ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 5, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

A STUDY OF CERVICAL LESIONS IN CORRELATION WITH HUMAN PAPILLOMAVIRUS ANALYSIS BY IMMUNOHISTOCHEMICAL ASSAY

P NEERAJA[1], C BHAKTHAVATSALA REDDY[2]*, C LAKSHMI KALAVATHI[1]

[1]Department of Pathology, A. C. Subba Reddy Government Medical College, Nellore, Andhra Pradesh, India. [2]Department of Cardiology, Narayana Medical College and Hospitals, Nellore, Andhra Pradesh, India. Email: bhakthadm@yahoo.co.in

Received: 12 February 2023, Revised and Accepted: 20 March 2023

ABSTRACT

Objective: It has become clear that human papillomavirus (HPV) plays a critical role in the pathogenesis of nearly all cervical squamous cell carcinomas. Because HPV causes a variety of cervix lesions, including benign, premalignant, and malignant lesions, early detection of this infection benefits the patient.

The goal of this study is to identify neoplastic and non-neoplastic cervix lesions, to study neoplastic and non-neoplastic cervix lesions caused by HPV in correlation with HPV analysis by immunohistochemistry (IHC), and to understand the significance of HPV in screening cervical lesions.

Methods: The current study evaluated and compared HPV cocktail expression in 100 samples collected from 100 cases with cervical lesions. A semi-quantitative method was used to determine the positivity of the HPV cocktail. Comparison of expression of HPV cocktail IHC and histopathology was carried out.

Results: Of the 100 cases, 30 were carcinoma cervix (CC), 4 were carcinoma *in situ* (CIS), 22 were squamous intraepithelial lesions (SIL), 20 were chronic cervicitis, 16 were chronic cervicitis with Koilocytic change, 7 were Koilocytic change, and 1 was Inflammatory polyp. SIL and CIS have a mean age of 46.8 and 44.5 years, respectively. There was a 42% incidence of carcinoma cases seen in the fourth decade, with a mean age of 45.9 years. The most common complaint presented by CC patients is AUB, with postmenopausal bleeding being the most common complaint. Seven patients with Squamous cell carcinoma present with postmenopausal bleeding. There were neoplastic lesions in 56% of the cases and non-neoplastic lesions in 44% of the cases. Premalignant cervix lesions include low grade squamous intra epithelial lesion, high grade squamous intra epithelial lesion, and CIS, which account for 46.42% of all cases. Non-neoplastic lesions such as chronic cervicitis account for 45.55% of all cases, followed by chronic cervicitis with Koilocytic change (36.36%), Koilocytic change (15.91%), and Polyp (2.27%). There were 28 Squamous cell carcinomas and 2 Adenocarcinomas among the 30 cases of CC. Squamous cell carcinoma accounted for 93.33% of all cases, while adenocarcinoma accounted for 6.67%. Moderately differentiated squamous cell carcinomas account for 86.66% of the cases, followed by well differentiated squamous cell carcinomas and poorly differentiated squamous cell carcinomas, which account for 6.66% of the cases. 26 (86.6%) of 30 squamous cell carcinomas tested positive for IHC. All two Adenocarcinoma cases (100%) and all CIS cases (100%) were positive. Out of the 22 SIL cases, 18 (81.81%) were positive. Out of 16 cases of chronic cervicitis with Koilocytic change, 14 (87.5%) were positive. Out of 20 chronic cervicitis cases, 12 (60%) were positive. 5 (71.43%) of the 7 cases of Koilocytic change were positive. Cervical lesions (benign, premalignant, and malignant) have a significant correlation with HPV cocktail IHC (p=0.01).

Conclusion: The expression of the HPV cocktail was associated with clinical and histopathologic parameters in benign, premalignant, and malignant cervical lesions. Periodic follow-up allows patients to be better managed by providing vital data on the incidence of HPV infection in various cervical lesions. Low-grade SILs and high-grade SILs with HPV +ve infection can be monitored by HPV once a year instead of every 6 months. Patients over the age of 30 years with HPV-positive non-neoplastic cervical lesions can be monitored once every three years rather than annually. HPV is a biomarker for a better prognosis in cervical cancer, regardless of age, International Federation of Obstetricians and Gynecologists stage, or histologic type. HPV-positive tumors have a better prognosis.

