

ROLE OF PROTEIN BIOMARKERS P15 AND P16 IMMUNE-STAINING IN EVALUATION OF CERVICAL DYSPLASIA PROGRESSION

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ABSTRACT:

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Background: Cervical cancer ranks as the third most prevalent cancer among women globally, having been the leading cause of cancer-related deaths half a century ago. Low-grade squamous intraepithelial lesions (LSIL) typically advance to normal or benign conditions, with only a small percentage progressing to high-grade squamous intraepithelial lesions (HSIL). HSIL is commonly associated with the development of cervical cancer. Currently, there are no reliable biomarkers for predicting HSIL progression in high-risk populations. Apoptosis plays a crucial role in preventing cancer formation, with cell cycle regulators like P15 and P16 being key in G1 cell cycle arrest and oncogene-triggered apoptosis.

Aim: To assess and contrast the expression profiles of the candidate biomarker proteins P15 and P16, and to determine their utility as predictive biomarkers for the advancement of cervical dysplasia.

Patients and Methods: This study included 50 cervical biopsy cases. Immunohistochemical staining was performed for P15 and P16. Tissue blocks and clinical data were collected from March 2021 to March 2023 in the Department of Obstetrics and Gynecology at the unit of Cancer Early Detection, and Department of Pathology, and Al-Zahra' a University Hospital.

Results: High expression of P15 and P16 in HSIL were higher than that in LSIL and NIL, with statistically significant P value of 0.003 for both markers. A P value of <0.0001 indicated a very strong positive correlation between P15 and P16 expression in all cases examined.

Conclusion: As cervical dysplasia degree increases, the expression of P15 and P16 increases. Detecting these two markers in combination has important potential for predicting HSIL. IHC expression of P15 and P16 is associated with the grade of histological dysplasia and malignancy, suggesting their prognostic and predictive value in the treatment of cervical lesions.

Keywords: Cervical pathology; LSIL; HSIL; Immunohistochemistry; P16; P15; Prognostic markers.

INTRODUCTION:

Cervical cancer stands unique among gynecological cancers, as it is the only one for which a screening test exists. The most effective method for detecting precancerous changes in the cervix is the Cervical Pap smear, which is optimally interpreted through a uniform and well-structured reporting system like the Bethesda system. Within this system, squamous intraepithelial lesions (SILs) represent a spectrum of squamous cell

carcinomas. This spectrum begins with precancerous lesions and progresses from low-grade SILs (LSILs) to high-grade SILs (HSILs), culminating in invasive squamous cell carcinomas⁽¹⁾.

Cervical cancer is the only malignant tumor in the world for which the cause and preventive treatment are well known, not like cervical intraepithelial neoplasia (CIN) I caused by widespread HPV infection as it is usually self-limiting and does not require

further treatment. However, some CIN I develop into CIN II or CIN III and eventually becomes squamous cell carcinoma⁽²⁾.

Cervical cancer ranks as the third most prevalent cancer among women globally, a significant decline from its status as the leading cause of cancer-related death in women half a century ago⁽³⁾. In 2018, there were about 570,000 cases diagnosed with cervical cancer led to the death of about two third of these cases⁽⁴⁾.

According to the latest statistics from the World Health Organization (WHO), 866 women are diagnosed with cervical cancer each year in Egypt, resulting in 373 deaths⁽⁴⁾. The main risk factors for cervical cancer include persistent infection with the oncogenic type of human papillomavirus (HPV) and lack of a regular screening program⁽⁵⁾.

The decline in cervical cancer incidence is primarily due to widespread systematic and opportunistic screening efforts⁽⁶⁾. However, the introduction of effective screening and treatment methods for precursor lesions has significantly reduced the mortality and morbidity of cervical cancer. However, cervical cancer remains the leading cause of cancer death among women in developing countries due to limited screening infrastructure and resources⁽⁷⁾.

Cervical intraepithelial neoplasia (CIN), a cancer precursor characterized by abnormal cell proliferation and nuclear changes, is usually stable or eliminated by the immune system without intervention. However, if left untreated, a small proportion of these lesions may progress to invasive squamous cell carcinoma (SCC), especially when associated with chronic infection with high-risk HPV strains, especially types 16 and 18.⁽⁸⁾

The categorization of these lesions has been streamlined into a two-tier model that differentiates between low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL)⁽⁹⁾.

The Pap test, a cervical cytology screening method, was pioneered by Papanicolaou more than 70 years ago⁽¹⁰⁾.

Pap smear can detect cell changes that indicate cervical cancer or CIN. Colposcopy is done to each woman with abnormal test results. Clinically, CIN2 and CIN3 lesions usually require treatment with large loop excision procedures or cone biopsy. As for patients having CIN1 lesions are more likely to be monitored using follow-up cytology⁽¹¹⁾.

