Prevalence of HPV Immunostaining in Benign, Preneoplastic & Neoplastic Cervical Lesions of Kurdish Women in Erbil City/Kurdistan of Iraq

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Abstract

Background: Human papillomavirus (HPV) has been linked to many types of cervical diseases, ranging from the relatively innocuous lesion to fatal invasive squamous cell carcinoma (SCC).

Objective: to investigate prevalence of Human Papillomavirus (HPV) expression in cervical biopsies by immunohistochemistry, (IHC) in Kurdish women in Erbil city.

Materials & Methods: During the study period between September 2012 and June 2013, a total of 57 biopsy samples of cervical tissue were retrieved randomly from the Pathology Department of Maternity Teaching Hospital & some private laboratories in Erbil city, Kurdistan of Iraq. They were categorized as: Benign cervicitis (12), cervical intrepithelial neoplasia CIN I (24), CIN II (7), CIN III (6), cervical squamous cell carcinoma ,SCC (5) and three cervical adenocarcinoma.

Results: None of the 12 samples of benign cervicitis were positive for HPV protein while 15 out of 24 CINI (62.5%) ,5 out of 6 CIN II (83.3%)and 5 out of 7 CIN III (71.4%) were positive for HPV. Also HPV positivity observed in all five cases of SCC (100%), mostly in sheets of less mature squamous cells and in 2 out of 3 (66.7%) cases of adenocarcinoma, mostly focal & in single cells.

Conclusion: The immunohistochemical staining technique revealed a significant detection of HPV protein in cervical intraepithelial neoplasia & cervical carcinoma in Kurdish women.

Keywords: Human papillomavirus (HPV), Cervical intraepithelial neoplasia (CIN), Cervical carcinoma.

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INTRODUCTION

Cervical cancer is the second-most common type of cancer among women, worldwide [1]. In developing countries, cervical cancer is an important public health problem for adult women and has a major impact as screening programs are not well established or effective [2, 3].In Iraq, cervical carcinoma constitute 1.16% from the total malignant cases in 2005 and the Crude Incidence Rate per 100,000 population was 0.63% [4].In Kurdistan in 2009 ,cervical carcinoma constitute 1.09% from the total malignant cases in females and the Crude Incidence Rate per 100,000 population was 0.6 [5]. The disease is caused by HPV, a sexually transmitted virus; other risk factors are immunosuppression, high parity, multiple sexual partners, and use of contraceptives [6].

During the last few decades accumulated epidemiological, clinical, and experimental evidence has revealed the important role of human papilloma virus (HPV), in the development of cervical carcinomas, an association almost unique in cancer epidemiology [7,8]. The virus is asymptomatic in the benign stage [9,10] and it clinically manifests as a neoplastic transformation[11].

Morphology remains the gold standard for lesion diagnosis since cytologic and /or histologic examination allow the recognition of viral cytopathic effects; however it can be hampered by inter- and intra-observer variability. To overcome these limitations, high sensitivity and specificity methods for detection of truly dysplastic or infected cells, in the form of an objective biomarker, is adopted [12]

The purpose of the present study was to assess the feasibility of staining paraffin sections for the presence of HPV with monoclonal antibodies (clone K1H8, IgG) raised against the major coat fusion capsid proteins and study the prevalence of HPV immunoexpression in benign, preneoplastic & neoplastic cervical lesions of Kurdish women in Erbil city.

MATERIALS & METHODS

This study included 57 cervical biopsies that were selected & retrieved randomly from the Pathology department of Maternity Teaching Hospital & some private laboratories in Erbil city,Kurdistan region, Iraq during the period from September 2012 to June 2013. Ethical approval was obtained from the Medical Research Committee at Hawler Medical University. The biopsy specimens were fixed in 10% buffered formalin and processed for routine paraffin section, using the conventional methods. The original histological diagnoses were obtained on the hematoxylin and eosin slides and were categorized as: Benign cervicitis (12), cervical intrepithelial neoplasia CIN I (24) , CIN II (7),CIN III (6), cervical squamous cell carcinoma ,SCC (5) and three cervical adenocarcinoma. Thin (4μm) sections were cut, placed on slides, and submitted for immunohistochemical techniques.