Keywords: Human papillomavirus, Histopathology, Oncogenesis, Immunohistochemical assay.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i5.47895. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in women worldwide [1]. Cancer in India In terms of cancer incidence and mortality, the uterine cervix ranks third. Despite the fact that the Pap test has successfully reduced the incidence and mortality rates caused by cervical cancer worldwide, the false positive and false negative rates of this test are significant [2], demonstrating the Pap test's diagnostic limitations as well as the possibility of under or over-treatment. Even among a panel of pathologists reviewing the same slides, there could be intra-observer and inter-observer diagnostic discrepancies in histopathology of cervical biopsies [3]. In histopathology, different immunohistochemistry (IHC) with biomarkers are being evaluated to differentiate pre-cancerous lesions and uterine cervical carcinoma. Infection with genital human papillomaviruses (HPVs) is the leading cause of cervical squamous intraepithelial lesion (SIL) and invasive cervical cancer [4]. There is strong evidence that the Pap and HPV tests are effective at detecting cancer and precancerous cells. Screening for high-risk HPV is more sensitive in detecting precancerous cervical lesions than cytology screening [5,6]. DNA similar to that found in the HPV has been found in cervical dysplasia and carcinoma *in situ* (CIS), both precursor lesions, as well as invasive cancers and lymph node metastases [7]. A review of cytology slides after subsequent cervical biopsy reports revealed a decrease in cervical cytology accuracy. The most common test for detecting cervical diseases is histopathological examination of biopsies of cervical lesions [8].

Hence, the current study aimed to differentiate neoplastic and non-neoplastic lesions of cervix, and to identify the significance of HPV in screening cervical lesions.

METHODS

From October 2018 to March 2020, 100 cervical biopsy or hysterectomy specimens were collected at the ACSR Medical College, Nellore''s Department of Pathology.

Patients with complaints of abnormal vaginal bleeding who underwent cervical biopsy or hysterectomy were included in the study.

Clinical data were obtained from the department's records. Following arrival, the specimens were adequately fixed in 10% neutral buffer formalin before being evaluated for gross features. The gross details of a specimen submitted for malignancy evaluation were observed and recorded.

The representative tissue from the hysterectomy specimen and the entire tissue from the cervical biopsy specimen were routinely processed for paraffin embedding. 4-5 μ thick sections were cut from paraffin embedded blocks, stained with hematoxylin and eosin, and studied. All tissue bits were taken from the original blocks and a tissue microarray was assembled for the study of IHC markers.

RESULTS

Of the 100 cases, 30 were Carcinoma Cervix (CC), 4 were CIS, 22 were SILs, 20 were chronic cervicitis, 16 were chronic cervicitis with Koilocytic change, 7 were Koilocytic change, and 1 was Inflammatory polyp.

The age range of patients with cervical lesions was 28–86 years, with the highest incidence (42%) occurring in the fourth decade. The remaining cervical lesions were 27% in 31–40 years, 17% in 51–60 years, 8% in 21–30 years, 5% in 61–70 years, and 1% in 81–90 years, respectively.

The highest incidence of premalignant cervix lesions; SIL, CIS in 41–50 year, and a total of 26 cases were included in this category, with 22 SIL cases and 4 CIS cases, respectively. SIL and CIS have a mean age of 46.8 and 44.5 year, respectively.

In total, 45 cases of cervical lesions with complaint of AUB were present, followed by white discharge in 23%, pain abdomen in 17%, pelvic bleed in 11%, 8% of mass per vaginum, mennorhagia in 13%, abnormal uterine bleeding in 6%, cervical growth in 5%, irregular bleeding in 5%, post coital bleeding in 2%, and blood tinged discharge in 1% (Table 1).

