistent. Annual positive rates of HPV infection decreased over the 5-year follow-up period [year 1: 73% (24/33); year 2: 43% (6/14); year 3: 36% (9/25); year 4: 50% (9/18); and year 5: 25% (6/24)] (Table 3).

Table 1 Changes in ThinPrep cytology test and human papillomavirus genotyping of the 10 patients who were followed up for 5 consecutive years

No.	Age	2011 TCT	2011 Biopsy	2011 HPV genotyping	2012 TCT	2012 HPV genotyping	2013 TCT	2013 HPV genotyping	2014 TCT	2014 HPV genotyping	2015 TCT	2015 HPV genotyping	2018 Biopsy
1	33	ASC-US	0	0	ASC-H	0	AGC-NOS	All negative	LSIL	0	AGC-NOS	0	
2	47	ASC-US	0	0	ASC-US	0	ASC-US	All negative	AGC-NOS	All negative	AGC-NOS	All negative	
3	20	ASC-US	0	0	AGC-NOS	0	ASC-H	16, 18, 43	AGC-NOS	All negative	AGC-NOS	16	
4	43	ASC-US	0	51, 43, 52	AGC-NOS	0	AGC-NOS	All negative	AGC-NOS	0	AGC-NOS	52, 53, 59	
5	41	ASC-US	0	0	AGC-NOS	All negative	AGC-NOS	All negative	AGC-NOS	5, 11	LSIL	All negative	
6	46	LSIL	0	2	ASC-H	0	AGC-NOS	0	AGC-NOS	0	AGC-NOS	0	
7	38	LSIL	NILM	0	ASC-US	0	AGC-NOS	All negative	AGC-NOS	0	AGC-NOS	All negative	
8	36	LSIL	NILM	0	AGC-NOS	0	HSIL	58	AGC-NOS	8	AGC-NOS	53, 56, 81	
9	40	LSIL	NILM	16	AGC-NOS	16	AGC-NOS	58	AGC-NOS	0	AGC-NOS	All negative	LSIL
10	27	LSIL	LSIL	0	AGC-NOS	0	AGC-NOS	0	AGC-NOS	0	AGC-NOS	All negative	

AGC-NOS: Atypical glandular cells not otherwise specified; ASC-H: Atypical squamous cells highly indicating the existence of high-grade cervical lesions; ASC-US: Atypical squamous cells of undetermined significance; HPV: Human papillomavirus; HSIL: High-grade squamous intraepithelial lesions; LSIL: Low-grade squamous intraepithelial lesions; NILM: Negative for intraepithelial lesion or malignancy; TCT: ThinPrep cytology test.

Twenty-one of the 101 patients received a biopsy from January 2011 to December 2019, with a positive rate of 29% (6/21). Four cases occurred in the 2nd year including two cases of NILM. One of these NILM cases was LSIL, and ASC highly indicating the existence of high-grade cervical lesions was detected *via* TCT in the 3rd year. The biopsies of the other two cases indicated AGC-NOS and ASC highly indicating the existence of high-grade cervical lesions. In the 3rd year they received TCT without a biopsy, which showed that both had AGC-NOS. Two cases were biopsied in the 6th year (one case of LSIL whose biopsy in the 1st year indicated NILM and one case of NILM whose biopsy in the 1st year showed LSIL). Five cases were biopsied in the 7th year. All biopsies detected NILM. One case had squamous cell cancer, whose biopsy in the 7th year indicated NILM. Seven cases were biopsied the 8th year including a case of LSIL, whose biopsy in the 1st year showed NILM, a case of LSIL, whose previous biopsy showed AGC-NOS, and another case of LSIL. They remaining cases were NILM. Two cases were biopsied in the 9th year and showed NILM (one case previously showed AGC-NOS and one cases previously showed LSIL).

DISCUSSION

Epidemiological and molecular biological information suggests that HPV infection can result in LSIL

Table 2 Changes in the ThinPrep cytology test of the 101 ThinPrep cytology test-positive patients during the 5-year follow-up

Year	Upgrading of squamous intraepithelial lesions	Downgrading of squamous intraepithelial lesions	AGC-NOS unchanged	Change from AGC-NOS to ASC	Change from squamous intraepithelial lesions to AGC-NOS, n (%)	Total, n
2011	50	31	20	0	0 (0)	101
2012	3	11	9	1	24 (50)	48
2013	3	9	8	1	27 (56)	48
2014	2	9	4	1	28 (68)	41
2015	1	4	1	1	17 (70)	24

The table only accounts for the proportion of cases with the change from squamous intraepithelial lesions to atypical glandular cells not otherwise specified. AGC-NOS: Atypical glandular cells not otherwise specified; ASC: Atypical squamous cells.

