Human Pabilloma Virus (HPV) and Cervical Cancer in Al-Najaf Governorate

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Abstract

Background: Human Papilloma virus causes benign and malignant tumours of squamous cells, many different types of HPV well known to infect a different portion of the body, also many HPV strains cause carcinoma of cervix. Objective To find the role of the virus in the cervical tumour and the extend of the effect of some tumour markers in the virus and its absence. Methods Seventy three cases of cervical cancer were obtained from Histopathological archival of central laboratory of AL-Sadder Hospital and out laboratory in Najaf AL-Ashraf Governorate. Clinical data were analysed from the medical records and formalin fixed, paraffin embedded tumour tissue were examined by Immunohistochemistry technique to the detection of the L1 protein of HPV in cervix tissue blocks. Results The expression of HPV in tissues, women patients with cervical cancer in the present study was 69.86% (51 out of 73), where strong correlation was found between expression of HPV and women patients with cervical cancer. Also found relationship between women state, residency and differential ratio between five years ago (2012-2016) in AL Najaf City. Conclusion Based on the results of the current study, Human Papilloma virus infection plays a major role in the pathogenesis of cervical cancer.

Keywords: Cervical cancer, Human Papilloma virus, Human Papilloma virus L1 protein, Immunohistochemistry technique.

Introduction

Cervical cancer is the second senior cancer betwixt women worldwide, It's an ailment caused by pathogenic strains from Human Papilloma virus (HPV) [1].

Human Papilloma virus causes benign tumours of squamous cells (papillomas), many different types of HPV well known to infect a different portion of the body, also many HPV strains cause carcinoma of cervix. Epidemiologic studies shown the interaction between of genital human papilloma virus (HPV) with cervical cancer is powerful, Independent of other risk factor [2].

Human Papilloma viruses (HPV) are modeled member of the papovaviridae family, include slightly larger genome (8 Kbp), duplicated strand from DNA, ringed by icosahedral capsid comprise of structural proteins and this virus from non-envelope virus [3]. HPV have sixth of the early protein genes include E1, E2, E4, E5, E6 and E7, the two last early protein E6 and E7 are concerned in carcinogenesis, the main risk factor of cervical cancer is infection with Human papilloma virus(HPV), About 40 kinds of HPV infect the genital tract, more common HPV kinds in patients frequently types 16, 18, 31, 33, 52, 58 and others [4].

Cancer of the cervix uteri is the cancer happened in the inferior one third from uterus that it's consist of dense fibro muscular tissues queued by two kinds of epithelium, columnar and squamous epithelial tissue, the delimitations font between columnar and squamous is called squamocolumnar junction (SCJ), the site of the unique SCJ change with woman's hormonal status, age, pregnancy status, history of birth trauma and use of oral drugs that prevent pregnancy, When exposure to the acidic conditions in the vagina, the usual replacement process called squamous metaplasia that its accrue and stretches to new SCJ,[5].
The detail that occurrence of cervical cancers in emerging countries that is highest consequences from the absence of actual screening programmes, Which are used for noticing and treating cancer precursor lesions before they converted into aggressive cancer [6]. HPV infections are the greatest commonly diagnosed as sexual transmitted viral diseases between man and women, Most sexually active men and women will be became infection with one type HPV at least through life time [7]. HPV infected patient that is able to carry virus many years without any symptoms, Certain infected persons problematic know the cause or time of infection, Also diagnosed HPV by detecting HPV DNA in biopsy specimens and cervical scraping that was done blindly in central laboratories with use polymerase-chain-reaction(PCR) based assays, and established the role of HPV in causation cervical cancer [8, 9].

Materials and Methods

All tissue blocks samples were collected during period from 5 March (2016) to 31 December (2016). take 650 tissue blocks to total 110 women patients which rips their uterus (hysterectomy) or only biopsy from cervix without rips their uterus. Collection of samples perform in two way, the first collection tissue blocks to five years ago (2012-2016) from the Histopathological archive of central laboratory in AL-Sadder Hospital and out laboratory in AL-Najaf Governorate, and the second collection tissue samples from patients came to the same hospital during the period of the action of the practical part of the research, so worked the preparation to tissue samples and completed the rest of the steps as if they were blocks ready, as in the first method. In the first collection 600 paraffin-embedded blocks to 90 women patients with ages 20-65 years, Which are makes surgical removal of them uterus or has injuries in the womb, while in the second collection include fresh tissue to 20 women patients through three months period 5 March 2016 to 31 December 2016 and prepared to them 50 paraffin-embedded tissue blocks.

