Immunohistochemical Evaluation of the Frequency of Human Papillomavirus in Cervical Lesions in a Sample from the North Iraqi Population

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ABSTRACT:

BACKGROUND:

Human papilloma virus has been linked to many types of cervical lesions, ranging from the relatively innocuous lesions to fatal invasive squamous cell carcinoma. There is growing evidence of Human papillomavirus being a relevant factor in other anogenital cancers (anus, vulva, vagina and penis) as well as head and neck cancers.

OBJECTIVE:

To assess the feasibility of immunohistochemical staining paraffin sections for the presence of Human papilloma virus with monoclonal antibodies (clone K1H8, IgG) raised against the major coat fusion capsid proteins and to detect the frequency of human papilloma virus immunoexpression in benign, preneoplastic and neoplastic cervical lesions in patients living in Erbil city (North of Iraq).

MATERIALS AND METHODS:

A total of 75 paraffin blocks samples of cervical tissue were retrieved retrospectively from the Pathology Department of Maternity Teaching Hospital and some private laboratories in Erbil city, during a period spanning from September 2013 to June 2014. They were categorized as: Benign cervicitis (10) samples, cervical intraepithelial neoplasia, CIN I (33) samples, CIN II (10) samples, CIN III (13) samples, cervical squamous cell carcinoma (6) samples and three samples with cervical adenocarcinoma Immunohistochemistry was performed on those samples using the avidin -biotin-peroxidase complex in which primarily monoclonal anti Human papilloma virus antibodies was used.

RESULTS:

None of the 10 samples of benign cervicitis were positive for Human papillomavirus protein while 21 out of 33 (63.6%) samples of CINI, 9 out of 10 (90%) samples of CIN II and 9 out of 13 (69.2%) samples of CIN III were positive for HPV. Also Human papillomavirus positivity observed in all six samples of squamous cell carcinoma (100%), mostly in sheets of less mature squamous cells and in 1 out of 3 (33.3%) samples of adenocarcinoma , mostly focal and in single cell.

CONCLUSION:

The immunohistochemical staining technique revealed a significant detection of HPV protein in cervical intraepithelial neoplasia and cervical carcinoma.

KEYWORDS: human papillomavirus, immunohistochemistry, cervical intraepithelial neoplasia, cervical carcinoma.

INTRODUCTION:

Worldwide, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women [(1)]. In Iraq cervical cancer ranks by Iraqi Cancer Registry as the 12th most frequent cancer among all women and the 10th most frequent cancer among women between 15 and 44 years of age [(2)]. In the Northern part of Iraq (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 [(3)]. Recently, it was found several human papillomavirus (HPV) genotypes among infected Iraqi women [(4)]. Furthermore, a link between breast cancer pathogenesis and HPV infection was found in Iraqi

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patients [5]. During the last few decades accumulated epidemiological, clinical, and experimental evidence has revealed the important role of HPV in the development of cervical carcinomas, an association almost unique in cancer epidemiology [6]. This virus is asymptomatic in the benign stage [7] and it clinically manifests as a neoplastic transformation[8] with infection by a "high risk" types of HPV, such as 16, 18 appears to be involved in the development of more than 90% of cases but only 2% of cervical HPV infections will develop into cervical cancer [9]. The factors that may modify the risk for HPV DNA positive women include immunosuppression, high parity, multiple sexual partners, previous exposure to other sexually transmitted diseases, smoking and the use of contraceptives [9, 10, 11, 12].

Morphology remains the gold standard for lesion diagnosis, despite the fact that it can be hampered by inter- and intra-observer variability [13]. Additionally, the contribution of morphology in the field of HPV research cannot be overemphasized, since cytological and/or histological examinations allow the recognition of viral cytopathic effects, and, with the aid of immunohistochemical (IHC) and other in situ techniques, may reveal the exact cells, in which some main interactions take place. Thus, the correlation of cellular alterations with new sensitive methods of detection either for HPV nucleic acids or for HPV-related intracellular interactions might lead both to the identification of different groups of lesions according to their clinical significance, as well as to the correct application of current morphological criteria [14].