The progression of precancerous cervical lesions such as LSIL and HSIL to invasive cancer is variable⁽¹²⁾. It is estimated that 1% to 2% of women develop CIN2 each year, and the risk of progression to invasive cancer or CIN3 is approximately 5% and 20%, respectively⁽¹³⁾. It may be difficult to distinguish cervical dysplasia from non-malignant disease simply by evaluating H&E-stained sections. In such cases, biomarkers can help make an accurate diagnosis⁽¹⁴⁾.

Cervical cancer screening tests by conventional cytology have raised a notable knowledge of the decline in both cervical cancer morbidity and mortality in recent past decades. Despite these advancements, conventional cytology is limited in sensitivity and specificity. As a result, histological screening tests were suggested, yet the histological diagnosis of cervical biopsies still presents certain challenges⁽⁷⁾.

P15 is a member of the cyclin-dependent kinase inhibitor family, which functions by inhibiting cyclin-dependent kinase 4 and 6 to negatively regulate the cell cycle, impacting cell cycle regulation⁽¹⁵⁾. P15 (INK4b) is also a cyclin-dependent kinase inhibitor (CDKI), located centromeric at the p16/p14 locus P14 (ARF), and is a tumor suppressor and transforming growth factor β by causing cell cycle arrest⁽¹⁶⁾.

P16, as a cyclin-dependent kinase inhibitor, obstructs the phosphorylation of different cyclins. Upon HPV infection of

cervical cells, the virus generates E7, which binds to and deactivates the tumor-suppressor role of pRb. In dysplastic cervical cells, overexpression of P16 functions as a negative feedback response to counteract the inactivation of pRb⁽¹⁷⁾.

P16 (INK4a) serves as a tumor suppressor protein and cyclin-dependent kinase inhibitor that plays a role in cell cycle regulation. It inhibits cyclin-dependent kinases involved in Rb phosphorylation, thus slowing down the cell cycle. The expression of P16 is influenced by the phosphorylation status of Rb during human papillomavirus (HPV) infection. The HPV oncogenes E6 and E7 can block the retinoblastoma protein (pRb) and lead to increased P16 expression. Consequently, the overexpression of P16 serves as an indirect indicator of HPV infection⁽¹⁸⁾.

P16, also known as Cyclin-dependent kinase inhibitor 2A (CDKN2A), is a gene found on chromosome 9 that produces a protein responsible for suppressing cyclin-dependent kinases 4 and 6 (P16), which act as inhibitors of retinoblastoma proteins. This leads to the reactivation of retinoblastoma proteins and the halting of the cell cycle in the G1 phase. Consequently, the detection of p16 protein expression in cancer cells could suggest a positive prognosis⁽¹⁹⁾.

AIM OF THE WORK:

The aim of this study is to assess and compare the expression profiles of the candidate biomarker proteins P15 and P16, and to investigate their potential as predictive biomarkers in the advancement of cervical dysplasia.

PATIENTS AND METHODS:

Our study is a prospective comparative study that was conducted upon fifty women with abnormal cervix in the Obstetrics and Gynecology Department at the early cancer

detection Unit and Pathology Department, at Al-Zahra'a University Hospital, Al-Azhar University during the period from to March 2021 and March 2023.

Study population:

In a test for agreement with our study using the kappa statistic, sample size of at least 45 subjects achieves 95% power to detect a true kappa value of 0.80 in a test when there are two categories with frequencies equal to 0.50 and 0.50.

Patient's selection criteria:

Inclusion criteria:

Ladies are included if they are; Aged 20 -75 years, with intact uterus, and abnormal suspicious areas in the cervix with colposcopy examination

Exclusion criteria:

Ladies will be excluded from our study if they:

Have surgical removal of the cervix, history of cervical, endometrial, vulvar or ovarian cancer, cervical masses, and pregnant patients.

Study tools and procedure:

The study was conducted in many steps:

- This study was revised to assess demographic data related to cervical dysplasia, patient symptoms, medical history, past medical history (medical and surgical), stage of disease, and treatment received.
- After a brief targeted physical examination, a visual examination of the cervix is performed. To obtain a proper view of the cervix, cervical mucus was initially removed using a saline-moistened cotton swab⁽²⁰⁾
- Colposcopy: Prepare the patient by treating any associated infections and instruct the patient to avoid douches and avoid sexual intercourse for 48 hours before colposcopy. The patient lay in the

lithotomy position, and the vulva and perineum were thoroughly examined, and any abnormal areas noted. The vagina and cervix were examined using a Cusco speculum.

