Incidence of HPV in Pre-Invasive and Invasive Cervical Cancer among Patients in Galaa Hospital

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ABSTRACT
Background: A significant contributor to cervical carcinogenesis is the human papillomavirus (HPV), which alters genetic structure and function. There are two main histological subtypes of cervical cancer: adenocarcinoma (AC) and squamous cell carcinoma (SCC).
Objective: To examine the correlation between human papillomavirus (HPV) and cervical cancer in women who attended the Galaa Maternity Hospital's Early Cancer Detection Clinic in the Gynecology Department.
Patients and Methods: In this prospective analytic study, a total of 100 patients with histologically confirmed pre-invasive and invasive cervical lesions were analysed. The HPV detection and genotyping were performed using a commercial PCR kit referred to General Organisation for Teaching Hospitals and Institutes (GOTHI) Early Cancer Delectation Clinic of Gyn-oncology Department, Under supervision of El-Galaa Maternity Teaching Hospital.
Results: Patients who had HPV-positive status had a significantly higher mean age (55.32 years) compared to HPV-negative patients (46.10 years), with a p-value of <0.0001, indicating a strong association between older age and HPV positivity. In the crosstabulation of HPV status and pathology types, the findings revealed significant associations between HPV positivity and certain pathologies. Adenocarcinoma: All cases (100%) were HPV-positive, indicating a very strong association between adenocarcinoma and HPV infection (p < 0.0001). CIN 3: A higher proportion of CIN 3 cases were HPV-negative (69.2%), but 30.8% were HPV-positive, reflecting a stronger association with HPV as the lesion grade increases. Squamous cell carcinoma (SCC): The majority (85.7%) of SCC cases were HPV-positive, reflecting a strong correlation between HPV infection and SCC.
Conclusion: There is poor correlation between HPV and cancer cervix and further national multicentre studies with wide scale of patients are recommend to prove the correlation between cancer cervix and HPV in Egyptian ladies.
Keywords: Human papillomavirus, Cervical cancer, Pre-invasive and invasive.

INTRODUCTION

Adenocarcinoma (AC) and squamous cell carcinoma (SCC) are the two main histological subtypes of cervical cancer, which is a malignant tumor of the cervix [1].

The occurrence rate of SCC is 70%, making it more common [2]. In contrast to SCC, which develops from squamous cells lining the external portion of the cervix that opens to the ectocervix, AC begins in the glandular cells that line the cervical canal, also known as the endocervix. A majority of tumors begin in a specific area called the transformation zone, which is home to both squamous and thin, flat glandular cells [3].

The human papillomavirus (HPV), which causes changes in genetic structure and function, is one of the main risk factors for its carcinogenesis. Other factors include: smoking, immunodeficiency, oral contraceptives, high parity, smoking, HIV, and other sexually transmitted diseases [3]. Squamous cell carcinoma (SCC) accounts for 69% of all cases and adenocarcinoma (AC) for 25%. Each kind has its own unique immunologic tumor microenvironment and oncogenic alterations. Various other forms, comprising less than 6% of cases, encompass adenoid cystic, neuroendocrine, mixed epithelial-mesenchymal, and undifferentiated carcinoma tumors [4].

Early disease often does not cause any symptoms and is often found by chance during screenings or routine pelvic exams (20% of women). The most common symptoms include heavy or irregular vaginal bleeding, bleeding after sexual relations, and a vague vaginal discharge that is typically mistaken for simple vaginitis or cervicitis due to its mucoid consistency, purulent nature, and unpleasant odor [5].

This study aim was to examine the correlation between human papillomavirus (HPV) and cervical cancer in women who attended the Galaa Maternity Hospital's Early Cancer Detection Clinic in the Gynecology Department.

PATIENTS AND METHODS

In this prospective analytic study, a total of 100 patients with histologically confirmed pre-invasive and invasive cervical lesions were analyzed. The HPV detection and genotyping were performed using a commercial PCR kit.