The aim of the present study is to assess the feasibility of immunohistochemical staining paraffin sections for the presence of HPV with monoclonal antibodies (clone K1H8, IgG) raised against the major coat fusion capsid proteins and to detect the frequency of HPV immunoexpression in benign, preneoplastic and neoplastic cervical lesions in patients in Erbil city (North of Iraq).

MATERIALS AND METHODS:

A total of 75 paraffin blocks samples of cervical tissue were selected and retrieved retrospectively from the Pathology department of Maternity Teaching Hospital and some private labs. in Erbil city, during the period spanning from September 2013 to June 2014. Ethical approval was obtained from the Medical Research Committee at Hawler Medical University, College of Pharmacy. 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 (10) samples, CIN I (33) samples, CIN II (10) samples, CIN III (13) samples, cervical SCC (6) samples and three samples with cervical adenocarcinoma. Thin (4μm) sections were cut, placed on slides, and submitted for IHC techniques.

Immunohistochemistry was performed on those 75 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. Known positive control sections were included in each run to ensure proper immunostaining while negative control slides were prepared from the same tissue block, but incubated with TBS instead of the primary antibody [15].

Immunohistochemical expression of HPV was scored using a semiquantitative composite scoring system as follows: (1) staining intensity, defined as 0 for negative, 1+ for weak, 2+ for moderate, and 3+ for strong; (2) positive area, defined as the (10 X) fraction of stained tumor cells in the entire tumor; and (3) expression score, defined as the staining intensity multiplied by the positive area. The highest possible score was 30.

Over expression was defined as a score >20[16].

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 too. The average age of patients was 47± 1.1 years and ranged from 25 years to 69 years. The clinical presentations were mostly vaginal bleeding for more than month. None of the 10 samples of benign cervicitis were positive for HPV protein while 21 out of 33 (63.6%) samples of CIN I, 9 out of 10 (90%) samples of CIN II and 9 out of 13 (69.2%) samples of CIN III were positive for HPV. It was evidently positive in areas with epithelial dysplasia, mainly in basal and parabasal cells in CIN I and CIN II as shown in Figure (1) and in whole layers in CIN III, as shown in Figure (2). For malignant cervical carcinoma, IHC study showed HPV positivity in all six samples of SCC (100%), mostly in sheets of less mature squamous cells, as shown in (Figure 3), and

in 1 out of 3 (33.3%) samples of adenocarcinoma (ADC), mostly focal and in single cell, as shown in Figure (4).

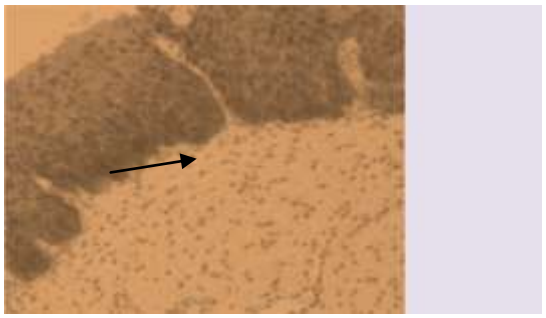

Figure 1: Positive HPV immunstaining in CIN II. The nuclei and cytoplasm of the basal and parabasal cells stained brown in color, (X 400 IHC).

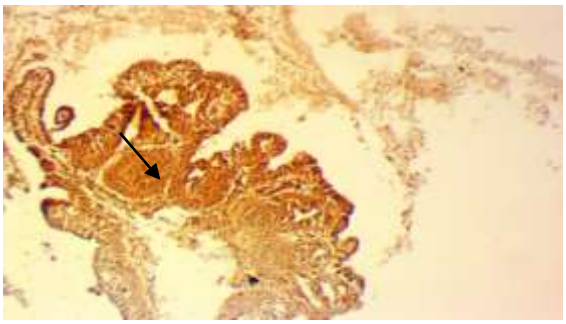

Figure 2: Positive HPV immunstaining in CIN III. The nuclei and cytoplasm of cells of the whole epithelial thickness stained brown in color, (X 100 IHC).