- A pap smear will be taken then 5% acetic acid solution will be placed on the cervix then visual inspection with acetic acid will be performed and will be positive if there is any aceto-white lesion. followed by painting the cervix with Lugol's iodine then visual inspection with iodine and will be positive if any part of the cervix doesn't stain.
- In participants with abnormal colposcopic findings like acetowhite changes, coarse punctation, and mosaic or atypical vessels, directed biopsy was obtained; 2–3 biopsies from the observed acetowhite lesions were obtained.
- Multiple punch biopsy specimens were taken from area of maximum colposcopic change and then sent for histopathological examination.
- Protein biomarker expression was also examined in Thin Prep slides exhibiting mild, moderate, and severe dyskaryosis.
- Intensity was assessed using a 0-3point system. All formalin-fixed and paraffin wax-embedded sections that showed strong nuclear or cytoplasmic staining were considered positive.
- A certified pathologist then qualitatively graded all sections using the following arbitrary scale: 0 (no positive dysplastic cell staining), 0a (basal layer staining), 1 (basal layer staining), staining + 10% positive staining of dysplastic cells), 2 (10%, but positive staining of 50% of dysplastic cells) and 3 (positive staining of 50% of dysplastic cells).
- Patients or their relatives were contacted by telephone after data analysis to assess the results.
- The final histopathological diagnosis was made based on the highest grade observed on biopsy or pap smear results.

- According to the pap smear and cervical biopsy results, these patients were classified as into 3 groups:

Group I: Normal (NIL): 25 cases.

Group II: Low grade Squamous Intra epithelial Lesion (LSIL): 21 Cases.

Group III: High grade Squamous Intra epithelial Lesion (HSIL): 4 Cases.

Tissue Specimens:

Tissue Specimens Processing: Three sections of paraffin blocks, each measuring 5-micron thickness, were obtained. One section was stained with hematoxylin & eosin for diagnostic evaluation, while the remaining two sections were mounted on positively charged slides and subjected to immunostaining using antibodies against P15 (INK4b) and P16 (INK4a).

Immunohistochemistry Procedure: Positively charged slides were prepared from each paraffin block and immune-stained with primary antibodies, including a rabbit polyclonal antibody against P15 and a rabbit monoclonal antibody against P16. Immunohistochemical reactions were carried out using the Labeled Streptavidin-Biotin2 System-Horseradish Peroxidase (LSAB2 System-HRP) methodology, utilizing Diaminobenzidine (DAB) as the chromogen reagent.

Controls and Evaluation: Phosphate buffer-treated tissue sections served as negative controls, while squamous cell carcinoma sections were utilized as positive external controls for both markers. Immunostaining evaluation involved assessing the intensity and extent of staining, with positive staining observed as brown in the nucleus. The percentage of positively stained cells was graded from 0 to 3 based on the proportion of cells staining positive, while intensity was graded on a scale of 0 to 2. The product of percentage and intensity scores determined the expression levels, with values above 2 classified as high expression and

values equal to or below 2 considered low expression for each marker⁽¹⁶⁾.

The assessment for both markers included an evaluation of both staining intensity and the proportion of cells with positive staining. The percentage of positively stained cells was categorized on a scale of 0 to 3: Grade 0 for less than 5%, Grade 1 for 5 to 25%, Grade 2 for 26 to 50%, and Grade 3 for 50% or more. Intensity of staining was graded from 0 to 2: Grade 0 for negative to weak intensity, Grade 1 for weak to moderate intensity, and Grade 2 for moderate to strong intensity. The product of percentage and intensity scores determined the expression levels, with values below 2 indicating low expression and values above 2 indicating high expression for each marker⁽¹⁶⁾.

Statistical analysis:

The collected data were analyzed using the Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented as mean ± standard deviation (SD), while qualitative data were expressed as frequency and percentage. The analysis included the following statistical tests: The Chi-square (χ^2) test for comparing proportions among qualitative variables, and Pearson’s correlation coefficient ® to assess the relationship between two sets of variables. A confidence interval of 95% and a margin of error of 5% were applied. Statistical significance was defined as follows: P-values < 0.05 were considered significant, P-values < 0.001 were regarded as highly significant, and P-values > 0.05 were deemed insignificant.

Ethical considerations:

The study received approval from the Ethics Committee of the Department of Obstetrics and Gynecology at the Faculty of Medicine, Al-Azhar University, with reference number 202103703. Informed written consent was obtained after providing a clear explanation of the study’s objectives and procedures to the participants. Data presentation is based on diagnosis rather than patient names to ensure confidentiality and enable easy patient tracking.

RESULTS:

Our study is a prospective comparative study that was conducted at Al-Zahra’a University Hospital, Al- Azhar University in the Obstetrics and Gynecology Department at the early cancer detection Unit and Pathology Department on fifty women with abnormal cervix on colposcopy examination during the period between March 2021 and March 2023. Colposcopic examination followed by Pap smear and multiple punch biopsies from the suspicious areas in the cervix were done. The final histopathological diagnoses were made based on the highest grade of the lesion observed in biopsy or in the Pap smear results.

