

Clinicopathological Correlation Can Improve Early Detection of Cervical High-Grade Intraepithelial Lesions and Cervical Squamous Cell Carcinoma

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Abstract

Worldwide, cervical intraepithelial dysplasia common lesions in adult women, particularly after the age of 30 years. The high grade lesions are considered as pre-malignancy and could give raise to in situ and invasive squamous cell carcinoma of uterian cervix. These cancerous transformations significantly can be reduced by screening program of the cervix, including: cytological examination of the cervix, HR-HPV test, and colposcopy. women with cervical cytology that showed morphology of intraepithelial dysplastic changes, especially high grade lesions, HR-HPV test should be done for these women. High prevalence of HPV infection will not predicate the risk of malignant transformation in these affected women, thus additional triage test, including immunochemical markers for transformed cells can be used to detect women need further evaluation, follow up and treatment. In current study, p16/Ki-67 dual biomarkers staining, were used for cytological smears to detect dysplastic cellular changes and can improve the diagnosis of pre-malignant lesions and squamous cells carcinoma.

Aim of the study: To identify the role of p16/ki-67 dual immunostaining in predicting the presence of significant cervical lesions in women with mild cellular atypia.

Patients and method: A prospective study of 214 women, with average age 38 years, over a period of January 2018 to December 2019, in Bagdad city. Women were referred from private gynecological clinics to private Al-Baraa lab and Lab of Dr. Inas Abd Al Majed Rasheed. All cervical cytological smears of the patients that were showed dysplastic changes, were submitted for HR-HPV-detection (by PCR), and p16/ki-67 dual biomarker of cytology followed by colposcopy-guided biopsy were done for 62 women.

Keywords: p16/ki-67 dual immunostaining, ASC-US, LSIL, squamous cell carcinoma, LBC, colposcopy guided biopsy.

Results: Positively staining slides for p16/Ki-67 dual biomarkers were showed dark red –red brown nuclei, with brownish or clear cytoplasm. Positive staining slides are identified by presences of \geq one cervical epithelial cells that expressed simultaneously for both biomarkers (p16/Ki-67).

It has been shown the sensitivity and specificity of immunostaining in diagnosis of CIN2/CIN3 for ASC-US and LSIL lesions were (100 and 75.2%), and (100 and 89.3%) respectively. Dual p16/ki-67 biomarker staining positivity also increased with severity of cellular dysplasia, in corresponded to histological morphology.

Conclusion: In addition to cervical cytology and PCR – testing for high risk -HPV in women with cervical intraepithelial lesions, p16/ki-67 dual biomarkers staining are of useful value in triage of ASC-US and LSIL lesions, according to high sensitivity and specificity.

INTRODUCTION

Worldwide,cervical intraepithelial dysplasia are common lesions in women after the age of 30 years, caused by infection with HPV, as this viral infection cause disordered development (dysplasia)of the squamous epithelial lining of uterine cervix.The dysplasia of cervical epithelial lining can be divided into 3 grades: grade I (low grade), grade II and III (high grade lesions). Severe dysplasia which involve the entire thickness of the lining epithelia, is considered carcinoma in situ of uterine cervix, and give rise invasive squamous cells carcinoma (1,2).

The cytological smears that showed cellular morphology of dysplasia,then those women should tested for HR-HPV, (3,4).

Regarding HPV genotypes, more than 100 different known genotypes had been found, and grouped as low risk and high risk types. The most prevalent worldwide include HR-HPV genotype 16&18, as they infect mucous membrane and cutaneous, causing wart lesions and dysplasia of the cervical epithelial lining (4,5).

Colposcopy is recommended for all women with dysplastic cytology &/ or positive HR-HPV test (5,6). To reduce over-referral colposcopy, p16/Ki-67 dual biomarker could be used to stain and detect dysplastic cells in cytology or histology of punch biopsy, and improve early diagnosis of squamous cells carcinoma (7).

P16 is a cell cycle regulatory protein that induce arrest of cell cycle when DNA damage have been occurred, so prevent abnormal cell division and proliferation (tumor suppressor gene), while Ki-67 is a cell proliferative marker.Normally, p16 and Ki-67 don't co-expressed in same cervical epithelial cell. The co-expression of both biomarkers indicate a disordered, abnormal cell cycle, mediated by high risk -HPV infection.

The co-expression of both p16/Ki-67, can be identify by using monoclonal antibodies against both markers..Immunostaining for p16 alone, resulting in brownish nuclei and cytoplasm, while red nuclei expressed by Ki-67 immunostaining alone.