Inclusion criteria: All cases with confirmed premalignant or malignant cervical lesions and preparing for radical surgery.

Exclusion criteria:
1- Pregnancy.
2- Patient with advanced cervical cancer stages.
3- Inoperable cases.

All cases had been subjected to the following:

Every patient had to sign a permission form, accurate documentation of demographic information (age, sex, and clinical presentation), review the patient's medical history for any relevant tests, and laboratory investigations, review of prior histopathological findings (the study's gold standard) and radiological studies (US and triphasic CT) performed on study participants.

Detailed conceptional history, general examination, pelvic examination was done using speculum and bimanual pelvic examinations to determine the size, position and mobility of the uterus). After doing all investigations punch biopsy or cervical smear were taken in outpatient clinic without anesthesia.

Squamous cell carcinoma of the cervix typically presents with a continuous spectrum of aberrant epithelium in its preinvasive phase, which is typically split into three categories for the sake of diagnostic and therapeutic guidance. Multiple characteristics involving differentiation, nuclear alterations, and mitotic activity are required for the histological diagnosis of CIN and the differentiation of grades. Subjectivity is involved in CIN grading. It is generally believed that mild CIN will worsen into invasive carcinoma if left untreated, but this does not always appear to be the case. The likelihood of progression increases with the severity of CIN present at diagnosis, both in terms of overall severity and specific subtypes. On the flip side, remission is more probable when CIN is mild when diagnosed. Although accurate data on the frequency of CIN-to-invasive carcinoma development is unavailable, it is safe to assume that a considerable number of cases, if not the vast majority, are at risk of progression [4].

Occult invasive (Stage lb) carcinoma and microinvasive carcinoma are the subtypes of preclinical invasive carcinoma. We still don't have good descriptions for these lesions, but microinvasive carcinoma should be used to describe the largest tumors that can be treated conservatively because they don't have the capacity to spread. Histological classification of invasive squamous cell carcinoma is based on cell type and degree of differentiation; however, whether cell type correlates with prognosis is controversial. A variety of histological types have been identified for adenocarcinomas, which comprise 5-10% of cervical malignancies. The diagnosis of adenocarcinoma in situ is on the rise, frequently alongside squamous CIN. Although its relationship to adenocarcinoma is well-documented, the exact nature of AIS remains unknown[4].

