Hormozgan Medical Journal

January 2024, Volume 28, Number 1

Research Paper

Investigating Ki67 and p16 Positivity Pattern in Human Papillomavirus 16 and 18 Versus Other High-risk Human Papillomaviruses

Ali AtashAbParvar[1], Mahnaz Baniasadi[1*]

1. Department of Pathology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Use your device to scan and read the article online

Citation AtashAbParvar A, Baniasadi M. Investigating Ki67 and p16 Positivity Pattern in Human Papillomavirus 16 and 18 Versus Other High-risk Human Papillomaviruses. Hormozgan Medical Journal. 2024; 28(1):9-16. http://dx.doi.org/10.32598/hmj.28.1.1

http://dx.doi.org/10.32598/hmj.28.1.1

Article info:
Received: 23 Jun 2023
Accepted: 18 Aug 2023
Available Online: 01 Jan 2024

Keywords:
Ki67 antigen, p16, Human papillomavirus

ABSTRACT

Objectives: The expression of Ki67 and p16 has been used for the diagnosis of human papillomavirus (HPV) 16 and 18; however, limited research has been conducted on the comparison with other high-risk HPVs (HR-HPVs) in this respect. Accordingly, this study compares the positivity pattern of Ki67 and p16 in HPV16/18 with other HR-HPVs.

Methods: This descriptive study included women with positive screening test (Pap smear test) and positive HR-HPV referred to Shahid Mohammadi Hospital, Bandar Abbas, Iran, from 2018-2019 for colposcopy and cervical biopsy. Biopsy specimens were stained for Ki67 and p16. Data on age, education, and menopause status were also recorded.

Results: Of the 80 women included in this study with a mean age of 33.16±8.55 years, 48.8% had positive HPV16/18, and 51.2% were positive for other HR-HPVs. The positivity pattern of Ki67 and the type of HR-HPV (HPV16/18 vs other HR-HPVs) were significantly correlated (P=0.006). Full-thickness positive Ki67 was only observed in HPV16/18 positive specimens. Positive Ki67 in the upper and middle thirds were also significantly more frequent with HPV16/18. The correlation between positive Ki67 and HR-HPVs remained significant in women aged >30 years, with university education, and of childbearing age.

Discussion: The positivity pattern of Ki67 is significantly correlated with HPV16/18, and age, education, and menopause status are the influential factors. Meanwhile, the strongly positive p16 pattern was more frequent in HPV16/18 compared to other HR-HPVs; however, the relationship between the positivity pattern of p16 and the type of HR-HPV was not significant.

*** Corresponding Author:**
Masoumeh Baniasadi, MD.
Address: *Department of Pathology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.*
Tel: *+98 (913) 1575501*
E-mail: *mmahnazbaniasadi@gmail.com*

[DOI: 10.32598/hmj.28.1.1]

Introduction

In its recent consensus statements, the World Health Organization (WHO) has emphasized the evaluation of cervical cancer. To date, no single effective method has been recognized for the prediction of cervical intraepithelial neoplasia (CIN) and disease progression in cervical cancer [1, 2]. The current screening program includes periodical Pap smear (cervical cytology) and high-risk human papillomavirus (HR-HPV) testing based on the WHO and the American Society for Colposcopy and Cervical Pathology (ASCCP) protocols [3, 4]. Evidence shows that HR-HPV testing has been more effective than cytology for the reduction of CIN 3 and cervical cancer [5].

P16 is a down-regulator of proliferation in normal cells by reducing the activity of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 when the retinoblastoma protein is deactivated. HPV E7 can accelerate the progression of cancer cells by deactivating retinoblastoma protein leading to the overexpression of p16 in the injured cells. On the other hand, Ki67 is a nuclear protein and an indicator of cell proliferation. Accordingly, in normal cells, p16 and Ki67 are not concurrently expressed. The co-expression of p16 and Ki67 shows the reduction of cell cycle due to HPV oncoproteins which mainly occurs during viral persistency and indicates the potential correlation of p16 and Ki67 co-expression (p16/Ki67) with HPV infection [6].