Positive p16/ ki-67 dual immunostaining, showed dark red to red brownish nuclei with clear or brown cytoplasm in the same cells.Positive slides are detected by presences

of one or more cervical epithelial cells that expressed simultaneously expressed for both p16/Ki-67 markers.

PATIENTS, MATERIAL AND METHOD

A prospective study of 214 patients with average age of 38 years old, was done in Baghdad city in Iraq, over a period lasting from January 2018 to Decemper 2019.

The included women in current study, were referred from private gynecological clinics in Baghdad city to private Al-Baraa lab and Lab of Dr. Enas A Rasheed.All involved women were referred to cervical cytology (using liquid based cytology, LBC), PCR for HR-HPV-test, and p16/ki-67 dual biomarker cytology staining.

Inclusion criteria of the patients included in the study, are: over 30 years old, who were presented with post-coital bleeding and dyspareunia, offensive vaginal discharge, pelvic discomfort and pain, and intermenstrual bleeding. Exclusion criteria for cytology include: pregnancy,intrauterine device, hysterectomy, patients previously diagnosed as cervical neoplasia,

Using liquid based cytology (LBC), which is a technique used for cervical cytology, in which cervical epithelial cells are collected by a small brush, in a similar way as for a conventional cervical cytology, but the sample is admixed into a vial containing a preservative liquid (SurePath®Preservative Solution, TriPath Imaging Inc., Burlington, NC27215, USA). The preservative liquid allow to remove mucous, blood and other debris from the sample, before a layer of cells is applied to the slides. Then, the slide is prepared and stained (by H&E) manually.LBC have an advantages over conventional cytology, include limitation of inadequate samples and increased the sensitivity of the cytological smears due to clearance of the sample from mucous and extensive blood of the sample (provide by preservative fluid), and can be provide material for HPV –PCR test (7,9,10).

The prepared slides then examined by cyto-pathologist and final diagnosis and report for each case was made according to Bethesda system of reporting cervical cytology (BTS).

Another set of slides for cervical smear were prepared from harvested cells preserved in initial process of LBC

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technique for each patient with abnormal cytological smears. Dual p16/ki-67 staining of these slides was done, using CINtec® Plus Kit (Roche mtm laboratories Ag, Heidelberg, Germany) according to the manufacture instructions. The primary antibody cocktail include: a mouse monoclonal antibody (clone E6H4) against p16 protein, and a rabbit monoclonal antibody (clone 274-11CA3) against ki-67 protein. Using Alcohol-free hematoxylin for counterstaining.

The slides were reviewed by two pathologist, positive slide was identified by presence of one or more cervical epithelial cells that expressed simultaneous dark red-brown nuclei, and clear-brown cytoplasm, irrespective of cellular abnormalities. Slides with no or zero cells expressed dual staining were considered negative.

STATISTICAL ANALYSIS

Data analysis was made by using Chi-square test for independence, to evaluate the association of p16/ki-67 dual staining with cervical epithelial abnormality, with P value < 0.05, as statistical significance.

RESULT

The study included 214 women of mean age of 38 years old, who were met inclusion criteria, 15 of these patient were showed unsatisfactory smear, and they were excluded from the current study. Of the remainder 199 cases, 112 cases were showed as a nonspecific lesions (e.g. chronic non-specific cervicitis and erosion) need no further evaluation, 22 (25.28%) had ASC-US, 25 (28.74%) had LSIL, 32 (36.78%) had HSIL, 8 (9.19%) had invasive squamous cell carcinoma.

A total of 73 cases (83.90%) has positive p16/ki-67 dual immunostaining among 87 cases with cervical epithelial abnormalities. Those showed positive p16/ki-67 immunostaining (Fig-1), were underwent biopsy later in the course of management.

Among the cases with ASC-US, 15 (68.19%) showed positive staining for p16/Ki-67 markers, all were found to be CIN2/CIN3 in punch biopsy, 3 (13.63%) cases showed negative immunostaining despite they having CIN2/CIN3 in histology, 4 (18.18%) cases were found to be negatively stained and they were showed no dysplastic changes in corresponding histology.

Regarding LSIL, 18 (72.0%) cases were expressed p16/Ki-67 dual markers with CIN2/CIN3 in punch biopsy, while 3 (12.0%) of them were showed CIN2/CIN3, and 2 (8.0%) had CIN1 on histology, with negative immunostaining. The remaining 2 (8.0%) patients were showed negative staining for dual p16-Ki-67, and were showed no dysplastic changes in corresponding histology.