Ethical approval:
After approval of the local Ethics Committee at El Galaa Maternity Teaching Hospital, all women included in the study were briefed about the nature of the study and informed consent was obtained from all of them before inclusion in the study. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 15, developed by SPSS Inc. and available for Windows, was used to conduct all statistical calculations. Mean ± standard deviation (SD), and range were statistical ways to represent quantitative data. Frequencies (number of occurrences) and percentages were used to represent quantitative data. We used a one-way analysis of variance (ANOVA) test. We used the Chi-square (X^2) test to compare the categorical data. When the anticipated frequency was fewer than 5, an exact test was employed instead. Statistical significance was defined as a p-value less than 0.05.
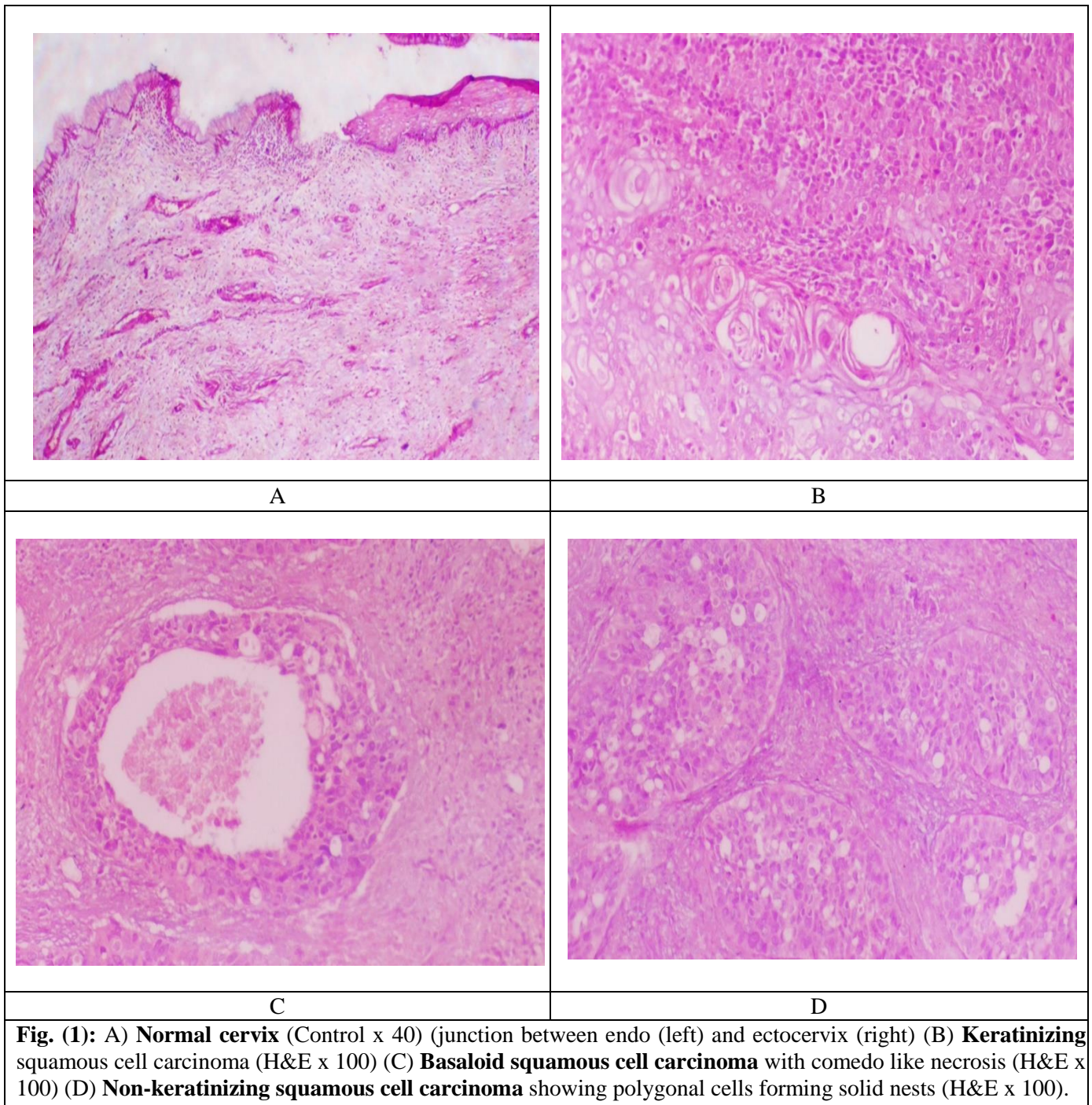
RESULTS

In this prospective analytic study, a total of 100 patients with histologically confirmed pre-invasive and invasive cervical lesions were analyzed (Figure 1). The HPV detection and genotyping were performed using a commercial PCR kit.

The mean age of the patients was 48.96 years, with a standard deviation of 12.21 years. The ages ranged from 29 to 70 years. The study's patient distribution by socioeconomic class shows that 43% belong to class B indicating a majority of middle-class participants. The distribution of parity by delivery type reveals that the highest proportion of participants (22%) had four normal vaginal deliveries (NVD). The findings highlight varying parity patterns, with a notable concentration in the mid-parity range, particularly among those with multiple NVDs (Table 1).

