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ORIGINAL ARTICLE

Immunohistochemistry Expression of Dual-Staining P16/KI-67 in Cytology in Cervical Cancers

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ABSTRACT

The cytologicalPapanicolaou test and primary human papilloma virus screening are often performed to detect cervical carcinomas and pre-carcinomic lesions, that is critical for cervical cancer restriction and therapy. ASCUS or LSIL patients should be continuously followed since some may develop CIN2+. Women who test positive for HPV may develop cervical dysplasia, reversible precancerous lesions, and, in the worst-case scenario, invasive cervical cancer. As a consequence, an effective biomarker must be developed to distinguish distinct individuals based on early screening findings. Cell growth is shown by Ki-67, whereas cell cycle arrest is induced by p16, a cell cycle regulator.They couldn't co-express in the identical cervical epithelial cells under normal circumstances. HR-HPV infection has disrupted the cell cycle, predisposing to the formation of high-grade cervical epithelial lesions, according to the co-expression of these two indicators. There's growing evidence that p16/Ki-67 dual staining cytology might be employed as an substitute biomarker for detecting high-grade CIN and cervical cancer with excellent sensitivity and specificity. The current research discusses the benefits of p16/Ki-67 dual staining, as well as how it may be used to screen for cervical cancer and precancerous lesions.

Keywords: human papilloma virus, cytology, Peshawar, cervical cancer screening, CIN, p16/Ki-67 dual-staining

INTRODUCTION

Cervical cancer prevention and therapy are now based on histopathology and cytopathology. Histopathology, the study of microscopic patterns of cell organisation in tissue slices taken from biopsy or surgical specimens, may help predict cancer and precancer therapy. However, morphological models of cervical cancer and precancer development are increasingly being replaced by viral and molecular data in the evaluation of emerging cervical cancer preventive approaches. Detection of cervical cancer by cervical cytopathology is the most often utilised tool in effective cervical cancer preventive programmes. It examines the cervix's exfoliated cells. Cervical cancer incidence has decreased significantly as a result of these methods (Anttila et al. 2004; Van den Akker-van Marle, van Ballegooijen, van Oortmarssen, Boer, &Habbema, 2002).

As a consequence of these changes, cervical cancer incidence has decreased significantly. In countries where cytological screening has been effectively adopted, incidence and death have been lowered by 70% or more. 2003 (van den Akker-van Marle et al.). In some countries, significant behavioural changes have resulted in an increase in human papilloma virus exposed individuals and other causative-factors, and these historical comparisons may understate the effect because they do not account for these increases in rates among younger females to whom less protection was provided. To save 4,500–6,000 lives each year, the United Kingdom's public health initiative begun in 1988 is expected to considerably cut mortality rates among women aged 30–34 years. (Peto, Gilham, Fletcher, & Matthews, 2004). Despite this, there are around 500,000 new cases of cervical cancer each year due to the technique's shortcomings and high costs. (Anttila et al., 2004).

In the past, histopathologists have detailed how cervical precancers and malignancies develop and evolve. Carcinoma-in-situ was first proposed by Rudolf Virchow in his work on cellular pathology in the early nineteenth century. He developed three phases of cervical intraepithelial neoplasia during the next several centuries, including less severe dysplastic changes. (2003); Wells & Herrington, 2003) Histotechniques are used to diagnosed different types of lesions and carcinomas i.e., non-invasive cancer and invasive cancer.

Cervical screening is performed using the cytopathology technique. Inventors Aurel Babes and Constantin Daniel created the term in 1927 (Tornesello et al., 2008; Papanicolaou et al., 1941). At 1941, a report written by George Papanicolaou and Herbert Traut in the United States on the cervical smear procedure received global attention. (Anderson et al., 1991). Epithelial Langerhans' cells are reduced in number in this case (Stanley, 2006). Based on a greater knowledge of the molecular pathophysiology of HPVs' involvement in the neoplastic transformation of cervical squamous epithelial cells, biomarker-based testing approaches have been created and clinically tested.

