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Follow-up study on 206 thin prep cytology test-positive patients of 12231 cases in tropical regions

Yun-Chun Chen, Chong-Nan Liang, Xiang-Feng Wang, Min-Fa Wang, Xu-Ning Huang, Jian-Dong Hu

Abstract

BACKGROUND

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

AIM

To observe the changes in thin prep cytology test (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 cases detected to be positive of 12231 cervical cancers screening cases in the preliminary research, and conducted an observational study on the TCT results based on the interpretation of The Bethesda System.

RESULTS

Proportions of the cases who were detected to have a tendency of glandular epithelial lesions against the 10 cases who continuously paid a follow-up visit for TCT over the five years among 101 cases: 0 (0/10), 60% (6/10), 70% (7/10), 90% (9/10), and 90% (9/10). (F = 166.252, P = 0.000). The differences among groups were statistically significant (P < 0.01). Proportions of the cases that were detected to have a tendency of glandular epithelial lesions against the cases that did not continuously pay a follow-up visit for TCT: 0% (0/101), 50% (24/48), 56% (27/48), 68% (28/41), and 70% (17/24). Annual positive rates of HPV infection: 73 % (24/33), 43% (6/14), 36% (9/25), 50% (9/18), and 25% (6/24). The positive rate of 21 patients detected *via* a biopsy over the nine years was 29% (6/21).

CONCLUSION

The follow-up study for five to nine 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 indicates a high negative conversion ratio of the viral infection. However, the follow-up cases were not found to have a persistent condition of high-risk HPV types, which might be one in the reasons for the result. Therefore, early intervention of cervical cancer screening is necessary. Low reexamination compliance, patient education and preventive measures should be enhanced.

Key Words: Cervical cancer; Thin prep cytology test screening; Human papilloma virus; Follow-up study; Screening; Tropical regions

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Core Tip: As shown in the statistics of the World Health Organization, it is estimated that approximately 75000 of the new cases of cervical cancer every year occur in China. In 2008, 33000 people died of cervical cancer in China[1]. Ermel,A.[2] proved that most women are at risk of cervical cancer. The progression from human papilloma virus (HPV) infection to cervical cancer can be several years or decades, which offers a unique opportunity to block cancer[3]. This paper carried out a follow-up study for five to nine years on 206 cases detected to be positivein 12231 cervical cancer screening cases in the preliminary research by the authors[4], and observed the changes in thin prep cytology test and HPV infection to find whether they recovered or deteriorated. The follow-up study for five to nine years revealed a tendency of change from squamous epithelial lesions to glandular epithelial lesions and an improvement of the disease (which had not been reported previously). The HPV test indicates a high negative conversion ratio of the viral infection. However, the follow-up cases were not found to have a persistent infection of high-risk HPV types, which might be one of the reasons for the result. Therefore, the early intervention of cervical cancer screening is necessary. Low reexamination compliance, patient education and preventive measures should be enhanced.

INTRODUCTION

As shown in the statistics of the World Health Organization, it is estimated that approximately 75000 new cases were of cervical cancer every year occur 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 papilloma virus (HPV) infection to cervical cancer can be several years or decades, which offers a unique opportunity to block cancer[3].

At present, the most important thing to pay attention to is the prevention and control of cervical cancer, which 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 (VIA/VILI), thin prep cytology test (TCT), HPV test, in chronological order. It can be divided into cytology tests and HPV test. It should be noted that the screening test is not a diagnostic test, still only a preliminary test and those who are positive or suspected to be positive must be further confirmed so that the necessary measures can be taken in those who are authorized. 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 the life course according to the natural history of the disease. At the national level, cervical cancer prevention and control programs will benefit from multidisciplinary collaboration and need to start 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 five to nine years on 206 cases were detected to be positive in 12231 cervical cancer

screening cases were in the preliminary research by the authors[4], and observed the c

MATERIALS AND METHODS

Materials

The 206 patients were positive cases in the research of the authors[4]. They were followed up for five to nine years (from January 2011 to December 2019). 101 cases were received more than one reexamination, wherein eight were hospitalized; 18 received a physical examination; and 75 were outpatients. They were between 20 and 72 years old, with an average age of 39. This study obtained informed consent from these patients.

Methods

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 Thin prep preservation solution. They were processed *via* the Thin prep system and filtered with an exact detailed filter. The number of cells was controlled to be less than 70000. And a smear with cells evenly distributed within a diameter of 2 cm was prepared. The cells were fixed with 95% and stained with HE. Mx3000P Multiplex Quantitative PCR System (from Strata gene, Germany) and Combi-H2 Hybridisation Machine (from 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 (RDB) was conducted for HPV genotyping.