Table (1): Distribution of Patients by Socioeconomic Class and Parity

Socioeconomic class	Frequency	Percent
A	21	21.0%
B	43	43.0%
C	36	36.0%
Parity	Frequency	Percent (%)
1 CS	16	16.0
1 Normal vaginal deliveries (NVD)	9	9.0
2 CS	5	5.0
2 NVD	9	9.0
3 CS	18	18.0
3 NVD	11	11.0
4 CS	2	2.0
4 NVD	22	22.0
5 NVD	2	2.0
6 NVD	1	1.0
9 NVD	1	1.0
Mixed (NVD + CS)	4	4.0

Fig. (1): A) **Normal cervix** (Control x 40) (junction between endo (left) and ectocervix (right) (B) **Keratinizing** squamous cell carcinoma (H&E x 100) (C) **Basaloid squamous cell carcinoma** with comedo like necrosis (H&E x 100) (D) **Non-keratinizing squamous cell carcinoma** showing polygonal cells forming solid nests (H&E x 100).

The distribution of delivery types in the study shows that 53% of patients had normal vaginal deliveries (NVD). This indicates a higher proportion of vaginal deliveries compared to cesarean sections among the participants (Table 2).

Table (2): Frequency of Deliveries by Delivery Type

parity	Frequency	Percent
CS	43	43%
NVD	53	53%
Mixed (NVD + CS)	4	4%

The relation between Age and the type of pathology:

The relationship between age and the type of pathology shows varying age distributions across different histological findings. CIN 3 cases had the highest mean age, while those with mild inflammatory changes had the lowest mean age. Adenocarcinoma and Squamous cell carcinoma cases had intermediate mean ages. Although there were differences in age distribution among pathologies, the p-value for adenocarcinoma suggests that the age difference for this specific pathology was not statistically significant, while the other pathologies suggest a wider age range without specific significance values provided (Table 3).

Table (3): Age Distribution by Pathology

Pathology	Frequency	Age (Mean ± SD)	Minimum	Maximum	P-value
Adenocarcinoma	4	52.50 ± 5.323	46	59	0.074
CIN 1	27	44.67 ± 12.884	29	69	
CIN 2	19	50.37 ± 10.494	29	69	
CIN 3	13	56.69 ± 10.578	39	70	
Endometrial atypia	13	50.77 ± 13.033	30	68	
Mild inflammatory changes	10	43.70 ± 10.594	30	63	
Squamous cell carcinoma	14	49.21 ± 13.291	30	70	

Classification of the pathology type:
The p-value of 0.708 indicates that there was no statistically significant difference in the age distribution between patients with invasive and pre-invasive pathologies (Table 4).

Table (4): Classification of the Pathology Type

Classification	Frequency	Age (Mean ± SD)	Minimum	Maximum	P-value
Invasive	18	49.94 ± 2.809	30	70	0.008
Pre-invasive	82	48.74 ± 1.362	29	70	

HPV Status
Patients with HPV-positive status had a significantly higher mean age compared to HPV-negative patients, with a p-value of <0.0001, indicating a strong association between older age and HPV positivity (Table 5).

Table (5): Age Distribution by HPV Status

HPV Status	Frequency	Age (Mean ± SD)	Minimum	Maximum	P-value
Negative	69	46.10 ± 11.448	29	70	<0.0001
Positive	31	55.32 ± 11.580	30	70	

The relation between HPV status and the type of pathology:
In the crosstabulation of HPV status and pathology types, the findings reveal significant associations between HPV positivity and certain pathologies: **Adenocarcinoma**: All cases (100%) were HPV-positive, indicating a very strong association between adenocarcinoma and HPV infection. **CIN 1**: The majority of CIN 1 cases (92.6%) were HPV-negative, showing a weak association between CIN 1 and HPV. **CIN 2**: A significant portion (78.9%) of CIN 2 cases were HPV-negative, indicating a moderate association with HPV. **CIN 3**: A higher proportion of CIN 3 cases were HPV-negative (69.2%), reflecting a stronger association with HPV as the lesion grade increases.