After Pap smear and cervical biopsies results, these patients were subdivided into 3 groups:

- Group I:** Normal (NIL); 25 cases
- Group II:** Low grade Squamous Intra epithelial Lesion (LSIL): 21 Case
- Group III:** High grade Squamous Intra epithelial Lesion (HSIL) 4 Cases

The age of the patients ranged from 44 to 71 years with a mean (58.2±9.01 years) Table (1).

Table 1: Different Cases Regarding Demographic Data.

Demographic data		Study Group (n= 50)
Age (y)	Mean ± SD	58.2±9.01
	Range	44-71
Age of marriage (y)	Mean ± SD	20.8±7.2
	Range	15-35

Parity	P0	N (%)	1 (2%)
	P1		2 (4%)
	P2		6 (12%)
	P3		16 (32%)
	P4		16 (32%)
	P5		4 (8%)
	P6		2 (4%)
	P7		1(2%)
	P9		2 (4%)
Smoking	Yes	N (%)	28 (56%)
	No		22 (44%)
Contraception	Injection	N (%)	3 (6%)
	IUD		8 (16%)
	Oral		4 (8%)
	Tubal		1 (2%)
	Negative		34 (68%)
Complain	Bleeding	N (%)	44 (88%)
	Infection		5 (10%)
	Wart		1 (2%)

Histopathological Groups of the Study:

According to histopathological results, patients were divided into 3 groups: Group I formed of 25 cases (50%) negative for intraepithelial lesions (NIL) including (normal cervical tissues or chronic cervicitis) as a control group, Group II formed of 21 cases (42%) of low-grade squamous intraepithelial lesions (LSIL) including (Condyloma or CIN1), and Group III formed of 4 cases (8%) of high-grade squamous intraepithelial lesions (HSIL) including (CIN2 or CIN3) Table (2).

Table 2: Histopathological Groups of the Study

Group	Study group (n=50)	
	N	%
Group I (NIL)	25	50
Group II (LSIL)	21	42
Group III (HSIL)	4	8

Correlation between age and different studied groups:

According to the relation between age in all studied groups, there was not a significant difference of age between NIL, LSIL and HSIL groups with a P-value 0.351 Table (3).

Table 3: Correlation between age among studied groups

Group	Age (y)		P-value
	Mean	SD	
Group I (NIL)	41.16	6.81	0.351*
Group II (LSIL)	41.33	5.72	
Group III (HSIL)	46.00	4.69	

*P > 0.05 is considered insignificant.

Correlation between age and P15 expression:

According to the relation between age and expression of P15, there was no a significant difference of age between high and low P15 expression with a P-value 0.625 Table (4).

Table 4: Correlation between age and P15 expression

P15	Age (y)		P-value
	Mean	SD	
High	42.55	5.27	0.625
Low	41.41	6.49	

*P > 0.05 is considered insignificant.

Correlation between age and P16 expression:

According to the relation between age and expression of P16, there was no a significant difference of age between high

and low P16 expression with a P-value 0.228 Table (5).

Table 5: Correlation between age and P16 expression.

P16	Age (y)		P-value
	Mean	SD	
High	43.11	5.40	0.228
Low	40.84	6.60	

*P > 0.05 is considered insignificant.

P15 expression in different studied groups:

Positive staining was indicated as brown color in the nucleus of the cells or nucleocytoplasmic staining in some cases. Nine cases showed high P15 expression (18.0%) and the remaining 41 cases showed low expression (82.0%). The ratio of high P15 expression in Group III (50.0%) was higher than that in Group II (33.3%) and Group I (0.0%) with a statistically significant P-value of 0.003 Table (6) Figure (1).

Table 6: P15 expression in different groups

		Group I NIL (n=25)		Group II LSIL (n=21)		Group III HSIL (n=4)		Total (n=50)		Chi-square Test	P- value
		N	%	N	%	N	%	N	%		
		P15	Low	25	100.0%	14	66.7%	2	50.0%		
	High	0	0.0%	7	33.3%	2	50.0%	9	18.0%		