Regarding patients with HSIL and squamous cell carcinoma, they were positive for p16/ki-67 dual staining in all (100%), corresponding with histology.

The current study had showed the sensitivity and specificity for p16/ki-67 dual immunostaining in identification of CIN2/CIN3 in patients had ASC-US, were 100 and 75.2% respectively, and cases with LSIL were

found to be 100 and 89.3% respectively.

Figure 1. Show positive p16/Ki-67 immunostaining.

DISCUSSION

Cervical intraepithelial dysplasia are common in women above 30 years old. The intraepithelial lesions are classified as low and high grade, according to dysplasia that occur in cervical lining epithelia. The high grade can give rise to carcinoma in situ and invasive squamous cell carcinoma. These lesions are mediated by HR-HPV infection that responsible to wart lesions and dysplasia of mucous membrane.

More than 100 HPV genotype has been found, and categorized into low risk and high risk - HPV. It has been found that HR-HPV 16 & 18 are the most prevalent subtypes. Sexual transmission is the main root for infection with these subtypes. The integration of viral particle into host cells, causing dysregulation of cell cycle, and bypass immune response of the infected host cells, resulting in abnormal cell division and proliferation. It has found that cervical carcinogenesis mediated by HR-HPV infection is a multi-steps process, ranging from chronic infection, to intra-epithelial lesions (dysplasia of various grades), and cervical squamous cell carcinoma.

As shown, high risk -HPV infection can produce heterogenous molecular changes and dysmutation in the infected host cells (3,6,7), and may difficult to be detected by single test, thus combination of cervical cytology, PCR test for HR-HPV of the cervical epithelia, and additional triage test by biomarkers, namely p16/Ki-67 dual staining has been develop to increase the detection of dysplastic changes early malignant transformation.

The cervical lesions of ASC-US and LSIL remain difficult and of great challenge in clinical management.

In current study, it has been revealed the prevalence of CIN2+ lesions in ASC-US women was 81.18%, which significantly higher than other studies, ranging from 5-22%, (3,15,16). These difference may be due to use of conventional cytological examination of the cervix as screening program and diagnostic tool in previous studies. Other studies have shown it to be around 53%, (7). Similarly, the prevalence of CIN2+ lesions in women with LSIL was found to be also higher than other studies and reached to 84%, while other studies were around 30-58.3% (3,7,17,18).

In current study, 73 (83.90%) of all cases with epithelial abnormalities, showed positive p16/ki-67 dual staining, which closely follow previously conducted studies (19,20,21). It has been showed the dual staining of cervical epithelia have high sensitivity (100%) and specificity (75.2%) in identification of CIN2+ lesions in ASC-US women, this follow previous study of Diya Das et al, which were (100 and 70%, respectively) (7), but other conducted studies have found a wide range of sensitivity (64-98%) and specificity (43-81%) for p16/Ki-67 dual immunostaining in identification of high grade intraepithelial lesions in ASC-US. (3,16,20,22).

Among women having LSIL in cytology, the sensitivity and specificity of p16/ki-67 dual immunoreaction to identify high grade lesions were 100 and 89.3%, respectively. Diya Das found the sensitivity 87.5% and specificity 100% (7), which close to current study. Consistent results have been achieved by other studies. (12,13,20,21,22,23)

The current study was limited by small population size, but the strengths lay in a well-defined population, with availability of histological diagnosis, and usage of same



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fresh cervical sample for cytological diagnosis (LBC), PCR test for HPV, and p16/ki-67 dual staining.

The p16/ki-67 dual staining could be a useful tool as a triage test for the ASC-US and LSIL group, as more studies have revealed a significant rate of immunostaining positivity with high sensitivity and specificity for identification of high grade intraepithelial lesions in these women.

In addition, over-referral colposcopy to women having abnormal cervical cytology, can be minimized by usage of p16/ki-67 dual immunostaining.

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Abbreviations

ASC-US: Atypical squamous cell of undetermined significance.

BTS: Bethesda system

CIN: cervical intraepithelial neoplasia.

CIN1: cervical intraepithelial neoplasia, grade 1

CIN2: cervical intraepithelial neoplasia, grade 2

CIN3: cervical intraepithelial neoplasia, grade 3

CIN2+: cervical intraepithelial neoplasia, high grade (2&3)

HPV: human papillomavirus.

HR-HPV: high risk human papillomavirus.

HSIL: high-grade squamous intraepithelial lesion

LBC: liquid -based cytology.

LR-HPV: low risk human papilloma virus.

LSIL: low-grade squamous intraepithelial lesion.

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