The most common complaint presented by CC patients is AUB, with postmenopausal bleeding being the most common complaint. 7 patients with Squamous cell carcinoma present with postmenopausal bleeding. 30 cases were CC, and the most common complaint with which CC patients presented was AUB, with post-menopausal bleeding being the most common complaint. In the study, the most common complaint presented by cervical carcinoma patients was postmenopausal bleeding, followed by vaginal bleeding and white discharge (Table 2).

There were neoplastic lesions in 56% of the cases and non-neoplastic lesions in 44% of the cases. Chronic cervicitis was the most common non-neoplastic lesion found in this study, followed by chronic cervicitis with Koilocytic change.

The current study included 30 cases of CC, 28 of which were Squamous cell carcinomas and two of which were Adenocarcinomas. Squamous cell carcinoma accounted for 93.33% of all cases, while adenocarcinoma accounted for 6.67%. Based on the International Federation of Obstetricians and Gynecologists (FIGO) guidelines, squamous cell carcinoma was histologically graded as well differentiated, moderately differentiated, and poorly differentiated. Moderately differentiated squamous cell carcinomas account for 86.66% of the cases, followed by well-differentiated squamous cell carcinomas and poorly differentiated squamous cell carcinomas, which account for 6.66% of the cases. Among the 22 cases of SIL, 20 were low-grade SILs (LSIL) and two were high-grade SILs (HSIL) (Tables 3 and 4).

Table 1: Presenting complaints of CIS, and squamous intra epithelial lesion

Presenting complaint	No of CIS cases	No of SIL cases
Pelvic white discharge	1	6
Pain abdomen	0	6
AUB	3	7
Pelvic mass	0	2
Cervical growth	0	1

CIS: Carcinoma *in situ*, SIL: Squamous intraepithelial lesions

Table 2: Different patterns of cervical lesions-non-neoplastic and neoplastic lesions

Characteristics	No of cases	Percentage
Pattern of lesions in cervix		
Chronic cervicitis	20	20
Chronic cervicitis with Koilocytic change	16	16
Koilocytic change	7	7
Polyp	1	1
SIL	22	22
CIS	4	4
Squamous cell carcinoma	27	27
Adeno carcinoma	2	2
Non-neoplastic lesions of cervix (n=44)		
Chronic cervicitis	20	45.45
Chronic cervicitis with Koilocytic change	16	36.36
Koilocytic change	7	15.91
Polyp	1	2.27
Neoplastic Lesion (n=56)		
Premalignant (SIL, CIS)	26	46.42
Malignant	30	53.57

CIS: Carcinoma *in situ*, SIL: Squamous intraepithelial lesions

Table 3: Histological patterns of cervical carcinoma, histological grade, and types of squamous intra epithelial lesions

Pattern/grade	Number of cases	Percentage
Histological pattern		
Squamous cell carcinoma	28	93.33
Adenocarcinoma	2	6.67
Histological grade		
Well differentiated	2	6.66
Moderately differentiated	26	86.66
Poorly differentiated	2	6.66
Histological pattern of SIL		
LSIL	20	90.90
HSIL	2	9.091

CIS: Carcinoma *in situ*, SIL: Squamous intraepithelial lesions,
LSIL: Low-grade squamous intraepithelial lesions, HSIL: High-grade squamous intraepithelial lesions

Table 4: Cocktail HPV expression in cervical lesions

Cervical lesions	No of cases	HPV cocktail (positive)	HPV cocktail (negative)
Squamous cell carcinoma	30	26 (86.66%)	4 (13.34%)
Chronic cervicitis	20	12 (60%)	8 (40%)
CIS	4	4 (100%)	0 (0%)
LSIL	20	17 (85%)	3 (15%)
Polyp	1	0 (0%)	1 (100%)
HSIL	2	1 (50%)	1 (50%)
Chronic cervicitis+Koilocytic change	16	14 (87.5%)	4 (12.5%)
Koilocytic change	7	5 (71.43%)	2 (28.57%)
Adenocarcinoma	2	2 (100%)	0 (0%)

HPV: Human papillomavirus, CIS: Carcinoma *in situ*, LSIL: Low-grade squamous intraepithelial lesions, HSIL: High-grade squamous intraepithelial lesions

In the study, 26 (86.6%) of 30 Squamous cell carcinomas tested positive for IHC. All two Adenocarcinoma cases (100%) and all CIS cases (100%) were positive. Out of the 22 SIL cases, 18 (81.81%) were positive. Out of 16 cases of chronic cervicitis with Koilocytic change, 14 (87.5%) were positive. Out of 20 chronic cervicitis cases, 12 (60%) were positive. 5 (71.43%) of the 7 cases of Koilocytic change were positive. Cervical lesions (benign, premalignant, and malignant) have a significant correlation with HPV cocktail IHC (p=0.01).