Pathogenesis of HPV-triggered neoplastic lesions: p16INK4a, a biomarker for changing HPV infections, has been found in both animal and human research. The most major risk factor for cervical cancer has been found as HPV

strains 16 and 18. In most cases, these infections in young people will go away on their own. 10. Only a small percentage of persons who are impacted acquire neoplastic tumours. (Tay and colleagues, 1987) The principal location of HPV-induced neoplastic tumours is the cervical squamo-glandular junction, which is infected with the virus. According to Solomon (years ago),

Squamous intraepithelial lesions (LSILs) and ASCs of unknown significance (ASCUS) in the cervical area may develop to more severe cervical intraepithelial neoplasia (CIN2+). Carpenter, El-Mofty, and Lewis are cited as sources in a 2011 work (2011). HPV DNA testing is being utilised to sort out even the slightest cytology abnormalities. Due to the less sensitivity of human papilloma virus DNA testing, many women, particularly those under 30 with high HPV prevalence, may be subjected to colposcopy. Carpenter et al. discovered an inventive solution to a problem (2011). Head and Neck-HPV incidence ranged between 80 and 85 percent in females with LSIL. For these people, a colposcopy or cytology is recommended (El-Mofty, 2014). Women who tested positive for HPV16 or HPV18 received prompt colposcopy assessment in accordance with FDA recommendations, whereas women who tested positive for HPV but not for HPV16 or HPV18 had a cytological examination. A follow-up exam will be done in a year if the cytology findings are negative. Plummer, Peto, Franceschi, and Cancer are all characters in the film Plummer, Peto, Franceschi, and Cancer (2012, Plummer, Peto, Franceschi, and Cancer). Because cytology cannot identify the presence of the virus, many women who get a positive HPV test may need to undergo cytology follow-ups. Consequently, Given its excellent sensitivity and specificity, the p16/Ki67 dual stain is increasingly being used as another biomarker for diagnosing elevated-grade CIN. (Tay and colleagues, 1987) In this research, the use of p16/Ki-67 dual staining as a screening tool for cervical cancer and precancerous lesions will be reviewed, as well as how it may be used to do so.

Feature and function of Ki-67: Ki-67 is a cell proliferation marker named for its hometown (Kiel) and the number of initial clones (Wang et al., 2004). Wang et al. (2004) describe Ki-67 as a nuclear non-histone protein that is encoded by the MKI-67 gene and expressed throughout the cell cycle with the exception of the G0 phase. Cell cycle progression 33: Ki-67 is involved in several aspects of cell cycle progression. The ki-67 is a proliferative marker used to indicate cancers with malignancy. In many cases, Ki-67 immunohistochemistry is often used on paraffinized sections and is a valuable indicator for cancer prognosis and prediction in many cases. Ki-67 detection is commonly employed in the supplemental diagnosis of cervical malignancies and precancers (Wang et al., 2004).

p16/Ki-67 dual-staining cytology and its implication: In contrast to p16, which acts as an anti-tumor inhibitor, Ki-67 is a tissue growth indicator. Ki-67 expression is not mutually exclusive with P16 overexpression and does occurred in different cervical epithelium cell under normal circumstances Due to its association with cell cycle dysregulation, the presence of p16/Ki67 may signal that head and neck human papilloma virus prompted cell alterations and the development of high-grade CIN lesions may be predicted. C. J. s. p. s. m. D. Bergeron Jenkins et al., 1986). Use antibodies that specifically target both p16 and Ki-67 for co-expression detection. Only p16 staining divulged the brown cytoplasm/nuclear signal, whereas only Ki-67 staining unveiled the red nuclear signal. Positive p16/Ki-67 dual-stainers exhibited dark red to red brown nuclear p16 expression and brown cytoplasmic staining, which indicated that p16 and Ki-67 were found inside one cell. Even if a single cervical epithelial cell labelled for p16 and Ki-67 was present, the slide was considered positive. D. & other members of Jenkins' team (D. Jenkins and colleagues, 1986).

Table.01: p16/K-67 staining performance in the screening of carcinomas and its links with human papilloma virus