Immunohistochemistry

Immunohistochemistry was performed on those 57 samples using the avidin -biotin-peroxidase complex in which primarily monoclonal anti HPV antibodies , that raised against a major structural capsid protein broadly expressed among different HPV (HPV 6, 11, and 18) (clone K1H8), were used & according to Dako Cytomation EnVision®+Dual link system-HRP(DAB+) staining protocol for immunostaining. Briefly, thin (4μm) sections of formalin-fixed, paraffin-embedded tissues were cut and placed on clean microscopic slides. The sections were dewaxed in xylene, rehydrated in graded alcohol, and rinsed in water. For antigen retrieval, the sections were immersed in 0.01M of citrate buffer, pH 6.0, in a high pressure cooker for 20 minutes, the tissue sections were cooled under tap water for 10 minutes. A peroxidase block reagent was applied on the specimen according to the tissue size and it was incubated for 5–10 minutes at room temperature. Next, a power block reagent was added, after draining out the slides, and incubated for 15 minutes at room temperature. An appropriate volume of mouse monoclonal anti HPV, clone K1H8 was added for one hour on the specimen and then with an appropriate volume of a secondary antibody (super enhancer reagent), it was incubated for 30 minutes, followed by rinsing with Tris buffer saline, pH 7.4 to 7.6 for 10 minutes. Poly horse radish peroxidase was added to it and it was incubated at room temperature for 30 minutes followed by rinsing with Tris buffer saline for 10 minutes. The slides were drained and blotted around the sections, to which an appropriate volume of substrate (3, 3′-diaminobenzdine) solution was added, and they were incubated for 40–50 minutes at room temperature followed by rinsing thrice in Tris-buffer saline. Finally, the sections were counterstained in a Mayer's hematoxylin bath for 1-10 minutes and then rinsed with tap water. Known positive control sections were included in each run to ensure proper immunostaining whereas the negative control consisted of the same section where the diluents without primary antibody were applied. The slides were tilted fully to cover the tissue by placing it in the horizontal position and allowed the coat to burden it, and then the cover slips were placed. It then viewed under the light microscope at 400X for the final and fine magnification.

RESULTS

The presence of viral infection was evidenced by a strong brown nuclear expression of viral infection marker K1H8 coat fusion/capsid protein in affected cells, occasionally the cytoplasm of cells was observed to be positive. The average age of patients was 48± 6.7yearrs and ranged from 26 years to 70yreas.The clinical presentations were mostly vaginal bleeding for more than month. None of the 12 samples of benign cervicitis were positive for HPV protein while 15 out of 24 CINI (62.5%) ,5 out of 6 CIN II (83.3%)and 5 out of 7 CIN III (71.4%) were positive for HPV. It was evidently positive in areas with epithelial dysplasia, mainly in basal & parabasal cells in CIN I &II and in whole layers in CIN III (Figure 1). For malignant cervical carcinoma,

Immunohistochemistry study showed HPV positivity in all five cases of SCC (100%) ,mostly in sheets of less mature squamous cells (Figure 2), and in 2 out of 3 (66.7%) cases of adenocarcinoma(ADC) , mostly focal & in single cells (Figure 3).

