

RESEARCH ARTICLE

Lack of HPV in Benign and Malignant Epithelial Ovarian Tumors in Iran

Farah Farzaneh¹, Seyed Alireza Nadji², Donya Khosravi^{1*}, Maryam Sadat Hosseini¹, Mohammad Hashemi Bahremani³, Mohammad Chehrazi⁴, Ghazal Bagheri², Afsaneh Sigaroodi², Zahra Haghghatian³

Abstract

Background: Ovarian epithelial tumors one of the most common gynecological neoplasms; we here evaluated the presence of HPV in benign and malignant examples. **Methods:** In this cross-sectional study the records of 105 patients with epithelial ovarian tumors (benign and malignant) referred to Imam Hossein University Hospital from 2012 to 2015 were evaluated along with assessment of the presence of the HPV infection using PCR. **Results:** Among 105 patients, comprising 26 (24.8%) with malignant and 79 (75.2%) with benign lesions, the factors found to impact on malignancy were age at diagnosis, age at first pregnancy, number of pregnancies and hormonal status. However, malignancies was not related to abortion, late menopause, and early menarche. In none of the ovarian tissues (benign and malignant) was HPV DNA found. **Conclusion:** In this study HPV DNA could not be found in any epithelial ovarian tumors (benign and malignant) removed from 105 women; more studies with larger sample size are needed for a definite conclusion.

Keywords: Epithelial ovarian cancer- human papillomavirus-Neoplasms

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Introduction

The malignant ovarian tumor is a prevalent and lethal neoplasm in the genital tract of females (Berrino et al., 2007). Epithelial ovarian cancer (EOC) is the fifth most prevalent women cancer (Ferlay et al., 2004; Berrino et al., 2007). A study in the United Kingdom reported that 6,500 new case and 4400 deaths per year are detected in the women in the UK due to EOC (Modder and Fitzsimons, 2010; Doufekas and Olaitan, 2014). In 75% of patients with the advanced or disseminated disease, a gradual invasion of the tumor to the surrounding organs or to the peritoneal cavity occurs, and the survival rate of patients with the extensive metastatic disease is about 20% (Russell, 2002). The symptoms of the EOC are not specific and some studies reported about 95% of women with EOC have nongynecologic symptoms, so, it is usually unrecognized for a period of time, when the tumor involved the distant areas (Friedman et al., 2005; Lataifeh et al., 2005). The formation of ovarian benign and malignant tumors is due to ovarian structural changes. The most common ovarian tumors originate from the ovary epithelial wall (serous, mucinous), germinal epithelium (teratoma) or connective tissue of the ovary (fibroma, sarcoma) and ovarian endometriosis (endometriomas)

can also changes to tumoral shapes. These tumors can be cystic or solid and the studies indicated that 20-30% of the ovarian tumors are malignant (Goff et al., 2004).

The inducer factors for ovarian cancer are still unknown. Among all oncogenic factors some researches show that viruses might be able to change the normal epithelial tissues to cancer. Papillomavirus is one of the oncogenic viruses which induce overgrowth of epithelial cells. Proteins which produced by HPV, are named as epithelial cell growth inducers (Berrino et al., 2007).

A study in the UK revealed that the rate of HPV infection is about 17.5%. In this study, the infection involvement was low in European people (4%) and people with Asian origin showed the highest rate of infection (31.4%) (Rosa et al., 2013). Moreover, some studies revealed a strong association between high-risk HPV and several cancers in the cervix, anus, vagina and oropharynx (Moscicki et al., 2006; Marur et al., 2010). Regarding EOC, the data are not consistent, some studies confirmed this correlation (Wu et al., 2003; Giordano et al., 2008; Shanmughapriya et al., 2012; Al-Shabanah et al., 2013) but some others emphasized that there is not any direct correlation between the HPV infection and EOC (Runnebaum et al., 1995; Anttila et al., 1999; Quirk et al., 2006; Idahl et al., 2010). Therefore, we conducted

¹Preventative Gynecology Research Center, ²Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), ³Department of Pathology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, ⁴Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran. *For Correspondence: dr.khosravi.d@gmail.com

this cross-sectional study to evaluate the trace of HPV in benign and malignant epithelial ovarian tumors taken from an Iranian female population who were referred to the Imam Hossein hospital.

Materials and Methods

Tissue samples and Genome extraction

In this cross-sectional study, the records of 105 patients with ovarian tumor (benign and malignant epithelial cancers) referred to Imam Hossein Hospital from 2012 to 2015 were recruited. A total of 105 blocks of paraffin-embedded tissue including 79 samples diagnosed as benign tumors and 26 malignant epithelial cancer samples were retrieved from archive of Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Iran.