DISCUSSION

Cervical intraepithelial lesions precede cervical cancer; in fact, it is one of the few cancerous lesions in which precursor lesions have been extensively researched and studied. Non- and pre-neoplastic lesions have a wide age distribution and frequently present late because most cases are asymptomatic. These lesions are treatable, and early detection of pre-neoplastic cases could significantly lower the incidence of cervical cancer. In recent years, it has become clear that HPV plays a critical role in the pathogenesis of nearly all cervical squamous cell carcinomas [9]. In the study, lesions were classified as non-neoplastic, premalignant, or malignant, as in previous studies [7,8,10-12].

There was a 42% incidence of carcinoma cases seen in the fourth decade, with a mean age of 45.9 years. Neoplastic lesions accounted for 56% of cases among 100 samples, with non-neoplastic lesions accounting for 44% of cases among cervical lesions. The mean age of CC in the Singh and Bannur study was 49.88 years [13]. In the study by Gupta and Basavaraj, the average age of invasive carcinoma was 48.5 years [14].

In our study, premalignant cervix lesions included low grade squamous intra epithelial lesion, high grade squamous intra epithelial lesion, and carcinoma *in situ*, accounting for 46.42% of the cases. This was similar to Jain *et al.* [10], Bagde *et al.* [12], who had 23% and 24.1%, respectively.

In our study, non-neoplastic lesions such as chronic cervicitis accounted for 45.55% of the cases, followed by chronic cervicitis with Koilocytic change (36.36%), Koilocytic change (15.91%), and Polyp (2.27%).

Chronic cervicitis accounted for 45.55% of total non-neoplastic lesions in our study. In the Nwachokor *et al.*'s [15] study, it is 43.5%, while in the Priyadarshini and Arathi [16] study, it is 48%.

In our study, 30 cases were CC, 28 cases were Squamous cell carcinomas, and 2 cases were Adenocarcinomas. Squamous cell carcinoma accounted for 93.33% of all cases, while adenocarcinoma accounted for 6.67%.

In the study of Yadav *et al.* [17], among the malignant cases, only two histological types were observed i.e., SCC which were 42 cases comprising 93.33% and 3 cases of adenocarcinoma comprising 6.67%.

Halle *et al.* [18] studied 401 cases, of which SCC was 282 cases accounting for 70%, adenocarcinoma was 90 cases accounting for 23%, and adenosquamous carcinoma was 12 cases accounting for 3%. Baythoon *et al.* [19] studied 42 tissue samples of invasive cervical carcinoma, 30 of which were squamous cell carcinoma and 12 of which were adenocarcinoma. Huang *et al.* found that out of 103 cases of cervical carcinoma, 95 were SCC, 5 were adenocarcinoma, and 3 were adenosquamous carcinoma [20].

In our study, moderately differentiated squamous cell carcinomas account for 86.66% of the cases, followed by well differentiated squamous cell carcinomas and poorly differentiated squamous cell carcinomas, which account for 6.66% each. Squamous cell carcinoma was classified into well, moderate, and poorly differentiated carcinomas at the time of initial diagnosis in the Kakati and Bhuyan [21] study. Out of 40 squamous cell carcinoma cases, 22 (55%) were moderately differentiated, 12 (30%) were well-differentiated, and 6 (15%) were poorly differentiated.

Thakrar and Mathur [22] found that well differentiated SCC was 3, moderately differentiated SCC was 76, and poorly differentiated SCC was 5. HPV infections are common, with 75% to 80% of sexually active people infected at some point in their lives. Other than these eight, HPV types are linked to <2% of cervical cancer cases worldwide. It is generally assumed that it takes several years to decades from the time of initial HPV infection to the development of a high-grade lesion and, eventually, the formation of a tumor [23]. HPV testing has several potential advantages over cytology-based screening.

