https://www.cytojournal.com/

CytoJournal

Co-editors-in-chief:
Lester J. Layfield, MD, (University of Missouri, Columbia, MO, USA)
Vinod B. Shidham, MD, FIAC, FRCPath (WSU School of Medicine, Detroit, USA)

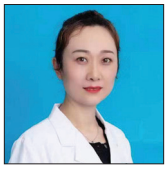
OPEN ACCESS
for HTML version

Research Article

Clinical utility of HPV typing and quantification combined with PAX1/ZNF582 methylation detection in accurate cervical cancer screening

Ying Wen[1], Hui Liang[2], Hui Zhang[2]

Departments of [1]Gynaecology, [2]Health Commission, Jiangsu Normal University, Jiangsu, China.



***Corresponding author:**
Ying Wen,
Department of Gynaecology,
Jiangsu Normal University
Hospital, Jiangsu, China.

lky571386@163.com

Received: 13 October 2022
Accepted: 19 April 2023
Published: 01 September 2023

DOI
10.25259/Cytojournal_46_2022

Quick Response Code:

ABSTRACT

Objectives: This article aims at exploring the clinical value of high-risk human papillomavirus (HPV) positive and paired boxed 1 (PAXI)/zinc finger protein 582 (ZNF582) gene methylation shunt as a new approach for accurate cervical cancer screening.

Material and Methods: Selecting 115 patients were treated in the Cervical Department of Xuzhou Matemal and Child Health Hospital from October 2018-October 2020. All patients underwent cervical exfoliated cell thinprep cytologic test (TCT) detection, HPV typing quantitative detection, and PAX1/ZNF582 gene methylation level Detection. Taking the biopsy pathological diagnosis under colposcopy as the gold standard, analyzing the test results statistically, and the sensitivity, specificity, and accuracy of the three screening methods alone and combined screening schemes were compared.

Results: Comparison of the three methods of cervical exfoliated cell TCT, HPV typing and quantification, and PAX1/ZNF582 methylation gene detection showed that the gene detection method has the highest specificity, 97.30%; The HPV typing quantitative detection has the highest sensitivity, 89.71%, but its specificity is poor; and the PAX1/ZNF582 gene detection has the highest accuracy.

Conclusion: For patients with high-grade cervical lesions and cervical cancer, PAX1/ZNF582 gene methylation level can be used as an important biomarker for the diagnosis and classification of cervical cancer. PAX1/ZNF582 methylation gene detection is effective in high-grade cervical lesions and cervical cancer. Screening has high clinical value and can become a new way of accurate cervical cancer screening.

Keywords: Cervical cancer, Human papillomavirus typing and quantification, Paired boxed 1 gene, Zinc finger protein 582 gene, DNA methylation

INTRODUCTION

Cervical cancer remains the fourth most common type of cancer in women. In 2020, there were an estimated 604,000 new cervical cancer cases worldwide and about 342,000 deaths from the disease.[1] Human papillomavirus (HPV) infection is widely recognized as one of the main causes of cervical cancer, which is generally manifested in four stages: latent infection, subclinical infection, clinical symptoms, and HPV-related tumors. However, differences in the specific type of HPV infection may have a different impact on the progression of the above staging.[2-5] DNA

methylation is one of the important molecular mechanisms of epigenetics, which, as studies have established, accumulates with the development of tumors. For example, the degree of methylation of paired boxed 1 (PAX1) and zinc finger protein 582 (ZNF582) is consistent with the progression of cervical intraepithelial neoplasia (CIN).[6-8] PAX1/ZNF582 thus can be used as a molecular biomarker for the early diagnosis of cervical cancer. Hence, this study attempts to establish a new triage, diagnosis, and treatment model for accurate screening and hierarchical management of cervical cancer based on HPV typing and quantification as a primary screening method as well as PAX1/ZNF582 methylation detection.