Multiple studies have used the expression of p16 and Ki67 for the diagnosis of HPV16 and HPV18. For instance, Nam et al. evaluated the relationship between p16 and Ki67 expression with CIN 3 and HR-HPV and found a positive correlation between the CIN grade with p16 and Ki67 expression [7]. In another study, Keating et al. reported a 91% positive predictive value with p16 expression and 82% with Ki67 for the diagnosis of HPV [8]. Moreover, Ordi et al. have recommended the assessment of p16 expression along with histologic examination of the cervical biopsy specimens from HR-HPV-positive women [9].

Although many studies have evaluated the expression of p16 and Ki67 for the diagnosis of HPV16 and 18, limited research has been done to compare the expression of these two markers between HPV16/18 and other HR-HPVs. Accordingly, this study compares the positivity pattern of Ki67 and p16 in HPV16/18 with other HR-HPVs.

Materials and Methods

Study participants

This descriptive study evaluated all women with positive screening test (Pap smear) and positive HR-HPV who had been referred to Shahid Mohammadi Hospital, Bandar Abbas, Iran, in 2018-2019 for colposcopy and cervical biopsy. The participants were selected through census sampling and all eligible patients were included in the study. Inappropriate cervical specimens for immunohistochemistry were excluded from the study.

Study design

The age, education, and menopause status of the patients were recorded. Cervical biopsy specimens underwent HPV genotyping using a polymerase chain reaction. The specimens were marked as HPV16/18 or other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69). Ki67 and p16 staining were performed using Sanatashkhis products (manufactured in Iran). The staining steps were as follows. At first, 100 µL peroxidase was added to each slide. Then, the specimens were incubated at room temperature and darkness for 10 min. Afterward, each slide was rinsed with distilled water three times for 5 min. In the next step, a 100 µL primary antibody amplifier was added to the specimens and remained for 15 min. Subsequently, each slide was rinsed three times for 5 min. Meanwhile, 100 µL master polymer plus horseradish peroxidase was added to each slide with specimens incubated at room temperature for 30 min followed by rinsing each slide 3 times for 5 min. The chromogen solution was added to each slide and was left for 5 min at room temperature followed by rinsing the slides 3 times for 5 min. Then, the 3,3'-diaminobenzidine enhancer was added to the slides and remained for 1 to 2 min at room temperature and then rinsed. For counterstaining, the specimens were covered with hematoxylin for 1 min and then rinsed with distilled water.

Ki67 positivity patterns included the lower third, the middle third, the upper third, and full-thickness, while p16 staining patterns were either weakly or strongly positive. Also, the CIN grades were determined.

Data analysis

The SPSS software, version 25 (Armonk, NY: IBM Corp., USA) was used for data analysis. Mean±SD, frequencies, and percentages were used to describe the variables. The Fisher exact tests were used to compare frequencies between groups. Meanwhile, $P \leq 0.05$ were considered statistically significant.

[DOI: 10.32598/hmj.28.1.1]

Results

Of the 80 women evaluated in this study (mean age of 33.16±8.55 years), 39(48.8%) had positive HPV16/18, and 41(51.2%) were positive for other HR-HPVs. Ki67 was positive in the lower third of the specimen in 60(75%), up to the middle third in 13(16.3%), up to the upper third in 5(6.3%), and full-thickness in 2(2.5%). Three specimens (3.8%) were negative for p16, while 52(65%) were weakly and 25(31.3%) strongly positive for p16. The general characteristics of the study population are demonstrated in Table 1.

Positive Ki67 up to the middle and upper third, and full-thickness positivity were significantly higher in positive HPV16/18 specimens compared to other HR-HPVs (P=0.006). Nevertheless, no significant correlation was detected between the p16 positivity pattern and HR-HPV type (P=0.163) (Table 2).