In our study, 26 (86.6%) of 30 squamous cell carcinomas were IHC positive. All two Adenocarcinoma cases (100%) and all CIS cases (100%) were positive. Out of the 22 SIL cases, 18 (81.81%) were positive. Out of 16 cases of chronic cervicitis with Koilocytic change, 14 (87.5%) were positive. Out of 20 chronic cervicitis cases, 12 (60%) were positive. 5 (71.43%) of the 7 cases of Koilocytic change were positive. Cervical lesions (benign, premalignant, and malignant) have a significant correlation with HPV cocktail IHC (p=0.01).

In the Siriaunkgul *et al.* study [24], HPV genotyping was performed using a linear array HPV ASSAY, and HPV was found in 96/99 (96.9%) of the cases. 75 (78.1%) cases had a single infection, while 21 (21.9%) had multiple infections. HPV 16 and HPV 18 were the most common subtypes, detected in 62/96 (64.4%) cases. Infections with HPV 52 AND HPV 58 were discovered in 17/96 (17.7%) of the cases. HPV-16 immunostaining was positive in 11 of 20 invasive CC cases studied by Rashed and Bekele [25].

CONCLUSION

The expression of the HPV cocktail was linked to clinical and histopathologic parameters in benign, premalignant, and malignant cervical lesions. Periodic follow-up allows patients to be better managed by providing vital data on the incidence of HPV infection in various cervical lesions. LSIL and HSIL with HPV +ve infection can be monitored by HPV once a year instead of every 6 months. Patients over the age of 30 with HPV-positive non-neoplastic cervical lesions can be monitored once every three years rather than annually. HPV-positive tumors have a better prognosis.

ACKNOWLEDGMENTS

Authors acknowledge to the technical staff of the department of pathology.

AUTHORS' CONTRIBUTIONS

Neeraja, Bhakthavatsala Reddy- conceptualization, methodology, formal analysis, writing - original draft, writing - reviewing and editing; Neeraja, Lakshmi kalavathi- data curation, writing - reviewing and editing; Neeraja, Bhakthavatsala Reddy, Lakshmi kalavathi- investigation, formal analysis.

COMPETING INTERESTS

There are no conflicts of interest.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90. doi: 10.3322/caac.20107, PMID 21296855
2. Koss LG. The Papanicolaou test for cervical cancer detection: A triumph and a tragedy. JAMA 1989;261:737-43. doi: 10.1001/jama.1989.03420050087046, PMID 2642983
3. McCluggage WG, Walsh MY, Thornton CM, Hamilton PW, Date A, Caughley LM, *et al*. Inter- and intra-observer variation in the histopathological reporting of cervical squamous intra-epithelial lesions using a modified Bethesda grading system. BJOG 1998;105:206-10. doi: 10.1111/j.1471-0528.1998.tb10054.x
4. Tornesello ML, Duraturo ML, Giorgi-Rossi P, Sansone M, Piccoli R, Buonaguro L, *et al*. Human papillomavirus (HPV) genotypes and HPV16 variants in human immunodeficiency virus-positive Italian women.