MATERIAL AND METHODS

Information and grouping of patients

Totally 115 patients treated at the Department of Cervical Surgery of Xuzhou Maternity and Child Health Care Hospital from October 2018 to October 2020 were selected according to the following inclusion and exclusion criteria. Inclusion criteria: A 20–65-year-old married women were included in the study. Exclusion criteria: History of cervical biopsy, history of reproductive system malignancy or immune system disease, menstruation, or pregnancy were excluded from the study. The study was approved by the Ethics Committee of the hospital, and all subjects signed informed consent. The diagnosis was based on pathological evaluation of colposcopy biopsy as the gold standard, and the patients were classified into four groups: Chronic cervicitis (inflammation, n = 21), low-grade cervical squamous intraepithelial lesion (LSIL, n = 20), high-grade cervical squamous intraepithelial lesion (HSIL, n = 41), and cervical cancer (n = 33). All the enrolled patients underwent thinprep cytologic test (TCT) of cervical exfoliated cells, HPV typing and quantification, and PAX1/ZNF582 methylation detection, and those who were positive for any of these tests were required to undergo pathological diagnosis of colposcopy biopsy.

Methods

Specimen collection

Subjects had no sexual activities, vaginal douching, or vaginal medication within 48 h before specimen collection. Biopsy specimens were collected during non-menstrual periods for examination and before the biopsy started. The cervix was exposed and the secretion as well as blood on the cervical orifice was cleaned before the cervical cytology brush for TCT, HPV and methylation tests was inserted into the cervical squamocolumnar junction and swirled clockwise or anticlockwise for 4–6 times to collect the cervical exfoliated cells. After that, the brush head was placed in a test tube containing the preservation solution marked with the subject code. Three specimens were used for TCT, HPV, and methylation tests. The specimens for typing and quantification of 21 HPV subtypes were collected and transported using the special specimen transport medium provided by Jiangsu Bioperfectus Technologies Co., Ltd.

TCT

Specimens were processed using an automated liquid-based cytology slide preparation system to make thin-layer smears. The cytology results were evaluated by The Bethesda System, and abnormal results had to be interpreted by a second pathologist, which included: Negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells-cannot exclude HSIL, LSIL, HSIL, and squamous cell carcinoma. A positive result of ASCUS and above was considered to be cytologically positive for follow-up analysis.

Typing and quantification of 21 HPV subtypes

21 HPV subtypes (HPV16+, HPV18+, HPV16/18+, 11 high-risk HPV$^+$, and 14 high-risk mixed HPV) could be quickly and accurately distinguished in the tested specimens within 2.5 h, and their viral loads could also be standardized and quantified using the standardized patented technology of cervical exfoliated cell sampling and multiplex quantitative fluorescent polymerase chain reaction (PCR) at the same time. In other words, the typing and quantification of 21 HPV subtypes (viral load value/10000 cells) could be achieved simultaneously.

Detection of gene methylation in cervical cancer

The real-time quantitative methylation-specific PCR was utilized to detect the methylation level of PAX1/ZNF582, which included two steps: (1) DNA extraction and bisulfite conversion: Cervical exfoliated cells were placed in a liquid-based cell preservation solution and stored in liquid nitrogen before DNA was extracted and treated with bisulfite. All procedures followed the instructions of the Cervi-M Detection Kits. (2) The methylation levels were detected by real-time quantitative PCR.

Statistical analysis

SPSS 20.0 was used for statistical analysis. The categorical data were tested by χ^2, and the quantitative data were expressed as (± s). The pairwise comparison was performed by the t-test, and $P < 0.05$ is statistically significant.

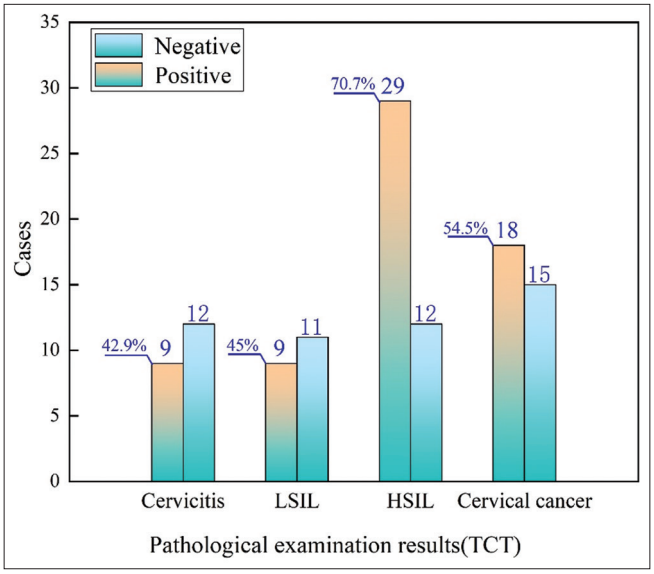


Figure 1: Histocytological examination results of the four groups (%).