The 5 µm-thick- tissue sections were cut from paraffin-embedded blocks on a microtome and put into sterile screw-cap tubes. The blocks were sectioned to several small groups at different time over a period of one week. New surgical blade was used for each sample. Also the filter tips were used both during extraction and PCR procedures. It is necessary to completely remove the embedding material before DNA extraction. The xylene and alcohol solutions were used to deparaffinization and rehydration of the tissue sections. For this purpose, the section-contained tubes were heated in dry oven at 55-60°C for 5 minutes, positioned so as to allow drainage of melting paraffin. Then Xylene (1 ml) was added to the tubes and incubated at room temperature for 2 minutes. The tubes were centrifuged at high speed and the Xylene was discarded. This was repeated once more in fresh xylene for 1 minute. For washing and rehydration, 1 ml of 100%, 95% 70% ethanol timely were added to the tubes for 2 minutes. Then the sections were immersed in 1X PBS for 2 minutes. The PBS was discarded from tube by centrifugation and pipetting. Then the sections were lysed by a tissue lysis buffer (containing EDTA 0.05 mM –Tris 0.01mM- SDS 0.2 %; pH 8.0) and Proteinase K (5 mg /ml). The samples were subjected to DNA extraction when the tissues were dissolved. The DNA was extracted from the lysed-tissue sample according to the company's instructions (RTP® DNA/ RNA Virus Mini Kit Procedure; Stratec Molecular, Berlin, Germany). The extracted nucleic acids were stored at -20°C until they underwent PCR. To monitor the contamination, a few negative controls (water samples) were used in each round of extraction.

Nested PCR

To exclude false-negative results, the adequacy of the DNA in each specimen for PCR amplification was determined by detection of a 110- or 268-base pair fragment of the b-globin gene after amplification using the PC03/PC04 and GH20/PC04 primer set, respectively (Saiki et al., 1986). For detection of HPV genome, nested PCR were performed using MY09-MY11 as outer and GP5+-GP6+ as inner primers (Sotlar et al., 2004). In the first round PCR with primers MY09-MY11 was performed in a final volume of 50 µl. Each PCR mixture

contained 4 mM MgCl₂, a 200 µM concentration of each dNTP, 2 U of Thermo Scientific Maxima Hot Start Taq DNA Polymerase and 1 µM of primers MY09 and MY11. Amplification were performed with the following cycling profile: Thermo Scientific Maxima Hot Start Taq DNA Polymerase activation was performed by incubation at 95 °C for 5 min followed by 40 cycles of 1-min denaturation at 94 °C, 1-min annealing at 55 °C and 1-min elongation at 72 °C in a PCR cycler (Gradient TurboCycler, BlueRay BioTech). The last cycle was followed by a final 5-min extension step at 72 °C. In the second round PCR with primers GP5+-GP6+, 5 µl of MY09-MY11 PCR product was used as template.

The nested PCR was performed in a final volume of 50 µl. Each PCR mixture contained 2.5 mM MgCl₂, a 200 µM concentration of each dNTP, 2 U of Thermo Scientific Maxima Hot Start Taq DNA Polymerase and 1 µM of primers GP5+ and GP6+. The cycling conditions were as follows: a 5 min Thermo Scientific Maxima Hot Start Taq DNA Polymerase activation step at 95 °C was followed by 40 cycles of 1-min denaturation at 95 °C, 1.5-min annealing at 40 °C and 1.5-min elongation at 72 °C. The last cycle was followed by a final 5-min extension step at 72 °C. Ten micro liters of the amplification products were analyzed by electrophoresis on 2% agarose gels and ethidium bromide staining.

Statistical Analyses

The data were analyzed using Statistical Package for Social Sciences version 22.0 (SPSS Inc, Chicago, Ill). Categorical data are presented as numbers (%), and continuous data as mean ± SD. We used chi square test and two independent samples t-test to compare categorical variables and continuous variables, respectively. Logistic regression model was built through Hosmer-Lemeshow algorithm to assess the relationship between epithelial ovarian tumors (Malignant versus Benign) as a binary outcome and HPV while adjusting the confounding variables. P<0.05 was considered significant.

Results

In this study 105 patients were evaluated including 26(24.8%) women with malignant and 79 (75.2%) women with benign tumors. The patients' age at diagnosis was significantly higher in patients with malignant tumor than woman benign groups (52.12±14.45 vs. 37.86±11.20, respectively=0.001). The mean age at first pregnancy, in the malignant group, was significantly lower than the benign group, moreover, the number of pregnancy in the malignant group was higher (P=0.001) (Table 1). The difference between two groups regarding abortion, early menstruation, and late menopause was not significant. The main part of the study regarding the HPV evaluation showed that all samples in two groups were negative for HPV DNA.