*P ≤ 0.05 is considered significant

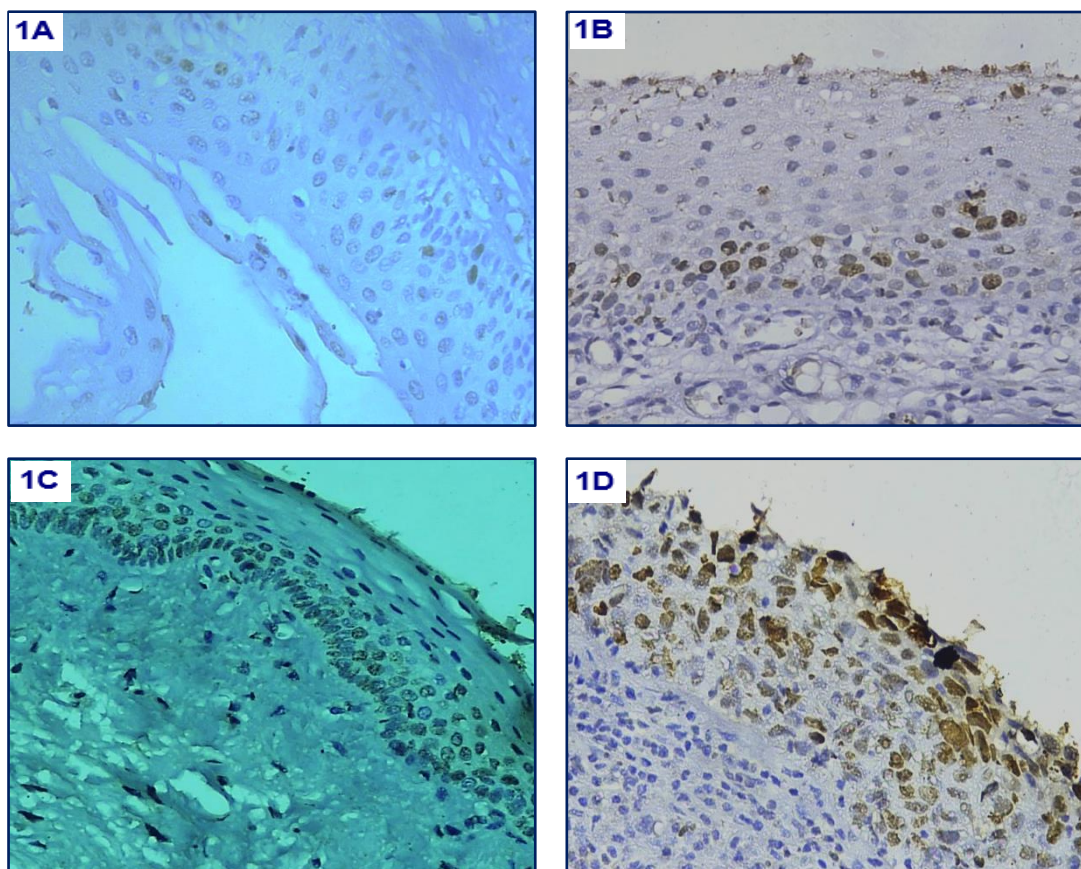


Figure 1: Immunohistochemical expression of P15 in different studied groups.

(A) Low P15 nuclear expression in normal cervix (× 200), (B) Low P15 nuclear expression in CIN1 (× 200). (C) High P15 nuclear expression in CIN2 (× 200), (D) High P15 nuclear expression in CIN3 (× 200).

P16 expression in different studied groups:

Positive staining was indicated as brown color in the nucleus of the cells or nucleocytoplasmic staining in some cases. 17 cases showed high P16 expression (34.0%)

and the remaining 33 cases showed low expression (66.0%). The ratio of high P16 expression in Group III (75.0%) was higher than that in Group II (52.4%) and Group I (12.0%) with a statistically significant P-value of 0.003 Table (7) Figure (2).

Table 7: P16 expression in different groups

		Group I NIL (n=25)		Group II LSIL (n=21)		Group III HSIL (n=4)		Total (n=50)		Chi-square Test	P- value
		N	%	N	%	N	%	N	%		
P16	Low	22	88.0%	10	47.6%	1	25.0%	33	66.0%	11.55	0.003*
	High	3	12.0%	11	52.4%	3	75.0%	17	34.0%		