The endo- and ectoscopy biopsy samples of the cervix were permanently in 10% buffered formalin; monotonous paraffin slices were occupied for treating and discolored with hematoxylin and eosin. The samples were graded as Normal cervicitis, Cervical intraepithelial neoplasia CIN I, CINII and CIN III, for unique diagnosis by pathologists. The additional sections were studied for the expression of HPV marker by immunohistochemistry.

Preparation of Tissue Sections for Immunohistochemistry

Immunohistochemistry for detection of HPV in paraffin embedded sections by using monoclonal mouse anti-human papillomavirus, clone K1H8 through the following steps:- (A) Paraffon embedded sections were cut 4µm thick, placed on slides at room temperature to dry. (B) The sections were placed in to a container with de-ionized or distilled water and preheated from 40 to 44 °C to relax the section from compression due to sectioning. (C) The section was placed on microscope slide as flat and wrinkle free as possible to optimize stain contact with tissue. (D) The tissue was placed on the slide with painted portion slide up. (E) Tissue sections were dried on the slides by heating in an oven at 60 °C for a minimum of 60 minutes to ensure that any moisture trapped under the tissue section is completely eliminated by melting of the paraffin and evaporation of the water droplets. (F) Chromagen reagent was prepared by adding appropriate volume of DAB Chromagen in a 1:25 ratio using an aspirate bottle. (G) Rinsing buffer was diluted twenty fold in deionized distilled water. (H) Primary antibody was diluted in IX dilution/blocking buffer.(I) Absolute ethanol was diluted in distilled water to prepare 95% and 70% concentration of alcohol. (J) Negative control was included for each run of immunohistochemistry. The negative control was obtained by replacing the primary antibody with PBS buffer. (K) Cervix slides infected HPV were used as a positive control (Dako).

Immunohistochemistry Procedure

Dewaxing: paraffin embedded sections were placed in hot air oven at 65 °C for 2 hours and dipped in xylene and ethanol containing jars,slides were washed in running water for 5 minutes, drained and put in citric acid [10] mm and placed in microwaves for 2 minutes at 96 °C. Sufficient amount of hydrogen peroxide 3% were placed onto the section and incubated for 20 minutes at 37 °C in a humid chamber, drained and blotted gently, the tissue sections were surrounded by a circle drawn by the PAP pen and allowed to dry for 2 minutes at room temperature.
The hydrophobic barrier made by the pen retains aqueous solutions within a defined area, eliminating the use of excess reagent. One hundred µl of a protein blocking reagent was placed onto the section and incubated for 30 minutes, then drained and blotted gently. One hundred µl of diluted primary antibody was placed onto the section and incubated for 1 hour at 37°C in a humid chamber. After incubation, the slides were drained and blotted gently. Slides were rinsed with a Rinse buffer from a wash bottle, then transferred to a bath Rinse buffer and incubated for 5 minutes.

One hundred µl of diluted secondary antibody was placed onto the section and incubated for 1 hour at 37°C in a humid chamber. Slides were rinsed with a Rinse buffer from a wash bottle, then transferred to a bath Rinse buffer and incubated for 5 minutes. One hundred µl of diluted streptavidin-alkaline phosphate conjugate was placed onto the section and incubated for 1 hour at 37°C in a humid chamber. One hundred µl of the DAB Chromagen was placed onto section and for 10 minutes at room temperature. Slides were in running water for 5 minutes. One hundred µl of hematoxylin was placed onto section and incubated for 2 minutes at room temperature. Slides were drained and blotted gently, the slides were washed in distilled water and dehydrated by placing them in ethanol and xylene jars. A drop of mounting medium (DPX) was placed onto xylene-wet section by using a xylene-moist cotton swab and section was quickly covered with a cover slip. The slide was let to dry and examined.

**Statistical Analyses**

Statistical analyses was done by using Graphed Prism software. The groups of patients women were analyzed by chi square and T test. Values of \( p < 0.05 \) were measured to be statistically significant.