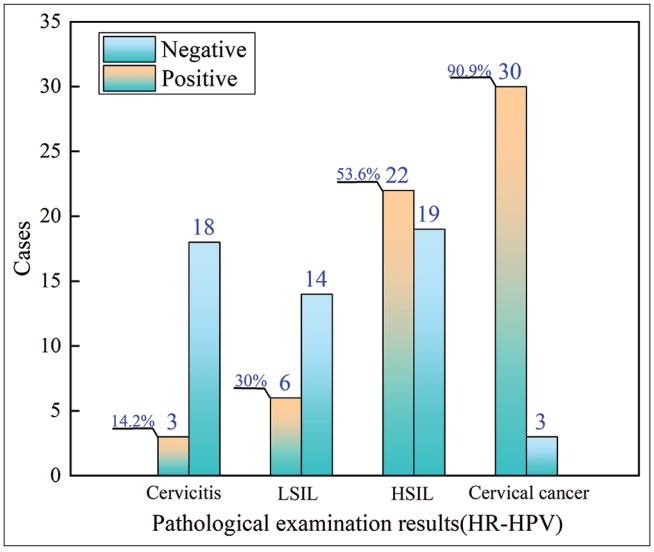


Figure 2: HP-human papillomavirus detection results of the four groups (%).

RESULTS

Histocytology

In TCT, the positive rates of chronic cervicitis, LSIL, HSIL, and cervical cancer groups were 42.9%, 45%, 70.7%, and 54.5%, respectively (sensitivity: 59.3%, specificity: 57.89%, diagnostic coincidence rate: 60.8 %), as shown in [Figure 1].

Helicobocton pyloni (HP)-HPV

In the HP-HPV test, the HPV genotypes with infection rates from high to low were HPV16, 52, 58, 33, 18, and 31, of which HPV16+ accounted for 41.3%. The positive rates of chronic cervicitis, LSIL, HSIL, and cervical cancer groups were 14.2%, 30%, 53.6%, and 90.9%, respectively (sensitivity: 89.71%, specificity: 18.92%, diagnostic coincidence rate: 65.2%), as shown in [Figure 2].

Correlation between viral loads and cervical lesions

According to the Bio perfectus multiplex real time (BMRT-HPV) test, there were statistically significant differences in HPV 16, 33, and 52 between different grades of cervical lesions ($P < 0.05$), and their viral loads increased with the grade of cervical lesions. The viral loads of HPV 16, 33, and 52 in high-grade lesions were significantly higher than those in low-grade lesions ($P < 0.05$). This indicated that the viral loads of HPV 16, 33, and 52 were positively correlated with the grade of cervical lesions, as shown in [Figure 3].

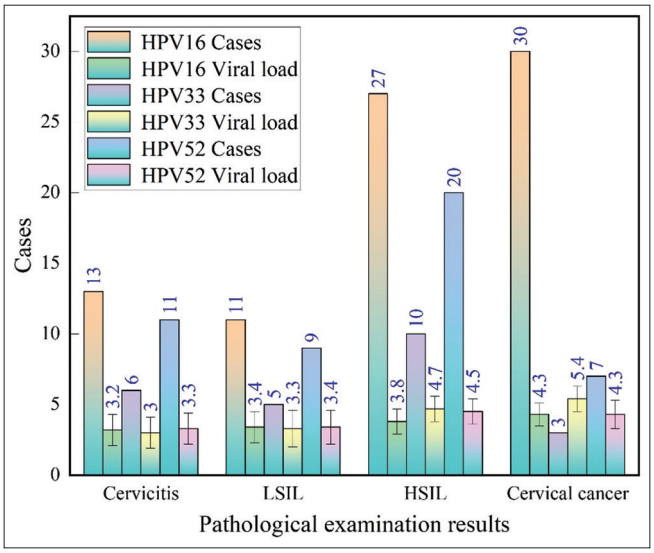


Figure 3: Correlation between the viral loads of HPV 16, 33, and 52 and the grade of cervical lesions. HPV: Human papillomavirus.