When the study participants were divided into different groups based on age, education, menopause status, and CIN grade, a significant relationship was found between Ki67 positivity pattern and HR-HPV type in women aged >30 years, those with university education, and women of childbearing age (Table 3). However, no significant correlation was observed between the positivity pattern of p16 and the type of HR-HPV in women of different age groups, education, or menopause status (Table 4).

Table 1. Study population characteristics

Variables		No. (%)
Age (y)	≤30	37(46.3)
	>30	43(53.8)
Education	High school diploma or less	46(57.5)
	University	34(42.5)
Menopause status	Childbearing age	74(92.5)
	Menopause	6(7.5)
HR-HPV	HPV16/18	39(48.8)
	Other HR-HPVs	41(51.2)
CIN	Negative	6(7.5)
	CIN 1	53(66.3)
	CIN 2	14(17.5)
	CIN 3	5(6.3)
	Carcinoma in situ	2(2.5)
Ki67 Staining	Lower 3rd	60(75.0)
	Middle 3rd	13(16.3)
	Upper 3rd	5(6.3)
	Full-thickness	2(2.5)
p16 staining	Negative	3(3.8)
	Weakly positive	52(65.0)
	Strongly positive	25(31.3)

Abbreviations: HPV: Human papillomavirus; HR-HPV: High-risk HPV; CIN: Cervical intraepithelial neoplasia.

[DOI: 10.32598/hmj.28.1.1]

Discussion

This study found a significant correlation between the positivity pattern of Ki67 with HR-HPV type (HPV16/18 and other HR-HPVs). Full-thickness positive Ki67 was only observed in HPV16/18 positive specimens. Also, positive Ki67 in the upper and middle third of the specimens were significantly higher with HPV16/18 compared to other HR-HPVs. However, although the strongly positive pattern of p16 was more frequent with HPV16/18 compared to other HR-HPVs, the difference was not statistically significant.

Cervical cancer is the fourth most common malignant tumor in women worldwide and HPV infection is considered its major etiology [10]. Specific HPV types, especially HPV16 and HPV18 can lead to cervical dysplasia which is a reversible precancerous lesion. Consistent cervical infection with HPV can cause irreversible alterations that can result in carcinoma in situ and consequently invasive cervical cancer [11]. Early diagnosis using different screening methods is the primary measure for the prevention and treatment of cervical cancer. Currently, three methods, including cervical cytology, HPV genotyping, and cytology together with HPV genotyping are used for cervical cancer screening [12]. Due to the low sensitivity of cytology, many HPV-positive women require multiple cytology follow-ups. Thus, more effective markers are required to triage HPV-positive women with normal cytology or without HPV16/18, or to differentiate women with high-grade CIN from low-grade squamous intraepithelial lesions and atypical squamous cells of undetermined significance. Evidence shows that cytology with p16/Ki67 dual staining can be a substitute biomarker with high sensitivity and specificity for high-grade CIN [13-17].

In line with our findings, Lewitowicz et al. also showed a significant correlation between HPV16 and positive p16/Ki67 [18]. Similarly, Liao et al. demonstrated that p16/Ki67 expression in HPV16/18 positive and other 12 HR-HPV positive specimens was significantly higher compared to specimens with negative HPV [19]. The results of this study were also consistent with ours; however, we only evaluated HR-HPV-positive specimens and showed significantly higher expression of Ki67 with HPV16/18 compared to other HR-HPVs. On the other hand, Jiang et al. reported an increase in p16/Ki67 positivity with increased HPV risk (HR-HPVs) from 65% in women with positive HPV51/39/68/35 to 88% in women with positive HPV16/18 [20]. Moreover, Yu et al. observed that the odds of positive p16/Ki67 in women with persistent HPV16/18 infection was approximately 4-fold compared to women with other HR-HPVs [6]. In addition, Dona et al. reported that the correlation between positive p16/Ki67 with HPV16/18 was twice stronger than its correlation with other HR-HPVs [21]. In previous studies, the concurrent expression of p16 and Ki67 has been evaluated, while this study assessed each marker separately and showed no significant correlation between positive p16 and HR-HPV type. Furthermore, in the previous studies, specimens were assessed regarding the positivity of these markers, i.e. each specimen was either positive or negative, while we also evaluated the pattern of positivity for each marker, which can be regarded as a major strength for the current study. By