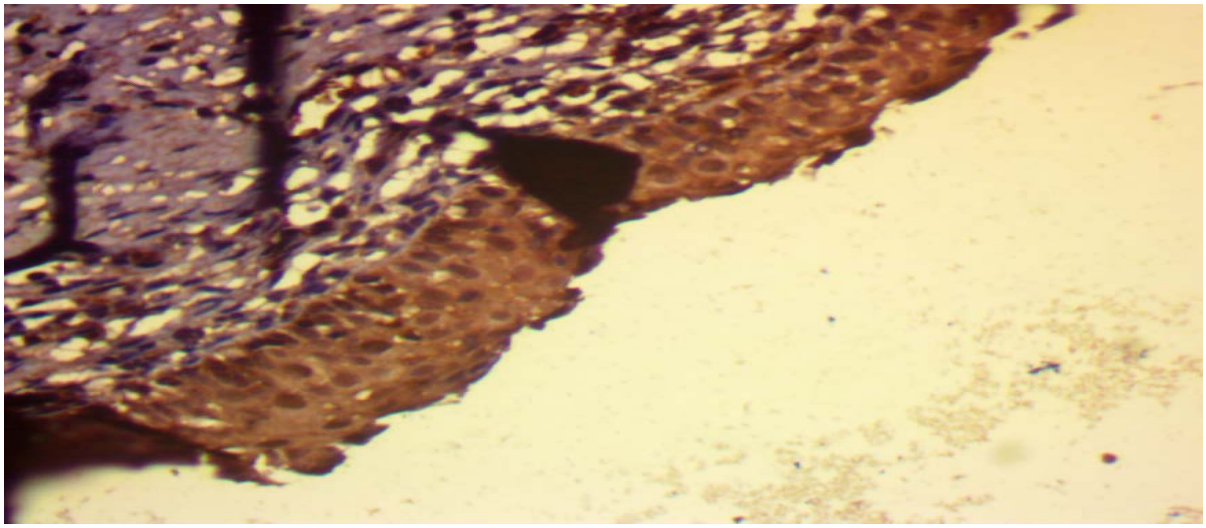

Figure 1: Positive HPV immunstaining in CIN III. The nuclei and cytoplasm of cells of the whole epithelial thickness stained brown in color. (X 400 IHC).

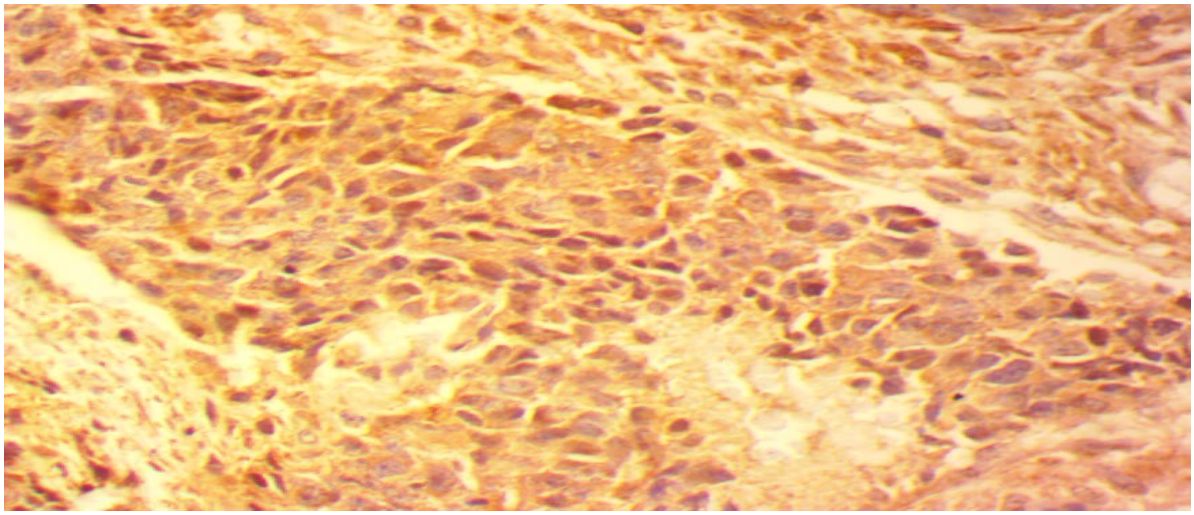

Figure 2: Squamous cell carcinoma of uterine cervix with positive HPV immunostaining, predominently in less mature cells. (X400 IHC).