*P ≤ 0.05 is considered significant

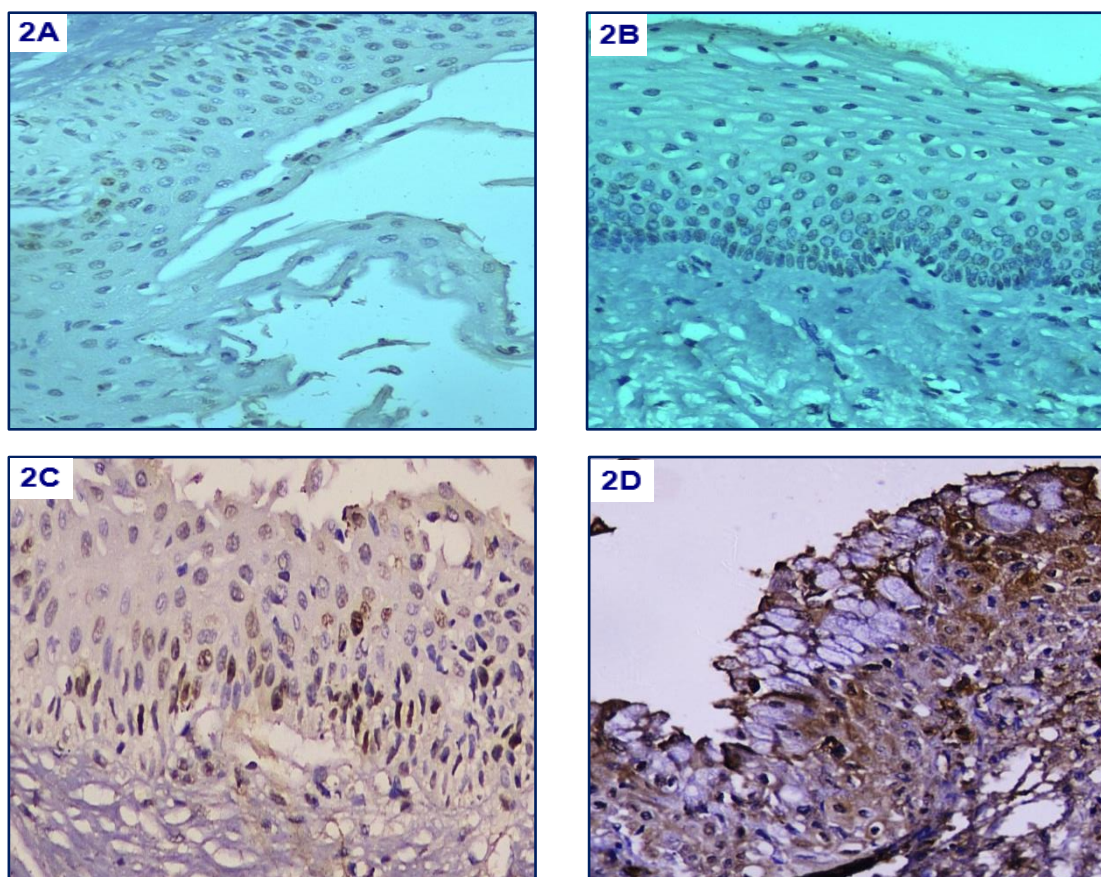


Figure 2: Immunohistochemical expression of P16 in different studied groups. (A) Low P16 nuclear expression in normal cervix (× 200), (B) Low P16 nuclear expression in CIN1 (× 200). (C) High P16 nuclear expression in CIN2 (× 200), (D) High P16 nuclear expression in CIN3 (× 200).

Correlation between P15 and P16 expression:

According to the relation between P15 and P16 expression in all studied cases, there was a statistically highly significant

direct positive relation between P15 and P16 expression in different studied cases with a P-value <0.0001. All nine cases with high P15 expression showed high expression with P16 Table (8).

Table 8: Correlation between P15 and P16 among all cases.

P16	P15				Total
	High		Low		
	N	%	N	%	
High	9	100.0%	8	19.5%	17 (34.0%)
Low	0	0.0%	33	80.5%	33 (66.0%)
Total	9	100.0%	41	100.0%	50 (100.0%)
Kappa	0.59				
P-value	< 0.0001*				

*P ≤ 0.001 is considered highly significant

DISCUSSION:

Early detection and proper management of cervical cancer are crucial in improving patient outcomes and reducing the impact of the disease. The detection of P15 and P16 proteins using an immunohistochemical method has become an important diagnostic tool in cervical cancer screening and diagnosis.

Understanding the role of P15 and P16 gene in cervical cancer provides insight into the molecular mechanisms underlying the disease and help in the development of therapies and diagnostic approaches. High expression levels of P16 are observed in precancerous and cancerous cervical cell, making it a useful biomarker for identifying cervical lesions. Its increased expression act as a defense mechanism against cell transformation and prevent the progression to malignant cell.

P16 and P15 are two genes that are important in the development and progression of cervical cancer. P15 gene also known as CDKN2B, encode for a protein called p15INK4b that play a role in controlling the cell cycle. This protein functions as tumor suppressor, inhibiting the cell cycle and preventing uncontrolled cell growth. On the other hands P16 also known as CDKN2A encodes for protein called P16INK4a also known as tumor suppressor and involved in regulating the cell cycle.

In the line we aimed to study the expression of P15 & P16 in different biopsies of normal tissue as well as premalignant lesions of the cervix, by immunohistochemistry to explore the possibility of P15 and P16 as diagnostic and prognostic markers in cervical lesions, also to clarify their role in carcinogenesis and progression of cervical cancer.

Our study a prospective comparative study that was conducted upon fifty women with abnormal cervix in the Obstetrics and Gynecology Department at the early cancer detection Unit and Pathology Department, Al-Zahra’a University Hospital, Al-Azhar University during the period from to March 2021 and March 2023.

Colposcopic examination followed by Pap smear and multiple punch biopsies from the suspicious areas in the cervix were done. The final histopathological diagnoses were made based on the highest grade of the lesion observed in biopsy or in the Pap smear results. After Pap smear and cervical biopsies results, these patients were subdivided into 3 groups: **Group I:** Normal (NIL) 25 cases, **Group II:** Low grade Squamous Intra epithelial Lesion (LSIL) 21 Cases and **Group III:** High grade Squamous Intra epithelial Lesion (HSIL) 4 Cases.

