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Original Article

Failure to Demonstrate the Role of High Risk Human Papilloma Virus in Epithelial Ovarian Cancer

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ABSTRACT

Background and Aims: **Ovarian cancer is one of most common causes of cancer related women's mortalities. Human papilloma virus is a known factor concerning cervical cancer but its role in causing ovarian cancer is not yet verified. A few studies also identified HPV DNA in ovarian carcinoma tissues. However, some studies did not detect HPV DNA in ovarian carcinoma tissues. In this article, we investigated the potential role of high risk HPVs in the ovarian epithelial carcinoma.**

Methods: **Fifty archived epithelial ovarian cancer paraffin blocks were collected. Then, 30 non-malignant ovarian blocks used as control. These samples were histopathologically were confirmed by a pathologist and the proper blocks for DNA extraction and PCR were sorted. PCR was conducted deploying highly specific primers for high-risk types of HPV (18 and 16) according to the instructions of manufacturer company.**

Results: **High-risk oncogenic sequences were identified in 4 (5%) of the 80 studied samples. Of the 4 HPV positive cases, there was 1 case with normal tissue, 1 case of mucinous cyst adenocarcinoma, and 2 cases of serous cyst adenocarcinoma**

Conclusion: **Surprisingly, our findings could not support any association between high-risk oncogenic human papilloma virus (18 and 16) and malignant ovarian epithelial cancer. Therefore, that HPV is highly unlikely to play any causal role in the pathogenesis of epithelial ovarian neoplasia.**

Keywords: **Human Papillomavirus 16, Human Papillomavirus 18, Epithelial Ovarian Cancer**

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Introduction

Ovarian cancer is the second most common genital malignancy in women as well as the most common cause of death among women with gynecological cancer. Every year nearly 21,650 new cases of ovarian cancer are diagnosed and 15,520 deaths related to it occur. Epithelial ovarian cancer is the most common type of ovarian malignancy. The average age at diagnosis is 55 years old (1).

Prognosis of patients depends on the early detection and diagnosis. Unfortunately there are no signs of this malignancy until a considerable progress, metastasis has occurred, and since there are no reliable methods for early detection, many patients are in the advanced stages of the disease when they notice their illness. Chances of an individual getting this disease in a lifetime are low and a five-year survival is less than 50% (2).

Epithelial ovarian cancer is created from the malignant transformation of the ovarian epithelial cells. The molecular events that lead to ovarian cancer are not clear, however the incidence of mutation and excessive oncogenes such as HER2 and c-myc and K-ras and tumor suppressor gene p53 are seen in sporadic cancers, and their events are determined (3).

Various hypotheses have been proposed for the pathogenesis of epithelial cancer, including repeated trauma to the epithelium during ovulation, followed by restoration and an opportunity for a mutation to occur in the genes (4). Other hypotheses include the role of various factors such as inflammation, increased stromal activity, and high androgen (5).

The recurrence rate of this neoplasm is high after hip surgery and chemotherapy. This cancer creates many clinical problems among patients and therefore, for better control of this disease, early detection is necessary. To achieve this objective, risk factors and causes need to be recognized.

Various risk factors have been found for ovarian cancer, including low number of pregnancies and infertility (6,7), early menarche and late menopause, family history of ovarian or breast cancer, history of endometrial cancer or non-polyposis colorectal cancer, familial (14) and environmental factors including smoking , obesity (8). Recently, some researchers suggest HPV as an environmental risk factor.

There are more than 100 species of human papillomavirus (HPV), which can contaminate the skin and mucosal tissue and approximately 15% of them are carcinogens.

HPV is involved in 45-95% of anal cancers, 60-65% of vaginal cancers, 40-60% of vulvar cancers, and 70% of the cervical cancers. Researches done in the past 10 years have been similarly conflicting . Many studies about the role of HPV tumorigenesis have been conducted, including prohibition of apoptosis and production of the inhibitor proteins P53 and Retinoblastoma genes that cause the production of cancer cells (9,10).