Table 2 shows the results of univariate logistic regression which age at diagnosis, first pregnancy age, number of pregnancy and hormone status were statistically significant. Since HPV test result was negative for both malignant and benign patients, the odds ratio for that

Table 1. The Difference of Patient's Characteristics Between Two Groups

	Malignant (N=26)	Benign (N=79)	P value
Age at diagnosis	52.12±14.45	37.86±11.20	0.001
First pregnancy age	20.43±1.80	23.14±3.23	0.001
Period without pregnancy (years)	4.90±1.97	3.21±1.57	0.001
abortion	10 (37%)	17 (63%)	0.18
Early menarche	2 (33.3%)	4 (66.7%)	0.63
Late menopause	2 (50%)	2 (50%)	1
HPV	0	0	NG
Hormone status			
menopause	14 (46.7%)	16 (53.3%)	0.004
Anti-gestation drug use	5 (21.7%)	18 (78.3%)	
none	7 (13.7%)	44 (86.3%)	

NG, Not Given

Table 2. The Results of Univariate Logistic Regression for Association Between Epithelial Ovarian Tumors and the HPV Result

Variables	OR (95% CI)	P value
Age at diagnosis	1.091 (1.049-1.134)	<0.0001
First pregnancy age	0.667 (0.529-0.842)	0.001
Number of pregnancy	1.718 (1.279-2.309)	<0.0001
abortion	2.192 (0.780-6.158)	0.136
Early menopause	1.562 (0.267-9.145)	0.621
Late menopause	1.312 (0.162-10.619)	0.799
Hormone status	0.423 (0.242-0.737)	0.002
HPV status	NG	NG

OR, Odd sRatio; CI, Confidence Interval; NG, Not Given

variable was not being able to compute. The outputs of the multivariable model showed just age at diagnosis and first pregnancy age were significant in associated to epithelial ovarian tumors. The odds ratio and relevant confidence intervals along with p values are shown in Table 3.

Discussion

The studies have revealed that the integration of viral genomes into the host genome and persistent infection with high-risk HPV are the most important causes of the malignant and premalignant cancers of the lower genital tract in the females (Lataifeh et al., 2005). Also, some others confirmed that the high-risk type of HPV infection may be related to the development of proliferative epithelial cells to the carcinoma (Goff et al., 2007). In current practice, we did not detect the HPV genome in benign or malignant ovarian tumors taken from 105 women with ovarian masses. Our practice was in agreement with a review by (Anttila et al., 1999). In 1999 that demonstrated no correlation between HPV and ovarian tumor (Anttila et al., 1999). Moreover, a study by (Alavi et al., 2012) in Iran on 50 patients with EOC did not prove any association between HPV 18 and 16 with ovarian cancer. Harmoniously, (Quirk et al., 2006) supported these results and denied a direct correlation between HPV 16,18,33 and ovarian tumors (malignant,

Table 3. The Results of Multivariate Logistic Regression Through Hosmer-Lemeshow Algorithm for Association Between Epithelial Ovarian Tumors and the HPV Result

Variables	OR (95% CI)	P value
Age at diagnosis	1.073 (1.024-1.125)	0.003
First pregnancy age	0.735 (0.595-908)	0.004

OR, Odd sRatio; CI, Confidence Interval

benign) in 20 patients. An experience by (Idahl et al., 2010) evaluated the possible correlation between several types of viruses including HPV and OEC, but could not find any association between benign epithelial ovarian tumors and viruses. These outcomes were repeated by (Ingerslev et al., 2016), they conducted a relatively large sample size study, and found only one patient was positive for HPV 18 among 191 patients with EOC and all patients were negative for HPV 16. However as opposed to our practice and the mentioned trials above, (Bilyk et al., 2014) in a small study on 20 patients with EOC and 10 controls, signified that the HPV 16,18 were correlated to EOC. These findings were repeated by (Chiang et al., 2015) on 33 patients, they demonstrated HPV is an important factor in the transformation of mature cystic teratoma (MCT) to the squamous cell carcinoma (SCC). The reasons for such discrepancy between the studies are not clear, however, different methods of the studies may be the main reason for this discrepancy, moreover, these studies, including current study, suffer from small sample size and their results should be considered with caution.

Regarding the demographic factors, in current practice the patients with malignant ovarian tumor were older at diagnosis time, lower age at the first pregnancy and the higher numbers of pregnancy that was in line with (Wasim et al., 2009) study in Pakistan. Moreover, several studies indicated that older age is a predictor of the malignant ovarian tumor (Rafiq et al., 2005; Sultana et al., 2005; Olsen et al., 2006; Shaikh et al., 2007; Yoshida et al., 2016).

Some limitations should be addressed in this experience. This was a retrospective study which the sample size was reduced due to unforeseen circumstances such as losing many laboratory sample blocks before

carrying out the study. A prospective study with larger sample size is performing to validate findings reported here.

In this study the HPV DNA could not be finding in all women with epithelial ovarian tumors (benign and malignant) removed from 105 women; more studies in larger sample size are needed to have the better conclusion.

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