PAX1

The positive rates of chronic cervicitis, LSIL, HSIL, and cervical cancer groups were 0%, 5%, 73.1%, and 84.8%, respectively (sensitivity: 79.41%, specificity: 97.3%, diagnostic coincidence rate: 85.71%), as shown in [Figure 4].

PAX1/ZNF582

The positive rates of chronic cervicitis, LSIL, HSIL, and cervical cancer groups were 4.7%, 15%, 80.4%, and 97%, respectively (sensitivity: 88.24%, specificity: 91.89%, diagnostic coincidence rate: 89.52 %), as shown in [Figure 5].

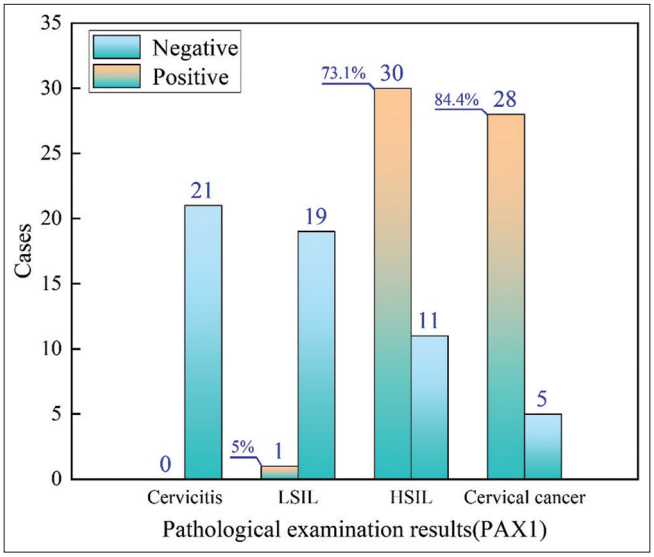


Figure 4: PAX1 detection results of the four groups (%).

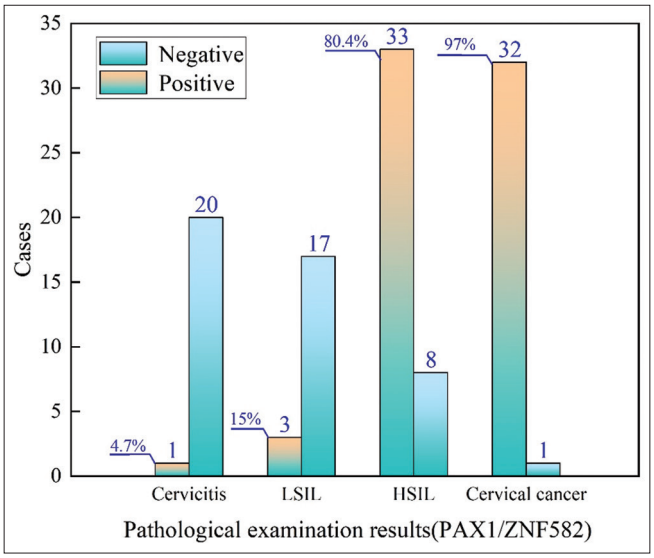


Figure 5: PAX1/ZNF582 detection results of the four groups (%).

DISCUSSION

The principle of cervical cancer screening should be to maximize screening benefits and minimize potential harms. Simply put, it is to identify precancerous lesions that may progress to infiltrating cancer, and avoid excessive examination and unnecessary treatment for transient HPV infections and their corresponding benign lesions. More than 90% of HPV infections will be cleared by the body within 2 years,[9] while infection with high-risk HPV for more than 2 years, if not treated, may progress to CIN. High-grade cervical lesions may further progress into cervical cancer.