Table 2. Ki67 & p16 patterns: HPV16/18 vs other HR-HPVs

Variables	Distribution	No. (%)		P*
		HPV16/18 (n=39)	Other HR-HPVs (n=41)	
Ki67 staining	Lower 3rd	23(59.0)	37(90.2)	0.006
	Middle 3rd	10(25.6)	3(7.3)	
	Upper 3rd	4(10.3)	1(2.4)	
	Full-thickness	2(5.1)	0(0.0)	
P16 staining	Negative	1(2.6)	2(4.9)	0.163
	Weakly positive	22(56.4)	30(73.2)	
	Strongly positive	16(41.0)	9(22.0)	

HPV: Human papillomavirus; HR-HPV: High-risk HPV.

*Fisher exact test.

[DOI: 10.32598/hmj.28.1.1]

Table 3. Ki67 pattern: HPV16/18 vs other HR-HPVs by age, education, menopause, CIN grade

Ki67 Staining	Distribution	No. (%)		P*
		HPV16/18	Other HR-HPVs	
Age(y) ≤30	Lower 3rd	11(55.0)	14(82.4)	0.157
	Middle 3rd	8(40.0)	3(17.6)	
	Upper 3rd	1(5.0)	0(0.0)	
Age(y) >30	Lower 3rd	12(63.2)	23(95.8)	0.021
	Middle 3rd	2(10.5)	0(0.0)	
	Upper 3rd	3(15.8)	1(4.2)	
	Full-thickness	2(10.5)	0(0.0)	
High school diploma or less	Lower 3rd	13(68.4)	24(88.9)	0.118
	Middle 3rd	3(15.8)	3(11.1)	
	Upper 3rd	1(5.3)	0(0.0)	
	Full-thickness	2(10.5)	0(0.0)	
University	Lower 3rd	10(50.0)	13(92.9)	0.015
	Middle 3rd	7(35.0)	0(0.0)	
	Upper 3rd	3(15.0)	1(7.1)	
Childbearing age	Lower 3rd	21(60.0)	35(89.7)	0.010
	Middle 3rd	10(28.6)	3(7.7)	
	Upper 3rd	4(11.4)	1(2.6)	
Menopause	Lower 3rd	2(50.0)	2(100.0)	0.467
	Upper 3rd	2(50.0)	0(0.0)	
CIN 1	Lower 3rd	20(100.0)	33(100.0)	-
CIN 2+	Lower 3rd	0(0.0)	2(40.0)	0.076
	Middle 3rd	10(62.5)	3(60.0)	
	Upper 3rd	4(25.0)	0(0.0)	
	Full-thickness	2(12.5)	0(0.0)	

Abbreviations: HPV: Human papillomavirus; HR-HPV: High-risk HPV; CIN: Cervical intraepithelial neoplasia.

*Fisher exact test.

solely taking positive and negative results into account, most probably we could have achieved similar results concerning these markers.

Another strength of our study was that we evaluated the correlation between the positivity pattern of Ki67 and p16 with HR-HPV types in women of different age groups, education levels, and menopause status and found a significant correlation between Ki67 positivity pattern with HR-HPV types in women aged >30 years, individuals with university education, and women of childbearing age. Previous studies lacked such analyses.