Table 3 Changes in human papillomavirus infection of the 101 ThinPrep cytology test-positive cases during the 5-year follow-up

Year	Re-examination, n	Positive, n	Positive rate %, positive (n)/re-examination (n)
2011	33	24	73% (24/33)
2012	14	6	43% (6/14)
2013	25	9	36% (9/25)
2014	18	9	50% (9/18)
2015	24	6	25% (6/24)

and cervical cancer. High-risk and persistent HPV infection is the most significant factor that predicts cervical cancer. High-risk HPV infection generates viral oncoproteins, wherein E6 and E7 inactivate or degrade the tumor suppressor genes of P53 and Rb of the host cell, respectively, and lead to cancer action through a series of molecular events. Recently, a growing number of young women have been diagnosed with cervical cancer. Statistics demonstrated that the proportion of young patients (< 35-years-old) among all cervical cancer patients climbed from 3.4% in 1960 to 24.9% in 2005[5]. The American Cancer Society estimated that 12820 cases of invasive cervical cancer are diagnosed in the United States each year. Approximately 79 million Americans were infected with HPV[6]. Ermel *et al*[2] observed that pathogenic HPV was detected in many women.

The positive cases in this study were processed in conformity with the conventional clinical therapy of gynecology in China. The patients in which high-risk HPV types 16, 18, 33, and 51 were detected received biopsy before treatment. Those in which other HPV types were detected received vaginal drug administration and then were re-examined later or placed under observation. If their biopsy results were normal, they received vaginal drug administration; otherwise, they received cold knife conization. The follow-up therapeutic scheme was based on the pathologic findings after cold knife conization. Nevertheless, most women had a short HPV infection period of 2-3 years. Generally, HPV infection is resolved in 8-10 mo. Approximately 10%-15% of women over 35-years-old are persistently infected, which increases the risk of cervical cancer.

Since 2018, HPV primary screening has replaced the cytological examination of women over 34-years-old (inclusive) in Norway[7,8]. The effectiveness and efficiency of this scheme are expected to improve. In this study, the number of patients who received HPV genotyping and the interval of HPV genotyping among the 101 patients during the 5 years were inconsistent. However, the annual positive rates of HPV infection were 73% (24/33), 43% (6/14), 36% (9/25), 50% (9/18), and 25% (6/24) (Table 3). The decline of the positive rate of HPV infection year-by-year implied a high negative conversion ratio of the viral infection. Recently, China has implemented cervical cancer screening nationwide. Unlike most other cancers, cervical cancer is the most easily preventable with primary and secondary preventive measures. The biggest influence screening lies in the precancerous changes detected, which can be treated before the progression to cancer. Most women treated for precancerous cervical lesions had a favorable prognosis and did not progress to cervical cancer[9,10].

Currently, there are three common strategies for cervical cancer screening at home and abroad: cytological screening; combined cytological and HPV screening [cytological screening for those between 25-years-old and 29-years-old; cytological + HPV screening for those above 30-years-old (inclusive)]; and HPV primary screening (12 high-risk HPV types, such as 16 and 18)[11,12]. Combined screening is the ideal strategy for individuals with an excellent financial profile. Those with a poor financial profile might only receive one screening throughout their lives[13]. In consideration of the national condition, it

is a challenge to provide combined cytological and HPV screening to the general public. Diversified strategies for cervical cancer screening are more suitable for China.

AGC patients should have histological assessment vigilantly, especially in postmenopausal or symptomatic patients[14-16]. AGC indicates lesions in multiple parts of the female genital tract. Its morphological manifestations are diversified but not unique. It is less often diagnosed than squamous epithelial lesions, with a detection rate lower than 0.5%. The survey of the College of American Pathologists in 2006 suggested that the average reporting rate of AGC of the labs adopting liquid-based cytology (547 labs) was 0.2%[17]. AGC implies an increased risk of cervical lesions, which is critical for regular follow-up examinations and visits of AGC patients. The guide to evidence-based medicine proposed by the American Society for Colposcopy and Cervical Pathology covers a complete set of measures, including: colposcopy and colposcopy biopsy, endocervical curettage, endometrial biopsy or dilatation and curettage, conization of the cervix, B-scan ultrasonography, repeated cytological smears, and follow-up visits[18-22].