The most common HPV genotypes in cervical precancerous lesions among the Chinese population are HPV16, 52, 58, 33, 18, and 31.[10] The higher the amount of HPV infection, the longer it takes the body to clear it.[11] Since the persistent infection of the virus is a necessary condition for the occurrence of cervical cancer, it can be considered that the higher the amount of HPV infection, the greater the risk of cervical cancer. The best screening concept at present is risk stratification and quantitative management. Although HPV is highly sensitive, the large number of transient infections in the results may increase the patients' unnecessary psychological stress and follow-up treatment. Therefore, given the high sensitivity and low specificity of primary HPV screening, certain measures should be taken to triage those who tested positive in primary screening.

HPV infection itself does not necessarily lead to the occurrence of cervical cancer, but some studies have shown that after HPV infection, the main risk factor contributing to the occurrence of malignant tumors is the abnormal expression of oncogenes, such as abnormal cell proliferation and division. The underlying mechanism is that the abnormal expression of methylation of tumor suppressor genes reduces their overall content, resulting in abnormal downstream genes and finally cervical cancer.[12,13] Studies have demonstrated that PAX1 can be used as a potential biomarker for the early diagnosis and screening of cervical cancer.[14] The methylation level of ZNF582 is also correlated with the occurrence and development of cervical cancer,[15,16] and the overall sensitivity and specificity of ZNF582 methylation detection are 70% and 82%, respectively.[17,18]

CONCLUSION

The present study reveals that the sensitivity and specificity of TCT are 59.30% and 57.89%, respectively; those of HPV16 and 18 are 72.06% and 78.38%, respectively; those of PAX1/ZNF582 are 88.24% and 91.89%, respectively, and the combination with PAX1/ZNF582 methylation detection can make up for the low specificity of HPV. PAX1/ZNF582 methylation detection enjoys several advantages. Firstly, DNA methylation is relatively stable and less affected by subjective factors. Secondly, the cervical exfoliated cells can be used for methylation detection, which is easy to obtain, non-traumatic, and easily accepted by the patients. Thirdly, the combined methylation detection of the two genes improves the sensitivity and specificity. Therefore, typing and quantification of HPV in primary screening, together with PAX1/ZNF582 methylation detection and shunting can make up for the deficiencies of methylation detection, HPV, or cytology alone, and can be considered as a promising means of accurate cervical cancer screening.

ACKNOWLEDGMENT

This paper is the phased results of the feasibility study on the application of deep learning-based artificial intelligence technology in the screening of cervical cancer in population (F202103), a key funded project of Maternal and child Health of Jiangsu Province.

COMPETING INTEREST STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

AUTHORSHIP STATEMENT BY ALL AUTHORS

YW: Conception and design of study, collection and interpretation of data, drafting of manuscript. HL: Collection and interpretation of data, literature search, intellectual input in drafting of manuscript. HZ: Collect and interpret of data, manuscript revisions. All the authors approved the final version of the manuscript.

ETHICS STATEMENT BY ALL AUTHORS

The authors collectively take the responsibility of the research work and mantainence of relevant documentation. The work was ethically approved by the institutional research committee.

LIST OF ABBREVIATIONS (In alphabetic order)

ASCUS - Atypical squamous cells of undetermined significance
BMRT - BioPerfectus multiplex real time
CIN - Cervical intraepithelial neoplasia
HP - Helicobocton pyloni
HPV - Human papillomavirus
HSIL - High degree squamous intraepithelial lesion
LSIL - Low degree squamous intraepithelial lesion
PAX1 - Paired boxed 1
PCR - Polymerase chain reaction
TCT - Thinprep cytologic test
ZNF582 - Zinc finger protein 582

EDITORIAL/PEERREVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through automatic online system.