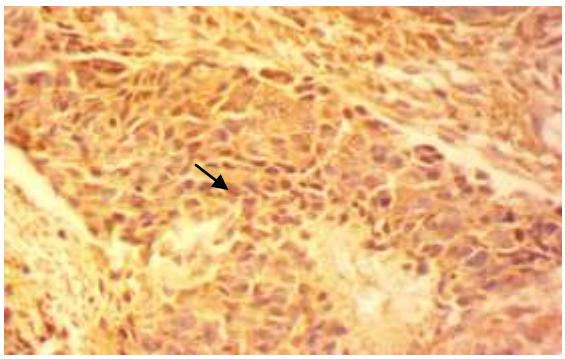

Figure 3: Squamous cell carcinoma of uterine cervix with positive HPV immunostaining in the nuclei and cytoplasm predominantly in less mature squamous cells, (X400 IHC).

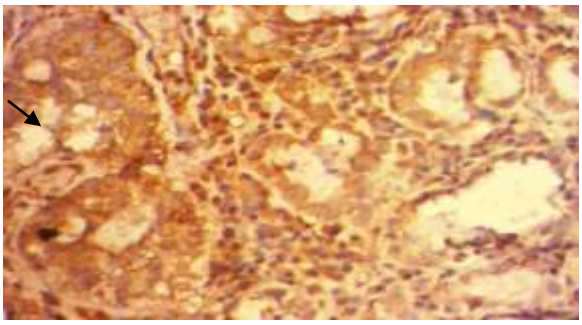

Figure 4: Adenocarcinoma of uterine cervix shows positive reactivity for HPV. The nuclei and occasionally cytoplasm of cells stained with brown color, (X 400 IHC).

DISCUSSION:

Despite the presence of interobserver variation in the histopathologic interpretation of cervical lesions, in more instances definite diagnosis is made by routine histopathological examination with the aid of IHC and other in situ techniques which are supportive in follow up of the patients. Human Papillomavirus 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 [17]. Nearly all cervical cancers are directly linked to previous infection with one or more of the oncogenic types of HPV [18, 19]. The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. [14].In the present study, all benign cervical tissues is negative for HPV immunostaining, which is similar to that observed by other [20]. 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 [19]. This finding is 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 of CIN 1 and CIN II in (63.6% and 90%) of the samples respectively, While the positive immunoexpression was observed in the whole thickness of cervical epithelium of CIN III in (69.2%) of the samples. This is nearly similar to that observed by other [21] and lower than an others [22, 23]. In a study done by Lulin etal. [24], 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 samples of normal cervicitis, 48 samples of CIN I, 46 samples of CIN II, and 52 samples of CIN III. The results showed that all 15 biopsies within normal limits were negative for $P16^{INK\ 4A}$. The positivity of $P16^{INK\ 4A}$ was 44% in CIN I. This was increased to 97% expression in CIN II and CIN III. SCC is the predominant type of cancer of the uterine cervix and infection with HPV is considered to be the principal causal agent in the development of SCC of the uterine cervix [19, 25, 26]. In the present study, all samples of SCC (100%) showed positive expression in for HPV in which the less differentiated areas showed a strong positive immunstaining, similar findings was noted by other studies [19,21], while higher than an others [20, 27, 28] .This variability in the frequency of HPV immunostaining in various studies may be attributed to the number of samples studies, IHC methodology used, sensitivity of the detection system and the determination of criteria for positive results used. Also differences in population groups, diversity of risk habits, variation of genetic predisposition and geographic factors may also contributed to this variation.

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 SCC at similar stages [20, 29]. Unlike SCC, however, the risk factors for ADC of the cervix are not well understood [26]. The etiopathogenesis of ADC 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 ADC of the cervix [29]. 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 [27]. In this study 1 out of 3 (33.3%) samples of ADC showed positive HPV immunreactivity, mostly focal and in single cell, which is similar to that observed by other study [30] but higher than another one [31]. In Iraq, although the incidence rates of cervical cancer are relatively low, the majority of the cases usually present in advanced stages with poor prospects of cure [32].

Therefore, many researchers [33, 34] recommended education; knowledge and awareness about HPV and its relation to cervical cancer are needed for Iraqi women to prevent HPV infection.

CONCLUSION:

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

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