Endometrial atypia: Most cases (76.9%) were HPV-negative, suggesting a moderate link between HPV and endometrial atypia.
Mild inflammatory changes: A large proportion (80%) of cases were HPV-negative, showing a minimal association.
Squamous cell carcinoma (SCC): The majority (85.7%) of cases were HPV-positive, reflecting a strong correlation between HPV infection and SCC (Table 6).

Table (6): HPV Status and Pathology Crosstabulation

Pathology	HPV status		Total	P-value
	Negative N (% within Pathology)	Positive N (% within Pathology)		
Adenocarcinoma	0 (0.0%)	4 (100%)	4 (4.0%)	<0.0001
CIN 1	25 (92.6%)	2 (7.4%)	27 (27.0%)	
CIN 2	15 (78.9%)	4 (21.1%)	19 (19.0%)	
CIN 3	9 (69.2%)	4 (30.8%)	13 (13.0%)	
Endometrial atypia	10 (76.9%)	3 (23.1%)	13 (13.0%)	
Mild inflammatory changes	8 (80.0%)	2 (20.0%)	10 (10.0%)	
Squamous cell carcinoma	2 (14.3%)	12 (85.7%)	14 (14.0%)	

DISCUSSION

Nearly eight percent of all cancer fatalities in women occur from cervical cancer annually, making it the fourth most common genital cancer in women globally. Although human papillomavirus (HPV) is a known cause of cervical cancer, it is important to note that not all cervical tumors are linked to persistent HPV infection. Additionally, the new classification of female genital tumors by the World Health Organization has further divided cervical squamous and adenocarcinomas into two groups: those associated with HPV and those that are not. This research aims to offer an overview of the association between HPV and cervical cancer based on this new evidence [3].

This study was a prospective study that aimed to investigate the link between HPV and cervical cancer in 100 patients at Galaa Maternity Hospital's Early Cancer Detection Clinic. The study used commercial PCR kits to extract DNA from cervical biopsy, excluding pregnant women, advanced cervical cancer patients, and inoperable cases.

Among diseases caused by HPV, cervical cancer is the most common. At least 99.7 percent of cervical cancer cases are caused by persistent high-risk genital HPV infection. Cervical cancer is among the most frequent malignancies in women globally, with 528,000 new cases reported in 2012. While most HPV infections clear up without treatment, anogenital and oropharyngeal cancer can develop from persistent infections with high-risk or oncogenic strains. Two effective preventative vaccinations against HPV infection are available at this time: viral particles derived from human papillomavirus types 6, 11, 16, and 18; and human papillomavirus types 16 and 18 [6].

In addition to being more sensitive than cytology as a primary screening, HPV testing is therapeutically beneficial in the triage of low-grade cytological abnormalities for the secondary prevention of cervical cancer. Thousands of lives may be saved if both wealthy and developing nations adopted these preventive measures [7].

The range of the patients included in the study varied from 29 to 70 years of age with a mean age of 48.96 years. According to **Kops et al.** [8], who included more than 7000 participants in her Brazilian first national study, no significant difference in HPV prevalence was found across socioeconomic groups. Also, the socioeconomic status in our paper found that the most socioeconomic class that is widely presented in the study was the middle class with 43% more patient distribution than the other classes as shown in table 1, whilst **Kops et al.** [8] found that the distribution of HPV by different socioeconomic groups has no significant difference in the prevalence of HPV, as it was nearly evenly distributed among all social classes according to the Brazilian national study.

The parity number and mode of delivery made a huge difference in the incidence of the pre-invasive and invasive cervical cancer patients as the more the normal vaginal delivery, the more the woman becomes susceptible to it. Also, that comes in coherence with the results published by **Tekalegn et al.** [9] in their systematic review of more than 6000 participants (3227 patients and 3458 controls) incorporated in their meta-analysis with the sum of 12 studies, concluded that it has very strong association with the high parity.