REFERENCES

1. Huang LY. Cervical Cancer can be Prevented and Controlled, and China's HPV Vaccine may Benefit African Countries. London: International Talent; 2021. p. 48-50.
2. Zhu Y, Huang Y, Liu M, Yan Q, Zhao W, Yang P, *et al*. Epigallocatechin gallate inhibits cell growth and regulates miRNA expression in cervical carcinoma cell lines infected with different high-risk human paillomavirussub-types. Exp Ther Med 2019;17:1742-8.
3. Shidham VB. Role of immunocytochemistry in cervical cancer screening. Cytojournal 2022;19:42.
4. Shidham VB. CellBlockistry: Chemistry and art of cell-block making - A detailed review of various historical options with recent advances. Cytojournal 2019;16:12.
5. Wang XY, Liang Y, Deng YJ. The correlation between changes in vaginal flora and cervical lesions. Chin J Microecol 2018;30:588-93.
6. Bhat S, Kabekkodu SP, Varghese VK, Chakrabarty S, Mallya SP, Rotti H, *et al*. Aberrant gene-specific DNA methylation signature analysis in cervical cancer. Tumour Biol 2017;39.
7. Nikolaidis C, Nena E, Panagopoulou M, Balgkouranidou I, Karaglani M, Chatzaki E, *et al*. PAX1 methylation as an auxiliary biomarker for cervical cancer screening: A meta-analysis. Cancer Epidemiol 2015;39:682-6.
8. Xu L, Xu J, Hu Z, Yang B, Wang L, Lin X, *et al*. Quantitative DNA methylation analysis of paired box gene 1 and LIM homeobox transcription factor 1 α genes in cervical cancer. Oncol Lett 2018;15:4477-84.
9. Rodríguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE, *et al*. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst 2008;100:513-7.
10. ICO IARC Information Centre. Key Data on HPV and HPV-related Cancers. Wycliffe House: ICO IARC Information Centre; 2018.
11. Elfgren K, Elfstr MK, Naucler P, Arnheim-Dahlström L, Dillner J. Management of women with human papillomavirus persistence: Long-term follow-up of a randomized clinical trial. Am J Obstet Gynecol 2017;216:264.e1-7.
12. Duan LF, Du H, Xiao AM, Wang C, Huang X, Zhao MF, *et al*. Correlation between type-specific viral load of high risk HPV reflected by the Ct value of Cobas 4800 HPV system. Zhonghua Fu Chan Ke Za Zhi 2019;54:458-63.
13. Shidham VB. CellBlockistry 101.United States: Cytopathology Foundation Inc.; 2021.
14. Lu H, Guo LH, Hu QW. Correlation between methylation status of pairing box family gene 1 and cervical tissue lesions. Chin Rural Health Serv Adm 2014;5:575-8.
15. Cheng SJ, Chang CF, Ko HH, Lee JJ, Chen HM, Wang HJ, *et al*. Hypermethylated ZNF582 and PAX1 genes in mouth rinse samples as biomarkers for oral dysplasia and oral cancer detection. Head Neck 2018;40:355-68.
16. Kamal MM, Bhatia JK. Uterine Cervix Cancer: The Bethesda System. London: Cytopathology Foundation Inc.; 2022.

17. Liou YL, Zhang TL, Yan T, Yeh CT, Kang YN, Cao L, *et al*. Combined clinical and genetic testing algorithm for cervical cancer diagnosis. Clin Epigenetics 2016;8:66.
18. Tian Y, Wu N, Liou YL, Yeh CT, Cao L, Kang YN, *et al*. Utility of gene methylation analysis, cytological examination, and HPV-16/18 genotyping in triage of high-risk human papilloma virus-positive women. Oncotarget 2017;8:62274-85.

How to cite this article: Wen Y, Liang H, Zhang H. Clinical utility of HPV typing and quantification combined with PAX1/ZNF582 methylation detection in accurate cervical cancer screening. CytoJournal 2023;20:26.

HTML of this article is available FREE at https://dx.doi.org/10.25259/Cytojournal_46_2022

NextGen CelBloking™ Kits

Frustrated with your cell blocks?
We have a better solution!

Nano

Nano NextGen CelBloking™

Cell block kit to process single scattered cell specimens and tissue fragments of **any cellularity**.

PATENT PENDING

Micro

Micro NextGen CelBloking™

For cellular specimens (more than 1 ml concentrated specimen with Tissuecrit more than 50%)

PATENT PENDING

www.AVBioInnovation.com