Studies	Subjects*	Sensitivityi%			Specificityi%			PPVi%			NPVi%		
		Dual	Cyto	HPV	Dual	Cyto	HPV	Dual	Cyto	HPV	Dual	Cyto	HPV
Ikenbergietal .2013	181 CIN2+/25577 screening	86.7	68.5	93.3	95.2	95.4	93.0	15.6	13.3	9.3	99.9	99.7	99.9
	100 CIN3+/25577 screening	87.4	73.6	96.2	94.8	95.1	92.7						
Wentzensen et al.2015	175 CIN2+/1509 HPV+	83.4	76.6		58.9	49.6		21.0	16.6		96.4	94.2	
	99 CIN3+/1509 HPV+	86.9	83.8		56.9	48.7		12.4	10.3		98.4	97.7	
	41 CIN2+/703 HPV+, Cyto-	70.7			70.8			13.1			97.5		
	16 CIN3+/703 HPV+, Cyto-	81.3			69.6			5.9			99.4		
Yui et al.2016	20 CIN2+/1079 screening	75.0	65.0	100.0	79.5	76.2	76.9	6.5	4.9	7.5	99.4	99.1	100.0
	6 CIN3+/1079 screening	83.3	83.3	100.0	78.8	75.8	75.9	2.2	1.9	2.3	99.9	99.9	100.0
	218 CIN2+/463 HPV+	92.7	94.5		52.7	53.5		63.5	64.4		89.0	91.6	
	178 CIN3+/463 HPV+	95.0	98.3		47.7	49.1		53.1	54.7		93.8	97.9	
	48 CIN2+/256 ASCUS, LSIL	87.5		91.7	66.4		55.8	37.5		32.4	95.8		96.7
	CIN3+/256 ASCUS,LSIL	89.7		89.7	62.1		51.5	23.2		19.1	97,9		97.5
Wright et al.2017	367 CIN2+/3467 HPV+	70.3	51.8		75.6	76.1		26.2	21.1		95.4	92.7	
	24iCIN3+/3467HPV+	74.9	51.9		74.1	75.0		18.5	14.0		97.4	95.2	
Tay et al.2017	63 CIN2+/97 Cyto+	93.7		85.7	76.5		14.7	88.1		65.1	86.7		35.7
	14 CIN2+/44 ASCUS,LSIL	92.9		85.7	76.7		16.7	65.0		32.4	95.8		71.4

Schmidt et al.2011	77 CIN2+/361 ASCUS	92.2		90.9	80.6		36.3						
	51 CIN3+/361 ASCUS	92.2		90.2	80.6		36.3						
	137 CIN2+/41 LSIL	94.2		96.4	68.0		19.1						
	72 CIN3+/415 LSIL	95.8		95.8	68.0		19.1						
Uijterwaal et al.2014	58 CIN2+/256iASC,LSIL,ASC-H,AGC	89.7		96.6	73.1		68.1	54.7		52.3	95.1		98.2
	27CIN3+/256 ASC,LSIL,ASC-H,AGC	100.0		96.3	64.4		57.6	28.4		24.3	100.0		99.1
Bergeron et al.2015	18 CIN2+/427 ASCUS	94.4		100.0	78.7		60.4	16.3		10.0	99.7		100.0
	14 CIN3+/427 ASCUS	100.0		100.0	78.2		59.8	13.5		7.8	100.0		100.0
	63 CIN2+/384 LSIL	85.7		98.4	53.3		15.6	26.5		18.6	95.0		98.0
	25 CIN3+/384 LSIL	88.0		100.0	49.3		14.2	10.8		7.5	98.3		100.0
White et al.2016	138 CIN2+/471 ASCUS, LSIL	75.4		92.8	88.3		48.9						
	48 CIN3+/471 ASCUS, LSIL	79.2		95.8	75.2		40.4	26.6		15.4	97.0		99.8
	CIN2+/206 ASCUS	71.9		94.7	87.9		64.4						
	CIN3+/206 ASCUS	71.4		100.0	78.7		56.9	17.8		14.8	96.5		100.0
	CIN2+/265 LSIL	77.8		91.4	88.6		35.3						
	CIN3+/265 LSIL	85.7		94.5	72.7		28.9	30.6		15.7	97.3		97.3
Petry et al.2011	37 CIN2+/425 HPV+,iCyto-	91.9			82.1								
	28CIN3+/425 HPV+,iCyto-	96.4			76.9								
Uijterwaal et al.2015	48CIN2+/762HPV+,Cyto-	68.8			72.8			25.2			94.6		
	15 CIN3+/762 HPV+,Cyto-	73.3			70.0			8.7			98.5		
Ordi et al.2014	378 HSIL,18 CC/1123 Colposcopy	90.9		96.0	72.1		41.4	63.9		47.1	93.6		94.9
	HSIL,CC/543 HSIL with Pap	94.5		96.1	73.4		51.9	81.3		71.7	91.6		91.0
	HSIL,CC/580 ASC,AGC,LSIL,HPV+	88.9		95.6	72.9		36.7	37.4		21.7	97.3		97.8