It was found that the proportion of cervical cancer among AGC patients from cervical screening was high[23-26]. The long-term risk of cervical cancer was high, especially adenocarcinoma. Clinical studies on the histological results of women diagnosed with AGC revealed glandular or squamous origins of benign changes and precursor cell lesions as well as invasive cervical cancer and other gynecological tumors. However, there are no studies on the long-term risk of cervical cancer in AGC patients from the perspectives of age, cytological results, and histopathological features of such cancers.

Unlike the above studies, 10 of the 101 cases received consistent follow-ups including TCT over a 5-year period. Their TCT results showed that the proportion of cases that improved to glandular epithelial lesions increased every year. Of the 206 positive cases, 101 cases received more than one re-examination over the 5-year period. However, the number of cases that received an examination declined every year, and the interval between re-examinations was inconsistent. The proportion of cases in which the squamous epithelial lesions improved to glandular epithelial lesions increased every year. The long-term follow-up study identified the improvement from squamous epithelial lesions to glandular epithelial lesions, which has not been previously reported. No further disease progression was detected. Twenty-one patients received a biopsy over a 9-year period. The positive rate was 29% (6/21), which was consistent with the TCT diagnosis. No deterioration was found. This might be associated with clinical intervention treatment, which will be tracked, investigated, and confirmed in the future. This follow-up study supports the principle of screening every 5 years[11,12] to prevent excessive screening.

Huang *et al*[27] assumed that education on combined cervical cancer screening strengthens patient compliance, followed by an increased screening rate, which is beneficial for the early diagnosis and treatment of diseases and prognosis. This follow-up study revealed low re-examination compliance and a lost-to-follow-up rate of 50% (105 lost to follow-up/206 positive cases) and suggested that promotion and guidance of screening should be enhanced in clinical practice.

The limitation of this study was that the detailed treatment plan of each individual was not collected during the follow-up of nearly 9 years. Follow-up work will be further strengthened, which is conducive to further study expansion.

CONCLUSION

In summary, this study followed 206 cervical cancer screening-positive cases out of a total of 12231 screened patients for a period of 5-9 years. This study revealed the improvement from squamous epithelial lesions to glandular epithelial lesions (which was not reported previously). No further disease progression was detected. Therefore, this study can serve as a reference for formulating strategies for cervical cancer screening in China. The HPV test indicated a high negative conversion ratio of the viral infection, and the follow-up cases did not have persistent infection of high-risk HPV. Therefore, early intervention in cervical cancer screening is necessary. Low re-examination compliance, patient education, and preventive measures should be enhanced.

ARTICLE HIGHLIGHTS

Research background

It is estimated that approximately 75000 new cases of cervical cancer occur every year in China. In 2008, 33000 people died of cervical cancer in China. It is proven that most women are at risk of cervical cancer.

Research motivation

The progression from human papillomavirus (HPV) infection to cervical cancer can be several years or decades, which offers a unique opportunity to prevent cancer.

Research objectives

To observe the changes in ThinPrep cytology tests (TCT) and HPV infection in patients who were positive *via* TCT screening of cervical cancer and to further explore the biopsy results.

Research methods

This paper performed a follow-up study on 206 cervical cancer screening-positive patients of 12231 total cases from our previous research. We conducted an observational study on the TCT results based on the interpretation of The Bethesda System.

Research results

The follow-up study of 5-9 years revealed a tendency to improve from squamous epithelial lesions to glandular epithelial lesions (which had not been reported previously).

Research conclusions

Early intervention of cervical cancer screening is necessary. Low re-examination compliance, patient education, and preventive measures should be enhanced.

Research perspectives

Women should have regular gynecological physical examinations to check for HPV infection. Early detection, accurate typing, and virus quantification of HPV infection are of great significance for the prevention and treatment of cervical cancer.

FOOTNOTES

Author contributions: Chen YC, Liang CN, and Hu JD conceived and designed the experiments; Wang XF and Wang MF performed the experiments; Huang XN analyzed and interpreted the data; Chen YC, Liang CN, and Hu JD contributed reagents, materials, and analysis tools or data and wrote the paper.

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