Diagnostic criteria of cervical cytology and histopathology

For the diagnostic criteria of cervical cytology(based on The Bethesda System cytological examination results are classified into: Negative for intraepithelial lesion or malignancy (NILM), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous cell cancer (SCC), atypical squamous cells (ASC), ASCs of undetermined significance (ASC-US), atypical squamous cells highly indicating the existence of high-grade cervical lesions (ASC-H), atypical glandular cells not otherwise specified (AGC-NOS), AGCs-favor neoplasia (AGC-FN), and adenocarcinoma insitu (AIS)/adenocarcinoma.

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

SPSS 18.0 statistical software for statistical analysis and data processing

Group design was adopted, and ANOVA 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.

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 and confirmation that informed consent was obtained.

RESULTS

During the nine-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). Proportions of the cases were which were detected to tend glandular

epithelial lesions against the 10 cases were who continuously paid a follow-up visit for TCT over the five years among 101 cases were: 0 (0/10), 60% (6/10), 70% (7/10), 90% (9/10), and 90% (9/10). Statistical values (SS = 231177.9, df = 4, MS = 57794.474, F = 166.252, *P* value = 0.000).The differences among groups were statistically significant (*P* < 0.01). See the Table 1.

Cases (*n* = 101) were received more than one reexamination over the five years among the 206 TCT-positive cases were diagnosed in 2011. The number of cases was who received an examination declined year by year, and the interval of reexaminations was inconsistent. The proportion of patients whose squamous epithelial lesions were transformed into glandular epithelial lesions rose year by year: 0% (0/101), 50% (24/48), 56% (27/48), 68% (28/41), and 70% (17/24). See the Table 2.

The number of patients who received HPV genotyping and the interval of HPV genotyping among the 101 patients during the five years were inconsistent. Annual positive rates of HPV infection fell year by year: 73% (24/33), 43% (6/14), 36% (9/25), 50% (9/18), and 25% (6/24). See the Table 3.

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 werein the second year (two cases wereof NILM: One cases wereof NILM in the second year, one cases wereof LSIL who was detected to have ASC-H *via* TCT in the third year. The biopsies of the two cases wereof AGC-NOS and ASC-H indicated LSIL, but only received TCT without a biopsy in the third year. The TCT showed that both had AGC-NOS). Two cases were in the sixth year (one case of LSIL whose biopsy in the first year indicated NILM and biopsy in the sixth year, LSIL. For the second cases were, the biopsy in the first year showed LSIL, while that in the sixth year, NILM). Five cases werein the seventh year (they had not received a reexamination. All their biopsies detected NILM. One of them had SCC, whose biopsy in the seventh year

indicated NILM). Seven cases werein the eighth year (The first cases werewas LSIL, whose biopsy in the first year showed NILM; and that in the eighth year, LSIL. The second cases werewas AGC-NOS, whose biopsy in the eighth year revealed LSIL. The third cases werewas LSIL, whose biopsy in the eighth year indicated LSIL. All the rest four cases werewere NILM.) Two cases werein the ninth year (one cases wereof AGC-NOS and one cases wereof LSIL. Their biopsies in the ninth year showed NILM).

DISCUSSION

Epidemiological and molecular biological information suggests that, HPV infection can result in LSIL and cervical cancer. High-risk and persistent HPV infection is the most significant factor that stimulates 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, 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 (ACS) estimated that 12820 cases were of invasive cervical cancer are diagnosed in America each year. Approximately 79 million Americans were infected with HPV[6]. Ermel *et al*[2]pointed out that many women were detected to have pathogenic HPV.

The positive cases were in this study were processed in conformity with the conventional clinical therapy of gynecology in China: The patients in high-risk HPV types 16, 18, 33, and 51 had a biopsy before treatment. Those in other HPV types had vaginal and then received a reexamination or were observed. If their biopsy results were normal, they would have vaginal administration; otherwise, they would receive cold knife conization (CKC).

The follow-up therapeutic scheme was based on the pathologic findings after CKC. Nevertheless, most women had a short HPV infection period of two to three years. Generally, HPV infection disappears in eight to 10 mo. Only approximately 10% to 15% of women over 35 years old are persistently infected. Such persistently infected women face a higher risk of cervical cancer. Since 2018, HPV primary screening has replaced the cytological examination of women above 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 five 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) (See Table 3). The decline of the positive rate of HPV inflection year by year implied a high negative conversion ratio of the viral infection. Along with its constant perfection of the disease prevention and control system and medical insurance system in recent years, 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 of the screening lies in the precancerous changes detected which can be treated before the progression to cancer, because most women whose precancerous changes of cervical cancer were treated had a favorable prognosis and did not have cervical cancer[9,10].