The mean age of participants in this current study ranged from 44 to 71 years, with a mean of (58.2 ± 9.01 years). The association between age and different cases was not statistically significant

(P value 0.351). These results are consistent with *Pandey et al.*,⁽¹²⁾ who reported that the mean age of all cases was 48.28 ± 8.9 years, with a range of 30–61 years.

Our results are also in close agreement with those of *Hu et al. 2019*,⁽⁷⁾ patients reported age ranging from 20 to 71 years (mean 40.6 years). According to *Chaloob et al.*,⁽⁸⁾ the mean age of LSIL cases was average (38.46 ± 2.3 years) in the range of 22 to 62 years, while the mean age of HSIL cases was average (43.32 years) in the range of 27 to 63 years.

Regarding the extent of squamous lesions, there were more LSIL cases (21 cases) than HSIL cases (only 4 cases) (Table 2). This finding is consistent with *Hu et al., 2019*⁽⁷⁾, who reported 380 patients with LSIL compared to 267 patients with HSIL. The case of HSIL. Also, *Pandey et al.*,⁽¹²⁾ reported that there were 16 cases of LSIL and only 3 cases of HSIL. In the same line *Chaloob et al.*,⁽⁸⁾ reported that there were 24 patients with LSIL and 28 patients with HSIL. Our results also differ from those of *Zhang et al.*,⁽¹⁶⁾ who reported that there were 18 cases of LSIL and 33 cases of HSIL.

In our current study, the high expression rate of P15 showed a statistically significant stepwise increase from NIL (0.0%) to LSIL (33.3%) to HSIL (50.0%), with a P value of 0.003. These results are in line with *Zhang et al., 2014*,⁽¹⁶⁾ who reported that the percentage of high P15 expression in CIN3 and CIN2 (100.0% and 62.5%, respectively) was significantly increased compared to CIN1 and normal cervix (both 0.0%). *al., 2014.* the P value is 0.000.

In the same way *Feng et al.*,⁽²¹⁾ also reported that the strong positive rate of P15 expression in CIN and normal cervix was (30.0% and 0.0%, respectively). These results highlighted that P15 expression increases with higher histological grade. Our study showed that the proportion of high P16 expression showed a statistically significant

stepwise increase from NIL (12.0%) to LSIL (52.4%) to HSIL (75.0%), with a P value of 0.003.

This is in line with the results of *Zhang et al.*,⁽¹⁶⁾ who showed a significant increase in high P16 expression from normal cervix (31.6%) to CIN2 (62.5%) over CIN3 (88.2%) to CIN1 (55.6%), with a P value of 0.004. Furthermore, *Hu et al.*,⁽⁷⁾ reported that the ratios of P16 positivity in normal cervix, LSIL, HSIL, and invasive cancer were 33.3%, 75.0%, 96.3%, and 100%, respectively. And showed that the intensity of positive expression of P16 increased depending on the severity of cervical lesions, and the ratio of strong positivity of P16 in normal, LSIL, HSIL, and invasive cancer was 0.0. I found that 10.7%, 92.6%, and 100.0%, respectively, and the P value is 0.00.

Kishore & Patil⁽²²⁾ showed that P16 expression in normal cervical epithelium was absent in all cases and increased from 25% of CIN 1 positive cases to 75% of CIN 3 positive cases. *Ta.* In semiquantitative evaluation, overexpression of P16 was observed in 25% of CIN-1 cases, 50% of CIN-2 cases, and 75% of CIN-3 cases. All cervical cancer cases positive for P16 expression, with P value <0.05.

According to a study by *Pandey et al. in 2018*,⁽¹²⁾ found that P16 was positive in 96% of invasive cervical cancers, 66.6% of HSILs, 37.5% of LSILs, but negative in all non-neoplastic controls. *Chaloob et al.*,⁽⁸⁾ also found a highly significant increase in P16 IHC expression from LSIL (37.5%) to HSIL (67.9%) to carcinoma (94.3%) but not in chronic cervicitis. (P<0.001).

The case showed positive immunoreactivity for P16, this study found no significant association between P15 and P16 expression and patient age with P values (0.625 and 0.228, respectively). This is consistent with the results of *Chaloob et al.*,⁽⁸⁾ who reported a non-significant association between P16 expression and age

of SIL patients with a P value of 0.378. Our study showed a highly significant direct association between P15 and P16 expression in the different cases studied with a P value <0.0001.

El sokkary⁽¹⁹⁾: “on the study of the prevalence of P16 immunohistochemistry positive staining and its correlation clinical and radiological staging of squamous cell carcinoma of the cervix” concluded that regarding the prevalence of p16 immunostaining, 34 cases (56.7%) were positive, whereas 26 cases (43.3%) were negative. Immunostaining and most of the late unresectable cases were P16 negative. There was a significant positive correlation between early resectable stage and positive P16 immunostaining, and a similar correlation existed between late unresectable stage and negative P16 immunostaining (p = 0.000).