[DOI: 10.32598/hmj.28.1.1]

Table 4. P16 pattern: HPV16/18 vs other HR-HPVs by age, education, menopause, CIN grade

Ki67 Staining	Expression Levels	No. (%)		P*
		HPV16/18	Other HR-HPVs	
Age (y) ≤30	Negative	0(0.0)	2(11.8)	0.150
	Weakly positive	11(55.0)	11(64.7)	
	Strongly positive	9(45.0)	4(23.5)	
Age (y) >30	Negative	1(5.3)	0(0.0)	0.228
	Weakly positive	11(57.9)	19(79.2)	
	Strongly positive	7(36.8)	5(20.8)	
High school diploma or less	Weakly positive	13(68.4)	21(77.8)	0.513
	Strongly positive	6(31.6)	6(22.2)	
University	Negative	1(5.0)	2(14.3)	0.223
	Weakly positive	9(45.0)	9(64.3)	
	Strongly positive	10(50.0)	3(21.4)	
Childbearing age	Negative	1(2.9)	2(5.1)	0.315
	Weakly positive	20(57.1)	28(71.8)	
	Strongly positive	14(40.0)	9(23.1)	
Menopause	Weakly positive	2(50.0)	2(100.0)	0.467
	Strongly positive	2(50.0)	0(0.0)	
CIN 1	Negative	0(0.0)	2(6.1)	0.827
	Weakly positive	18(90.0)	28(84.8)	
	Strongly positive	2(10.0)	3(9.1)	
CIN 2+	Weakly positive	2(12.5)	0(0.0)	1.000
	Strongly positive	14(87.5)	5(100.0)	

Abbreviations: HPV: Human papillomavirus; HR-HPV: High-risk HPV; CIN: Cervical intraepithelial neoplasia.

*Fisher exact test.

Since the CIN grade of each specimen was also available, this study assessed the correlation between Ki67 and p16 positivity patterns with HR-HPV types. According to the findings, regardless of the HR-HPV type, all CIN 1 specimens were positive for Ki67 in the lower third. Moreover, there was no significant correlation between the positivity pattern of Ki67 and p16 with HR-HPV types in CIN 2+ specimens; however, 4 and 2 HPV16/18 positive specimens with CIN 2+ were positive for Ki67 in the upper third and full-thickness, respectively, while Ki67 was positive in none of the CIN 2+ specimens that were also positive for other HR-HPVs. On the other hand, in CIN 1 and CIN 2+ specimens, the correlation between the p16 positivity pattern and HR-HPV types was statistically significant. Nonetheless, 18 and 2 HPV16/18 positive specimens with CIN 1 had weakly and strongly positive patterns of p16, which was higher compared to other HR-HPVs with CIN 1. Moreover, 2 HPV16/18 positive specimens with CIN 2+ had a weakly positive pattern of p16, while none of the specimens with other HR-HPVs and CIN 2+ had such a pattern. In this regard, Wentzensen et al. showed that for triaging HPV-positive women, p16/Ki67 staining can better classify the risk of CIN 3+ compared to Pap smear cytology. They reported that women with positive p16/Ki67 staining had

[DOI: 10.32598/hmj.28.1.1]

a higher risk of CIN 3+ compared to women with positive Pap smear [22]. Besides, in the study by Liao et al., the sensitivity of p16/Ki67 for the diagnosis of CIN 2+ and CIN 3+ was 94.1% and 92.9%, respectively [19]. Based on the results of the study by Stanczuk et al., the absolute sensitivity and specificity of p16/Ki67 for the diagnosis of CIN 2+ in women with positive HR-HPV were 85% and 76.7%, respectively [23]. Therefore, further studies are required to evaluate the relationship between the positivity pattern of Ki67 and p16 with HR-HPV types and its role in determining the CIN grade. The difference between previous studies and this research in this regard may rely on different demographic characteristics of the study populations, the concurrent evaluation of the two markers in other studies, and different study designs.

Conclusions

The present study showed a significant correlation between the positivity pattern of Ki67 with HPV16/18, with age, education, and menopause status as influential factors. Meanwhile, the strongly positive p16 pattern was more frequent in HPV16/18 compared to other HR-HPVs; however, the relationship between the positivity pattern of p16 and the type of HR-HPV was not significant.