**Results**

Detection HPV L1 Protein by Immunohistochemistry

The abnormal cervix tissue which contain viral particle that seen by nuclear staining kit and immunohistochemistry procedure. In the studied HPV immunoexpression was reported in 23 out of 73 cases of cervical carcinoma (31.5%) and in control cervix tissue none reveled positive immunoexpression for HPV in the nucleus showed in Figure(1).

![Image 1: Immunohistochemistry to abnormal tissues cervix show in more detail the localization of HPV DNA in the nucleus. (40 X magnification)](image-url)
Figure (2) shows the distribution of patients women which have cervix infection that which divided into six age groups with benign or malignant (with HPV or without HPV). Overall, there are significant differences in the distribution of these infections among different age groups and kinds of infection was 2.15 in $P=0.05$. However the age group A5 and A6 (52-60) years old has a highest percentage $2*16/73$ (21.9%) which contain in A5 groups 7 benign infections case (7 HPV and 0 without HPV) and 9 malignant infections case (9 HPV and 0 without HPV), whereas contain in A6 groups 10 benign infection case (5 HPV and 5 without HPV) and 6 malignant infections case (3 HPV and 3 without HPV) while the lowest percentage was among the age group A1 (20-27 years old) and A3 (36-43) $2*7/73$ (9.5%) which contain in A1 groups 6 benign infections case (1 HPV and 5 without HPV) and 1 malignant infection case (0 HPV and 1 without HPV), whereas contain in A3 groups 7 benign infection case (4 HPV and 3 without HPV) and 0 malignant infections case (0 HPV and 0 without HPV).

The distribution of cervix infection patients women according benign and malignant into two groups: first, benign with HPV and benign without HPV and second, malignant with HPV and malignant without HPV. The present study showed that benign with HPV percentage constituted $16/73$ (21.91%). And malignant with HPV percentage constituted $23/73$ (31.5%) while malignant without HPV percentage constituted $6/73$ (8.21%) all percentage shown in Figure (3).
The distribution of cervix infection women patients according malignant tumor into three type CINI, CINII and CINIII with HPV and without HPV. The total number of malignant tumor was 29/73 (39.72%). The percentage constituted from it with HPV 25/29 (86.2%) include CINI 17/29(58.62%), CINII 5/29(17.24%) and CINIII 3/29(10.34%) while the percentage constituted from it without HPV include CINI 4/29 (13.79%), CINII 0/29(0%) and CINIII 0/29(0%). This percentages shown in Figure (4).

Distribution of Women Patients According State

According to state of cervix women infection were divide into two groups married and unmarried (M and N), the present study showed that married patients group has the highest percentage constituted 67/73 (91.78%) patients ,while the unmarried patients constituted 6/73 (8.21%) patients as shown in Figure (5).
Figure 5: Distribution of patients women according state

M: Married women, N: Unmarried women

Distribution of Patients Women According to Residency

The distribution of cervix infection women patients according residency into two groups: Rural (R) and Urban (U) as shown in Figure (6).

The group R revealed highest percentage 42/73 (57.53%) women patients compared with group U constituted 31/73 (42.46%) Figure (6).

Figure 6: Distribution of women patients according to residency

- R: Rural residency group.
- U: Urban residency group.

Distribution of Women Patients According the Ratio of Infection to Five Years Ago

Figure (7) shows the distribution of women patients which have cervix infection to five years ago (2012-2016). The highest percentage was in 2016 year 18/73 (24.6%) while the lowest percentage was in 2013 year 11/73 (15%). All percentage to five years illustrate in Figure (7).

Figure 7: Distribution of women patients according the ratio of infection to five years ago
Discussion

In the present study, tissue blocks sample in the beginning collected and done preparation method to each blocks from sectioning to reach staining by Haematoxylin and Eosin stains, after complete the other steps will examination by multi heads light microscope. A total of 110 slides were obtained diagnosed as normal, benign and malignant (CIN I, CIN II and CIN III). In Galgano et al, method has several advantages, the first of which is the ability to identify relevant areas of Haematoxylin and Eosin stained tissue sections that pathologists examine using light microscopy, images are obtained from a standard Haematoxylin and Eosin stained slide without any other processing of the tissue and can be adopted by hospital laboratories without requiring extra-technical services, inherent variations from the Haematoxylin and Eosin staining are removed through our normalization process, thus staining done at various times or at different institutions can still be compared and evaluated[10].