Our results in the same line with *Liou et al.*,⁽²³⁾ who found that p16 was stained positively in 100% of HSIL, endocervical adenocarcinoma in situ, and invasive endocervical cases and was negative in all benign cervical samples.

Conclusions:

the present study demonstrated a positive correlation between P15 and P16 expression and cervical tumor extent. This suggests that the immune expression of P15 and P16 increases with the severity of cervical lesions and may be useful in classifying cases according to severity.

Neoplastic transformation is identified by overexpression of P15 and P16 in premalignant and malignant cases, which play an important role in the development of cervical cancer. Immunohistochemical detection of P15 and P16 expression may be a useful indicator of the progression of cervical dysplasia.

As the degree of cervical dysplasia increases, the expression of P15 and P16 increases. Detecting these two markers in

combination has important potential for predicting HSIL. IHC expression of P15 and P16 is associated with the grade of histological dysplasia and malignancy, suggesting their prognostic and predictive value in the treatment of cervical lesions.

Conflict of interest:

no conflict.

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دور دلالات البروتينين بي 15 وبي 16 بالتصبغ المناعي في تقييم تطور حالات الخلل النسيجي لعنق الرحم

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يعد سرطان عنق الرحم السبب الثالث لوفيات النساء ما حول سن الخمسين كما يتم تسجيل حالات سنويه من سرطان عنق الرحم الى مايزيد عن النصف مليون سنويا يتوفى منهم مايقرب من النصف نتيجة لهذا المريض.

كما ان انقاص نسبة الاصابة بسرطان عنق الرحم من اهم الاولويات في برامج الرعاية والهيئات الصحية بالاضافة ان سرطان عنق الرحم كمرض يمكن منعه عن طريق التشخيص المبكر للتغيرات الخلويه التي تحدث في عنق الرحم قبل الاصابه بالسرطان.

دراسه التغيرات الخلويه لعنق الرحم استطاعت تحديد العديد من المراحل التي تمر بها الخلايا مثل التغيرات الخلوية في المرحلة الاولى والثانية والثالية ما قبل الاصابة بالورم الفعلي والتي يمكن عندها منع تطور المرض الى مرحلة السرطان.

تعد دلالات البروتينين بي 15 و بي 16 من احد اهم الدلالات في تشخيص حالات الخلل النسيجي لعنق الرحم وسرطان عنق الرحم.

الهدف من البحث : لذا كان الهدف من هذا البحث هو دراسة وجود دلالات البروتينات بي 15 وبي 16 في حالات الاختلال النسيجي لعنق الرحم وتحديد دورها في تطور هذا الخلل

طريقة البحث: تم اختيار خمسين امراة للمشاركة في البحث بعد اكتمال معايير الاشتمال وهي :

- ♦ سيدات تتراوح اعمارهم ما بين العشرين الى خمسة وسبعين عاما , وجود الرحم كاملا.
- ♦ السيدات تعانين من نزيف بعد العلاقة الزوجية مع ايجابية مسحة عنق الرحم (الباسمير) .

معايير الاستبعاد: السيدات الاتي لديهن استئصال جراحي لعنق الرحم والسيدات ذات التاريخ المرضي لسرطانات عنق الرحم سرطانات الرحم و سرطانات العانة او المبيض والسيدات الحوامل

اجراءات البحث:

- ♦ تم اخذ التاريخ المرضي الكامل للمريضة
- ♦ سوف يتم عمل فحص شامل للمريضة كما تم متابعة الحالة المرضيه للمرضي
- ♦ عمل مسحة عنق الرحم (الباسمير) واختبار وجود اماكن محتمله للاختلال الخلوي بعنق الرحم عن طريق استخدام مادة الاستيك اسيد واللوجلز ايودين
- ♦ كما تم اخذ عينات متعددة من اماكن احتمالية الاختلال الخلوي
- ♦ تحضير العينات الايجابية لدراسة وجود دلالات البروتينين بي 15 وبي 16 وتقييم دورهم في تطور حالات الاختلال الخلوي

الدراسات الاحصائية: تم تجميع المعلومات وتحليلها احصائيا عن طريق الكمبيوتر

النتائج: زادت نسبة دلالات البروتينين بي 15 وبي 16 لدى حالات الاصابة بالتغير الخلوي العالى (حالات ما قبل سرطان عنق الرحم) عن حالات التغير الخلوي البسيط والحالات الطبيعية

الاستنتاجات: ان دلالات البروتينين بي 15 وبي 16 ذات اهمية في تشخيص حالات ما قبل سرطان عنق الرحم

التوصيات : يوصى باستخدام دلالات البروتينين بي 15 وبي 16 بالتصبغ المناعي في تقييم تطور حالات الخلل النسيجي لعنق الرحم