Study limitations

The primary limitation of the current study was its relatively small sample size which limits the generalizability of our findings. Future studies with a larger sample size are required to confirm our findings.

Ethical Considerations

Compliance with ethical guidelines

This study received ethical approval from the Ethics Committee of Hormozgan University of Medical Sciences (Code: IR.HUMS.REC.1398.421). It complies with the statements of the Declaration of Helsinki. Written informed consent was obtained from the participants.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization, study validation and supervision: Ali AtashAbParvar; Data analysis, data interpretation, writing and review: Masoumeh Baniasadi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors sincerely appreciate the dedicated efforts of the personnel of the Central Laboratory of Shahid Mohammadi Hospital, Bandar Abbas, Iran.

References

[1] Kanthiya K, Khunnarong J, Tangjitgamol S, Puripat N, Tanvanich S. Expression of the p16 and Ki67 in cervical squamous intraepithelial lesions and cancer. Asian Pac J Cancer Prev. 2016; 17(7):3201-6. [PMID]

[2] Possati-Resende JC, Fregnani JHTG, Kerr LM, Mauad EC, Longatto-Filho A, Scapulatempo-Neto C. The accuracy of p16/Ki-67 and HPV Test in the detection of CIN2/3 in women diagnosed with ASC-US or LSIL. PLoS One. 2015; 10(7):e0134445. [DOI:10.1371/journal.pone.0134445] [PMID]

[3] Alshenawy HA. Evaluation of p16, human papillomavirus capsid protein L1 and Ki-67 in cervical intraepithelial lesions: Potential utility in diagnosis and prognosis. Pathol Res Pract. 2014; 210(12):916-21. [DOI:10.1016/j.prp.2014.07.007] [PMID]

[4] Korolczuk A, Orzel M, Wozniak S, Smolen A, Caban K. P16/ki67 dual immunostaining in conventional cytology in women with positive papanicolau test. J Cytol Histol. 2015; 6(5):1-5. [DOI:10.4172/2157-7099.1000358]

[5] Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine. 2012; 30(Suppl 5):F88-99. [DOI:10.1016/j.vaccine.2012.06.095] [PMID]

[6] Yu LL, Guo HQ, Lei XQ, Qin Y, Wu ZN, Kang LN, et al. p16/Ki-67 co-expression associates high risk human papillomavirus persistence and cervical histopathology: A 3-year cohort study in China. Oncotarget. 2016; 7(40):64810-9. [DOI:10.18632/oncotarget.11705] [PMID]

[7] Nam EJ, Kim JW, Hong JW, Jang HS, Lee SY, Jang SY, et al. Expression of the p16 and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection. J Gynecol Oncol. 2008; 19(3):162-8. [DOI:10.3802/jgo.2008.19.3.162] [PMID]

[8] Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade BJ, Sun D, et al. Ki-67, cyclin E, and p16 INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Pathol. 2001; 25(7):884-91. [DOI:10.1097/00000478-200107000-00006] [PMID]

[9] Ordi J, Sagasta A, Munmany M, Rodríguez-Carunchio L, Torné A, del Pino M. Usefulness of p16/Ki67 immunostaining in the triage of women referred to colposcopy. Cancer Cytopathol 2014; 122(3):227-35. [DOI:10.1002/cncy.21366] [PMID]

[DOI: 10.32598/hmj.28.1.1]

[10] Venkatesulu BP, Mallick S, Rath GK. Patterns of care of cervical cancer in the elderly: A qualitative literature review. J Geriatr Oncol. 2017; 8(2):108-16. [DOI:10.1016/j.jgo.2016.12.004] [PMID]

[11] Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A, et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res 1999; 59(24):6132-6. [PMID]

[12] Wentzensen N, Schiffman M. Filling a gap in cervical cancer screening programmes. Lancet Oncol. 2014; 15(3):249-51. [DOI:10.1016/S1470-2045(14)70073-7] [PMID]