Regarding the assessment of the control group which includes normal cervical tissue (control group), some cases were negative for HPV Immunostaining without any expression of even faint cytoplasmic stain with significant difference from malignant cases. In the current study the results have clarified that 51 out of 73 cases of cervical carcinoma were expressing HPV Immunohistochemical nuclear staining in their histological sections.

However, malignant cases showed 23/29 (41.17%) positivity for HPV within this type of tissue and 100% within Immunostaining of HPV with a significant difference comparing with normal and benign cases (P<0.05). A high percentage of HPV overexpression was described in well and moderately discriminated cervical cancer, whereas the less percentage of HPV was described in poorly discriminated cervical cancer. These outcomes are in agreement with outcome of previous analogous study [11, 12].

In the present study, illustrate the cervical cancer women patients classify according benign and malignant into two groups: first, benign with HPV and benign without HPV and second, malignant with HPV and malignant without HPV. The present study showed that benign with HPV percentage constituted 28/73 (38.35%) while benign without HPV percentage constituted 16/73 (21.91%). And malignant with HPV percentage constituted 23/73 (31.5%) while malignant without HPV percentage constituted 6/73 (8.21%).the highest ratio constituted from it with HPV 28/44(63.63%) while the lowest highest ratio constituted from it without HPV 16/44 (36.36%). Also the malignant tumor with HPV or without HPV into three type CINI, CINII and CINIII, depend on the size and progressing of cancer, the total number of malignant tumor was 29/73 (39.72%), the ratio constituted from it with HPV 25/29 (86.2%) include CINI 17/29(58.62%), CINII 5/29(17.24%) and CINIII 3/29(10.34%) while the ratio constituted from it without HPV include CINI 4/29 (13.79%), CINII 0/29(0%) and CINIII 0/29(0%) and malignant cases without HPV was 4/31 (12.9%). Also in the present study show that cervix infection women patients which have HPV divided into benign and malignant tumor. The total number of HPV samples was 51/73 (69.86%), the highest ratio constituted from it was benign 28/51(54.9%) while the lowest ratio constituted was from malignant tumor 21/51 (41.17%). Too, the cervix infection women patients which haven't HPV divided into benign and malignant tumor, the total number of samples without HPV was 22/73 (30.13%), the highest ratio constituted from it was benign 16/22(72.72%) while the lowest ratio constituted was from malignant tumor 6/22(27.27%).

In current study, certain investigators ensure correlated with different degrees of CIN and HPV type have optional that CIN1, CIN2 and CIN3 are diverse manners, with CIN1 showing a self-limited sexually diffused HPV infection and CIN3 or CIN2
being the merely real cervical cancer precursor [13]. the natural history of cervical cancer as a nonstop single disease process developing gradually from mild cervical (CIN1) to more severe degrees of micro invasive lesions and neoplasia (CIN2 or CIN3) and lastly to aggressive disease has been the origin for finding, secondary defensive strategies, and therapeutic measures [14].

In the present study, the age of patients which ranged between 20-65 years. Most HPV infections occur in adults with a peak at 52-65 years of age. The cervix infections women classify into two state, married and unmarried so the total number was 73 women patients, the highest number 67 from it was married women which have one partner while lowest number 6 from it was unmarried women in Al Najaf Governorate. Also Rural contain high ratio (57.53%) as comparative with Urban (42.46%) because many causes lead to this ratios between them such environmental condition, Life style, and other factors.

The ratios of cervix infected women to five years ago (2012-2016) show the highest ratio was in 2016 year 18/73 (24.6%) while the lowest ratio was in 2013 year 11/73 (15%). In 2012 the ratio was 16/73(21.9%), and in 2014 the ratio was 15/73(20.5%) while in 2015 the ratio was 13/73(17.8%). This ratio differentiated depend on many factor such patients age, women status and residency. Also notice highest in the ratio to 2016 year as compared with years because Lack of health awareness is sufficient and not taking the necessary vaccines for the virus that causes this deadly disease.

**Conclusion**

Based on this study ,the following conclusions could be made :

The present study is concluded that the HPV patients women have a high risk to develop cervical cancer (benign or malignant).and present the relationship between women patients and it's age, the highest ratio is (52-65) years old. And the patients live in Rural are most infect with virus than in Urban

**References**