The type of pathology showed wide range of age distribution across different histological findings. CIN 3 cases had the highest mean age that was 56.69, while those with mild inflammatory changes had the lowest mean age 43.70 distribution respectively as shown in table 3. In **Wang et al.** [10] they found that the squamous cell carcinoma subtype has the most dominant subtype.

The invasiveness of cervical cancer differs according to age with a range of 30 to 70 years and that comes in comparison with **Raveinthiranathan et al.** [11] who demonstrated that the age range group varies from 29 to 70 years. Their findings are in line with ours as they noted that ICC incidence rates in British Columbia have decreased since 1971, but have not been able to attain the eradication target set by the World Health Organization. The rate of decrease was different for each age group and accelerated as people got older.

There was a strong association between the increase of age and the positivity of HPV in table 6, patients with HPV-positive status had a significantly higher mean age (55.32 years) compared to HPV-negative patients (46.10 years), and a cross-sectional study of over 3,000 women in Estonia, one of the three countries with the highest disease burden, was carried out by **Pärna et al.** [12] The results showed that the HPV genotype profile varied significantly with age, with a U-shaped pattern of age-specific prevalence of carcinogenic HPV strain. The highest values were found in the age groups of 30-33 and 67-70.

The findings revealed significant associations between HPV positivity and certain pathological types. All cases with adenocarcinoma were HPV positive and that comes with the recommendations and the findings of **Cleveland et al.** [13] which comes adjacent to our results that showed all adenocarcinoma (AC) cases were HPV-positive, confirming the strong association between adenocarcinoma and high-risk HPV strains.

According to pre-invasive lesions, the rate of HPV positivity increased with the severity of the lesion. While only 7.4% of CIN 1 cases tested positive for HPV, this rate rose to 21.1% in CIN 2 and 30.8% in CIN 3 according to table 6. This trend supports the hypothesis that persistent HPV infection drives the progression from mild to more severe pre-cancerous conditions and indicates that higher-grade lesions, such as invasive cancers, are more likely to be associated with high-risk HPV. These findings come in coherence with the results published by **Kalliala et al.** [14] which stated that women with CIN have an increased incidence of not only cervical, but of all HPV-related female genital tract cancers compared with the general population.

The findings revealed a minimal association between endometrial atypia and HPV status, and that comes in coherence with the findings published by **Olesen** *et al.* [15] who concluded that HPV seems to play a limited or no role in the genesis of endometrial cancer based on their systematic review and meta-analysis of 28 publications (29 studies) investigating the prevalence of HPV DNA in tumor tissue from endometrial cancer.

There was a strong correlation between SCC and HPV as many cases were HPV-positive, and these results come in coherence with that of **Gazzaz** *et al.* [16] who conducted a population-based study from Alberta-cancer registry, which found that women with HPV/p16+ OPSCC have a significantly higher risk of CC compared to the general population.

The results demonstrated that there was a strong association between the HPV positive status and the aggressiveness of the pathological type of cervical cancer, because among the 100 patients included in the study the findings showed that 31% were positive for HPV with a markedly higher prevalence in invasive cancers: 85.7% in SCC and 100% in adenocarcinoma as in table 6. Conversely, HPV positivity was much lower in patients with pre-invasive types, and these results come in coherence with the findings published about the importance of human papillomavirus (HPV) infection and the emergence of more aggressive pathological forms of cervical cancer as a result of genetic mutations in this varied population, which was highlighted by **Mohammed** *et al.* [17] who performed a comprehensive analysis of cervical cancer utilizing clinical and molecular datasets from four cohorts of patients across four continents.

CONCLUSION
The study reveals that there is poor correlation between HPV and cancer cervix and recommend further national multicenter studies with wide scale of patients to prove the correlation between cancer cervix and HPV in Egyptian ladies.

Conflict of Interest: None**.**
Financial Disclosures: None.

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