The PCR method is used for detection of HPV 16 and HPV 18. Recently vaccines have been produced for different high-risk type (11-13). The purpose of this study was to identify the high-risk human papillomavirus in tissues of epithelial ovarian cancer in 50 malignant samples.

Materials and Methods

This study was performed in Mashhad, Northeast of Iran, Mashhad University of Medical Sciences, in the Department of Pathology of Ghaem Hospital in 2011. Fifty paraffin blocks of patient with ovarian cancer along with 30 cases without malignant epithelial cancer including normal and benign epithelial neoplasm at nearly the same age of main group were collected. Other data were acquired out of patient's files.

PCR: In this method, DNA virus was looked for on paraffin blocks. The required primer was provided by Roche-inaolipa Labor company by line probe assay method.

PCR Procedures:

Before the cut, the microtome unit and the tools should be disinfected by HCL. 1.8-10 thick paraffin blocks were cut by the microtome

Deparaffinizing

In order to deparaffinize the samples, xylol was poured on them by sample and were then vortexed (three times and the surface solution was outpoured). During the third time, the sample was centrifuged by 1200 cycles for 5 minutes and again the surface solution was outpoured. Next, absolute and then 70% alcohol were added to the sample (like the previous phase, the sample was vortexed and the surface solution was outpoured) and it was rinsed by distilled water.

DNA extraction

The extraction kit No. 667327 extract Qia Gene Company including 3 buffers was used for extracting DNA:

A mixture of 1000 microliters of buffer 1 (RBCs lyses) and 300 microliters of buffer 2 (WBCs lyses) along with proteinase K were kept at 56^0C for 2 days.

Buffer 3 (for protein sedimentation) was added to the samples kept at 56^0C this time after centrifuging the surface solution was poured into a new tube and 500 microliters of absolute alcohol was added and centrifuged : the surface solution was outpoured 70% alcohol was added and centrifuged ;the solution was outpoured . Finally, the microtube was put under 37^0C so that the sample dried; then distilled water was added.

PCR thermal cycling program:

a. Predenaturation: 94^0C for 5 minutes : 1 cycle

b- Denaturation: 94^0C for 30 seconds: 40cycle

c- Annealing: 60^0C for 30 seconds : 40 cycle

d- Elongation: 72^0C for 30 seconds : 40 cycle

e – Post – elongation: 72^0C for 5 minutes: 1cycle

5- Electrophoresis in 2% Agarose gel; Voltage: IV

DNA bonds were observed under UV. We used SPSS to analyze statistically our data and *P* was assumed less than 0.05.

Results

The average age of 80 cases was 46/25±15/20, ranging from 20 to 80. Out of 50 malignant cases, 43 had serous adenocarcinoma and seven with mucinous adenocarcinoma. In control group, three displayed normal ovary, 12 had cyst adenoma, and 15 women had mucinous cyst adenoma (average age 40.27). PCR analysis revealed the presence of HPV DNA, in only four cases of 80 samples.

Among HPV positive cases, one had normal biopsy, one with muscinous adenocarcinoma, and two with serous cyst adenocarcinoma. Finally, our findings indicated that there was no correlation between HPV infection and ovarian epithelial cancer (Table 1).

Table 1- Characteristics of the patients

Variables		
Age (mean, range)		46.25 (20-80)
Histology		
Malignant	Serous cyst adenocarcinoma	43 (53.75%)
	Mucinous adenocarcinoma	7 (8.75%)
Benign	Non-tumoral	30 (37.5%)

To study any correlativity between HPV test results and type of the lesion, in terms of malignancy and benignity and pathologic specifications, we used Fisher's Exact Test as shown in Table 2. There was no significant difference in any of these cases.