J Gen Virol 2008;89:1380-9. doi: 10.1099/vir.0.83553-0, PMID 18474553

5. Arbyn M, de Sanjose S, Weiderpass E. HPV-based cervical cancer screening, including self-sampling, versus screening with cytology in Argentina. Lancet Glob Health 2019;7:e688-9. doi: 10.1016/S2214-109X(19)30067-1, PMID 31097266
6. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, *et al*. Efficacy of HPV-based screening for prevention of invasive cervical cancer: Follow-up of four European randomised controlled trials. Lancet 2014;383:524-32. doi: 10.1016/S0140-6736(13)62218-7, PMID 24192252
7. Fatima Q, Verma S, Bairwa NK, Gauri LA. Spectrum of various lesions in cervical biopsies in North West Rajasthan: A prospective histopathological study. Int J Res Prof 2017;3:104-11.
8. Ali MH, Ahmed HG, Salih RA. Histopathologic Pattern of Cervical Lesions at Omdurman Military Hospital, Sudan. Sch J App Med Sci 2015;3:2903-7.
9. Rosai J. Female reproductive system-uterus: Cervix. In: Rosai and Ackerman's Surgical Pathology. 10th ed., Ch. 19. Netherlands, MO: Elsevier Ltd.; 2011. p. 1436-76.
10. Jain A, Dhar R, Patro P, Sahu S. Histopathological study of cervical lesions. Int J Health Sci Res. 2018;8:82-7.
11. Kumari K, Umarani MK, Bharathi M. Histopathological spectrum of cervical biopsies- a 5 year retrospective study. Trop J Pathol Microbiol 2017;3:46-51.
12. Bagde S, Gupta R, Ganguly S, Bhardwaj A, Jogi S. Spectrum of cervical lesions in CIMS, Bilaspur: A 5 year retrospective study of 215 cases in a Tertiary Hospital of Central India. J Evid Based Med Healthc 2015;2:7505-10. doi: 10.18410/jebmh/2015/1014
13. Singh N, Bannur H. A cross-sectional study of p53 expression in patients with squamous cell carcinoma cervix: A hospital-based study. Indian J Health Sci Biomed Res 2017;10:203-7.
14. Gupta M, Basavaraj PK. Histopathological spectrum of premalignant and malignant lesions of uterine cervix. Natl J Lab Med 2018;7:PO19-26.
15. Nwachokor FN, Forae GC. Morphological spectrum of non-neoplastic lesions of the uterine cervix in Warri, South-South, Nigeria. Niger J Clin Pract 2013;16:429-32.
16. Priyadarshini D, Arathi CA. Histopathological spectrum of non-neoplastic uterine Cervical lesions in a tertiary Care Center. Ann Pathol Lab Med 2017;4:A303-9.
17. Yadav V, Jaiswal M, Pandey S. A comparative study of p53 expression in premalignant and malignant cervical lesions at a tertiary care institute of Rohilkhand region, India. Int J Med Sci Public Health 2018;7:216-21.
18. Halle MK, Ojesina AI, Engerud H, Woie K, Tangen IL, Holst F, *et al*. Clinicopathologic and molecular markers in cervical carcinoma: A prospective cohort study. Am J Obstet Gynecol 2017;217:432.e1-17.
19. Baythoon SJ, Ali HH, Qasim B. Immunohistochemical expressionof P53 invasive cervical carcinoma (A Clinicopathological Study). Iraqi J Med Sci 2008;6:90-102.
20. Huang LW, Chou YY, Chao SL, Chen TJ, Lee TT. p53 and p21 Expression in precancerous lesions and carcinomas of the uterine cervix: Overexpression of p53 predicts poor disease outcome. Gynecol Oncol 2001;83:348-54. doi: 10.1006/gyno.2001.6397:53
21. Kakati J, Bhuyan B. A histopathological study of neoplastic lesions of uterine cervix of peri and postmenopausal women. J Evid Based Med Healthc 2017;4:1294-8.
22. Thakrar M, Mathur K. Immunohistochemical study of p53 expression as proliferative index in neoplasia of the uterine cervix. Int Arch Integr Med 2017;4:16-25.
23. Burd EM. Human papillomavirus laboratory testing: The changing paradigm. Clin Microbiol Rev 2016;29:291-319.
24. Siriaunkgul S, Suwiwat S, Settakorn J, Khunamornpong S, Tungsinmunkong K, Boonthum A, *et al*. HPV genotyping in cervical cancer in Northern Thailand: Adapting the linear array HPV assay for use on paraffin-embedded tissue. Gynecol Oncol 2008;108:555-60.
25. Rashed MM, Bekele A. The prevalence and pattern of HPV-16 immunostaining in uterine cervical carcinomas in Ethiopian women: A pilot study. Pan Afr Med J 2011;8:21. doi: 10.4314/pamj.v8i1.71077, PMID 22121430