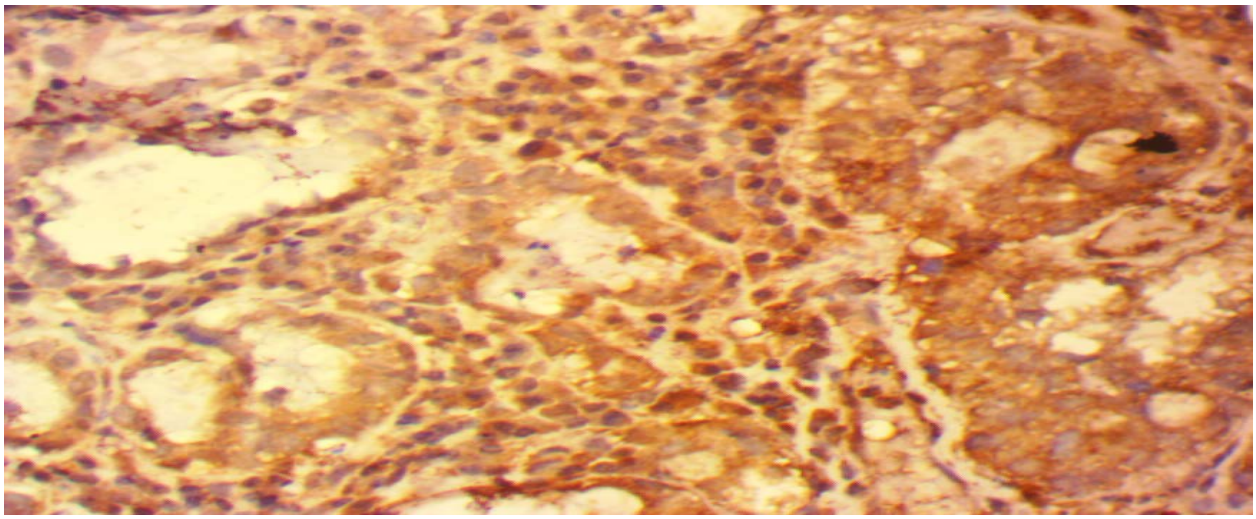

Figure 3: Adenocarcinoma of uterine cervix shows positive reactivity for HPV. The nuclei and occasionally cytoplasm of cells stained with brown color. (X 400 IHC).

DISCUSSION

Human papilloma virus has been considered as the most significant risk factor for cervical cancers and is recognized as a public health problem for its role as a critical factor in the pathogenesis of various cancers [(13)]. Nearly all cervical cancers are directly linked to previous infection with one or more of the oncogenic types of HPV [(14,15)]. The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. Since then, the link between HPV and cervical squamous cell carcinoma has become well established [(12)].

The aim of the present was to assess of the prevelance of HPV by immunostaining of paraffin sections using monoclonal antibodies (K1H8 clone,IgG), raised against the coat fusion proteins, and the results showed the possibility of detection of HPV in tissue section by using immunohistochemical staining technique .

All benign cervical tissues were negative for HPV immunostaining, which is similar to others [(16)]. It is known that during HPV infection, cells in the basal layer are infected first and the production of viral progeny takes place in differentiated cells in intermediate layers [(15)]. This finding was evident in the present study, in which the HPV positive immunoreactivity in the accompanied squamous dysplasia of the studied sections showed positive reaction in the basal and the parabasal epithelial cells in CIN I (62.5%) & II (83.3%) and in the whole thickness of cervical epithelium in CIN III (71.4%). This is nearly similar to others [(17)] and lower than others [(18, 19)]. In a study done by Lelin etal[(20)], another biomarker $P16^{INK\ 4A}$ had been adopted for the

detection of HPV in tissues of normal as well as different grades of CIN. Their study material consisted of formalin-fixed, paraffin-embedded blocks of cervical specimens from 161 adolescents. The specimen included 15 cases of normal cervicitis, 48 cases of CIN I, 46 cases of CIN II, and 52 cases of CIN III. The results showed that all 15 biopsies within normal limits were negative for $P16^{INK\ 4A}$. The positivity of $P16^{INK\ 4A}$ in CIN I was 44%. This was increased to 97% expression in CIN II and CIN III.

Squamous cell carcinoma is the predominant type of cancer of the uterine cervix and infection with Human Papillomavirus (HPV) is considered to be the principal causal agent in the development of squamous cell carcinoma of the uterine cervix [15, 21, 22]. In the present study, all cases of squamous cell carcinoma (100%) showed positive expression in which the less differentiated areas showed a strong positive immunstaining. This is similar to results of other studies [15,17] and higher than others[16,23,24] .This variability in the prevalence of HPV in various studies was attributed mostly to type of antibody used ,ethnic and geographic factors.

Adenocarcinoma, adenosquamous carcinoma, and small-cell carcinoma of the uterine cervix are reported to be low in incidence but clinically important. They usually exhibit a more aggressive biologic behavior and have a poorer prognosis than squamous cell carcinomas at similar stages [16,25]. Unlike SCC, however, the risk factors for ADC of the cervix are not well understood [22]. The etiopathogenesis of adenocarcinoma is not yet clearly understood. Recent studies have raised more controversy, rather than answering the question of whether specific HPV infection also plays a role in the development of adenocarcinoma of the cervix [25]. There have been several reports showing the presence of HPV DNA, predominantly HPV type 18, in ADC and ADSC in contrast to invasive SCC, in which the incidence of HPV 16 is very high [22]. In this study 2 out of 3 cases of adenoncarcinoma (66.7%) showed positive HPV immunreactivity, mostly focal & single cells, which is similar to other study[26] but higher than another one [27].

CONCLUSION

The results suggested that it was possible to detect HPV in the tissue sections, by using the immunohistochemical staining technique that revealed a significant detection of HPV in cervical intraepithelial neoplasia & cervical carcinoma in Kurdish women. This is recommending the further use of advanced techniques such as polymerase chain reaction for typing the virus.

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