DISCUSSION

High-grade CIN occurs in 3–7 percent of women who have normal Pap-smear and human papilloma virus positive. All three of these studies were published in 2015 (Wentzensen, Borghi, Ferro, and Mencarelli) and all three of these studies were published in 2015. p16/Ki-67 dual-staining was used to triage these patients, and positive findings were found in 25.4% of the 425 women tested, according to the researchers. There were 91.9 percent (34/37) and 96.4 percent (27/28) sensitivity. The year 2011 (Petry and colleagues). Compared to HPV16/18 genotyping, the CIN2+ genotyping test has much higher sensitivity, and the same findings were obtained again and again. Researchers (Uijterwaal and colleagues, 2015).CIN2+ and CIN3+ 5-year cumulative incidence rates (CIR) were 12.2% and 6.9%, respectively, in HPV-positive women with normal cytology in a study.Despite HPV16/18 genotyping being negative, CIN3+ had a 5-year CIR of 3.6%.

In the year of our Lord 2015 (Uijterwaal and colleagues). If these women were p16/Ki-67 negative, the 5-year CIR for CIN3+ would be reduced to 3.3%. HPV-positive women with normal cytology should be sent to colposcopy for p16/Ki-67 dual staining to identify individuals at high risk of CIN2+, according to this study. They include Schmidt, Denton, Ridder, and cytopathology as some of the best-known names in the field (Schmidt, Bergeron, Denton, Ridder, and cytopathology, 2011).There was a 2.6 percent risk of CIN3+ in women with ASCUS and LSIL, respectively, during a 5-year period. As a result, distinguishing between HSIL and ASCUS/LSIL is critical. P16/Ki-67 dual staining has been tested and shown to be effective. According to White and coworkers, (2016). P16/Ki-67 dual-staining for CIN3 identification has a specificity rate of 75.2 percent, compared to HPV testing's 40.4 percent, despite its lower sensitivity. To cite an example from the literature: p16/Ki-67 dual staining was shown to be more sensitive and specific than HPV detection in a number of subsequent investigations (C. Bergeron et al., 2015). It has also been shown that using the p16/Ki-67 dual staining method reduces the frequency of needless colposcopies, especially in women under 30. This study was conducted by Uijterwaal and colleagues (2014).Women who had merely tested positive for HPV DNA had a 15.6% chance of developing CIN3 (White et al., 2016).HPV-positive women were 27 percent more likely than HPV-negative women to have p16/Ki-67 and HPV DNA positive.

Using p16/Ki-67 dual staining and HR-HPV detection, it is possible to track the recurrence of CIN2+ (rCIN+). Studies show that certain individuals with chronic inflammatory disease 2 (CIN2) or 3 (CIN3) may relapse, and these patients should be constantly followed. In (C. Bergeron and colleagues, 2015). But cytological detection or an HPV/cytology combo has a low sensitivity. Women with CIN2/3 were studied for cytology, HR-HPV, and

p16/Ki-67 dual-staining for rCIN2+ sensitivity and specificity. The specificity of p16/Ki-67 was better than that of cytology (70.8%) and HR-HPV testing (76.2%), while the sensitivities were lower than those of cytology (82.1%), HR-HPV testing (84.6%) and 69.2 percent, respectively. However, there were significant differences in positive predictive value and fewer colposcopy referrals. Polman et al. (Polman et al., 2017). Due to the complexity in separating hyperplastic alterations from cancers , cytology of cervical glandular lesions is problematic (Nucci, 2002). p16/Ki-67 dual-staining was shown to be positive in 40 cases of cervical adenocarcinoma, while only one out of 16 cervical tissues free of glandular lesions was.

CONCLUSION:

Biomarkers may one day replace traditional morphological screening and diagnostic procedures for the identification and treatment of cervical cancer. Biomarker assessment requires knowledge of cervical histology and cytopathology theory and practice. Using 16/Ki-67 dual-staining cytology, precancerous lesions and cervical malignancies may be discovered and diagnosed. HPV-positive women with normal cytology may utilize it as a risk marker and to diagnosis high-grade CIN in women with ASCUS or LSIL who have ASCUS or LSIL.

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