Currently, there are three common strategies for cervical cancer screening at home and abroad, namely, cytological screening, combined cytological and HPV screening (cytological screening for those between 25 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 challenging to realize the combined cytological and HPV screening of the general public. Diversified strategies for cervical cancer screening are more suitable for China.

AGC patients should have histological assessment vigilantly, especially postmenopausal or symptomatic patients[14-16]. AGC is a vital message of cytological diagnosis, which 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 mostly lower than 0.5%. The survey of the College of American Pathologists (CAP) 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 the 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 (ASCCP) covers a complete set of measures, including: Colposcopy and colposcopy biopsy, endocervical curettage, endometrial biopsy or dilatation and curettage (D&C), 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 of cervical screening is high[23-26]. And the long-term risk of cervical cancer is high, especially adenocarcinoma. Clinical studies on the histological results of women diagnosed with AGC revealed the glandular or squamous origin 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 of AGC patients, from the perspectives of the age, cytological results, and histopathological features of such cancers.

Unlike the above studies, 10 of the 101 cases were continuously paid a follow-up visit for TCT over the five years. Their TCT results showed that the proportion of the cases were who were improved to glandular epithelial lesions increased year by year were: 0 (0/10), 60% (6/10), 70% (7/10), 90% (9/10), and 90% (9/10). The differences among groups were statistically significant ($P < 0.01$) (See Table 1). 101 cases were received more than one reexamination over the five years. The number of cases was who received an examination declined year by year. And the interval of reexaminations was inconsistent. The proportion of cases was whose squamous epithelial lesions were improved to glandular epithelial lesions rose year by year were: 0% (0/101), 50% (24/48), 56% (27/48), 68% (28/41), and 70% (17/24) (See Table 2). The long-term follow-up study identified the improvement from squamous epithelial lesions to glandular epithelial lesions. No further disease progression was detected. 21 patients received a biopsy in nine years. 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 probably supports the principle of screening every five years in the guide[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 reexamination compliance and a lost 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 is 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.

In summary, this study followed up on 206 positive cases were of 12231 cases were of cervical cancer screening for five to nine years. It 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 indicates a high negative conversion ratio of the viral infection. However, the follow-up cases were not found to have a persistent condition of high-risk HPV types, which might be one of the reasons for the result. Therefore, early intervention in cervical cancer screening is necessary. Low reexamination compliance, patient education and preventive measures should be enhanced.

CONCLUSION

The follow-up study for five to nine 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 indicates a high negative conversion ratio of the viral infection. However, the follow-up cases were not found to have a persistent condition of high-risk HPV types, which might be one in the reasons for the result. Therefore, early intervention of cervical cancer screening is necessary. Low reexamination compliance, patient education and preventive measures should be enhanced.

ARTICLE HIGHLIGHTS

Research background

The follow-up study for five to nine 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).

Research motivation

Excerpt from "Follow-up Study on 206 thin prep cytology test-Positive Patients of 12231 Cases in Tropical Regions". *Am J Obstet Gynecol* 2020.

Research objectives

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

Research methods

Proportions of the cases which were detected to tend glandular epithelial lesions against the 10 cases who continuously paid a follow-up visit for TCT over the five years among 101 cases: 0 (0/10), 60% (6/10), 70% (7/10), 90% (9/10), and 90% (9/10) ($F = 166.252$, $P = 0.000$).

Research results

The follow-up study for five to nine years revealed a tendency of change from squamous epithelial lesions to glandular epithelial lesions and an improvement of the disease (which had not been reported previously).

Research conclusions

The follow-up study for five to nine 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).

Table 1 Changes in thin prep cytology test and human papilloma virus genotyping of the 10 patients followed up for five 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 genot
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 ne
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
5	41	ASC-US	0	0	AGC-NOS	All negative	AGC-NOS	All negative	AGC-NOS	5, 11	LSIL	All ne
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 ne
8	36	LSIL	NILM	0	AGC-NOS	0	HSIL	58	AGC-NOS	8	AGC-NOS	53, 56
9	40	LSIL	NILM	16	AGC-NOS	16	AGC-NOS	58	AGC-NOS	0	AGC-NOS	All ne
10	27	LSIL	LSIL	0	AGC-NOS	0	AGC-NOS	0	AGC-NOS	0	AGC-NOS	All ne

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

Table 2 Changes in thin prep cytology test of the 101 thin prep cytology test positive patients during the five-year follow-up study

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 (%: Positive *n*/total *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.

Table 3 Changes in human papilloma virus infection of the 101 thin prep cytology test positive cases during the five-year follow-up study

Year	Reexamination, *n*	Positive, *n*	Positive rate %, positive (*n*)/reexamination (*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)

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