Table 2- Correlativity between HPV test results and pathologic specifications of the cases in the study

		Case		
		Serous Carcinoma	**Mucinous Carcinoma**	**Normal**
HPV test	Negative	41	6	30
	Positive	2	1	1
		$P = 0.118$		

Discussion

HPV is a DNA virus capable of infecting different parts of our body, especially the tissues with squamous epithelium (rectum, anus, vagina, cervix, etc.). This virus leads to cytopathologic and cytoproliferative lesions in infected places similar to giant condyloma, epithelial dysplasy, and cervical carcinoma. Different genes in the virus have been identified with the possibility of inducing oncogenic changes in human cells, including the gene products E6 and E7. It has been proven that by creating interference in intercellular proteins Rb and TP53, it will result in disruption of the cell cycle control and reconstruction of the DNA after mutations. The role of the virus in cervical, anal, and other cancers has been determined. However, up to now, the role of human papillomaviruses (HPVs) in ovarian epithelium (either normal or neoplastic) remains controversial.

For the first time in 1987, Kaufman *et al.* found a virus in ovarian cancer cells, however, a year after they withdrew their results . After that year, many studies took place to detect the virus in ovarian tissue. In 1992, Lai Chan and his partners found the DNA of HPV-16 virus in 50% of ovarian cancers (9/18) and in 4.44% of endometrial cancer (8/18). In this study, PCR was used to obtain the DNA of the virus (14).

Wu *et al.* used immunohistochemistry (IHC) and in situ hybridization (ISH) on 54 malignant samples. In 36-52% of the patients, infection by HPV 16 was found (15).

SM and his associates were successful in finding six cases (1 case of HPV-18 and 5 cases of HPV-16) from 60 patients, suffering from epithelial ovarian cancer (16). From the six positive results, six were in stage 1, and 1 in stage 3 of the disease. In the opinion of the diagnostic experts, there was one case of clear cell adenocarcinoma, one case of mucinous adenocarcinoma and the remaining four cases had intermediate malignant mucinous tumors. Konidaris *et al.* also reached similar conclusions as the previous studies (17).

In Turkey, in 8 (5.8%) of the patients, HPV virus was found (in 6 patients type 16 and in 2 HPV 33). In addition, no correlativity between this virus and ovarian tumor was found (18). Chen *et al.* failed to find the DNA E6, E7 in HPV 16, 18(19). A systematic also reached the conclusion that there was no association between this virus and ovarian cancer (20, 21). In 2006 in Jamestown Community College, no evidence of HPV 16, 18, or 33 was found (22).

Giordano *et al.* did a study for the first time in Italy in 2008. Accordingly, only three (4.22%) out of 71 samples of ovarian cancers showed evidence of HPV. Only one (3.57%) out of 25 patients with serous cancer, 1 (7.69%) out of 13 patients with an intermediate serous tumor and one (12.5%) of eight patients with intermediate mucinous cancer showed evidence of HPV. None of the mucinous cancers had a positive result. Just like our research that found no significance correlation between malignant lesions or pathologic lesions and the presence of the HPV virus, this study also found no relationship between infection by this virus and type of malignancy as well as grade and stage of tumor and age of patient. It seems that the result of this study in comparison to other studies in other parts of the world has

the most similarity with our research in that HPV was found in about 4% of their cases of ovarian cancer and at a rate of 5% in our research.

Since our main objective is assessment of correlation of prevalence of ovarian cancer with that of HPV infection and this work is being done in Iran for the first time, antigens, which were being assessed, were chosen from both low risk and high risk groups, of researcher's own accord, letting further discriminations to be performed in the future.

Pending further studies, PCR will be performed to determine the specific types of virus. In this study, 80 tissue samples were obtained, 50 of which were of ovarian malignant epithelial tumors and 30 of non-tumoral ovarian tissues. From the 80 samples, four HPV infected samples were found; of which one was normal and three were malignant, among them 2 were related to serous adenocarcinoma and 2 of mucinous adenocarcinoma. In this study, no relationships between HPV and different types of pathologic ovarian tumors were found.

Conclusion

There are no similar studies done to show the correlation between age and type of pathologic ovarian lesions. As indicated, it is shown that in cases of malignancy the average age of the patients was higher and this difference was statistically significant and deserved mentioning. Due to the few positive results of HPV and low sample sizes, these results should not be carried out toward etiologic role of HPV upon ovarian cancer and this research needs other samples with higher volumes of data.

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