[13] Ikenberg H, Bergeron C, Schmidt D, Griesser H, Alameda F, Angeloni C, et al. Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: Results of the PALMS study. J Natl Cancer Inst. 2013; 105(20):1550-7. [DOI:10.1093/jnci/djt235] [PMID]

[14] Petry KU, Schmidt D, Scherbring S, Luyten A, Reinecke-Lüthge A, Bergeron C, et al. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. Gynecol Oncol. 2011; 121(3):505-9. [DOI:10.1016/j.ygyno.2011.02.033] [PMID]

[15] Schmidt D, Bergeron C, Denton KJ, Ridder R; European CINtec Cytology Study Group. p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL Papanicolaou cytology: Results from the European equivocal or mildly abnormal Papanicolaou cytology study. Cancer Cytopathol. 2011; 119(3):158-66. [DOI:10.1002/cncy.20140] [PMID]

[16] Tay TKY, Lim KL, Hilmy MH, Thike AA, Goh ST, Song LH, et al. Comparison of the sensitivity and specificity of p16/Ki-67 dual staining and HPV DNA testing of abnormal cervical cytology in the detection of histology proven cervical intraepithelial neoplasia grade 2 and above (CIN 2+). Malays J Pathol. 2017; 39(3):257-65. [PMID]

[17] Wentzensen N, Schwartz L, Zuna RE, Smith K, Mathews C, Gold MA, et al. Performance of p16/Ki-67 immunostaining to detect cervical cancer precursors in a colposcopy referral population. Clin Cancer Res. 2012; 18(15):4154-62. [DOI:10.1158/1078-0432.CCR-12-0270] [PMID]

[18] Lewitowicz P, Nasierowska-Guttmejer A, Rokita W, Adamczyk-Gruszka O, Gluszek S, Chrapek M, et al. HPV genotyping and p16/Ki-67 test significantly improve detection rate of high-grade cervical squamous intraepithelial lesion. Arch Med Sci. 2020; 16(1):87-93. [DOI:10.5114/aoms.2018.80697] [PMID]

[19] Liao GD, Kang LN, Li J, Zeng X, Chen W, Xi MR. The effect of p16/Ki-67 and p16/mcm2 on the detection of cervical intraepithelial neoplasia: A prospective study from China. Int J Clin Exp Pathol. 2018; 11(8):4101-8. [PMID]

[20] Jiang MY, Wu Z, Li T, Yu L, Zhang SK, Zhang X, et al. Performance of HPV genotyping combined with p16/Ki-67 in detection of cervical Precancer and Cancer among HPV-positive Chinese women. Cancer Prev Res. 2020; 13(2):163-72. [DOI:10.1158/1940-6207.CAPR-19-0144] [PMID]

[21] Donà MG, Vocaturo A, Giuliani M, Ronchetti L, Rollo F, Pescarmona E, et al. p16/Ki-67 dual staining in cervico-vaginal cytology: Correlation with histology, Human Papillomavirus detection and genotyping in women undergoing colposcopy. Gynecol Oncol. 2012; 126(2):198-202. [DOI:10.1016/j.ygyno.2012.05.004] [PMID]

[22] Wentzensen N, Clarke MA, Bremer R, Poitras N, Tokugawa D, Goldhoff PE, et al. Clinical evaluation of human papillomavirus screening with p16/Ki-67 dual stain triage in a large organized cervical cancer screening program. JAMA Intern Med. 2019; 179(7):881-8. [DOI:10.1001/jamainternmed.2019.0306] [PMID]

[23] Stanczuk GA, Baxter GJ, Currie H, Forson W, Lawrence JR, Cuschieri K, et al. Defining optimal triage strategies for hrHPV screen-positive women-an evaluation of HPV 16/18 genotyping, cytology, and p16/Ki-67 cytoimmunochemistry. Cancer Epidemiol Biomarkers Prev. 2017; 26(11):1629-35. [DOI:10.1158/1055-9965.EPI-17-0534] [PMID].

[DOI: 10.32598/hmj.28.1.1]