Research Article

Immunological marker of human papillomavirus type 6 infection in epithelial ovarian tumor before and after paclitaxel drug treatment in Al-Najaf Governorate, Iraq

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ABSTRACT

Background: Ovarian cancer is the most common type of female cancer. The etiology of ovarian cancer is still unclear. Infection with different microbes may induce carcinogenesis. Chronic inflammation caused by infection may play a role in carcinogenesis, progression, and metastasis. **Materials and Methods:** A total of 21 patients with different types of malignant epithelial tumor included in this study. Serum samples from patients were collected from patients diagnosed as epithelial ovarian tumor. All the patients had provided their medical history and had undergone clinical and ultrasound examination. The average age of this group was 45.8 years and the range was 10–80 years. The blood sample was dispensed in a plain tube and left for 20 min at room temperature (20–25°C) for clotting. Then, it was centrifuged to collect serum. The serum was divided into aliquots (0.3 ml) and stored in the freezer (−20°C) until use for the measurement of interleukin-6 and tumor necrosis factor (TNF). **Results:** The study showed that ovarian cancer usually occurred in patients aged more than 50 years 15 (71%) ovarian cancer patients lived in urban areas 12 (57%), while in rural areas 9 (42%). High percentage of patient with ovarian cancer were in stage III. Six cases of 9 (Stage III) malignant surface epithelial tumors were human papillomavirus (HPV) positive which is statistically significant. A total of ovarian carcinomas were categorized as TNF-α negative $5.7 ± 1.6$ and $28.3 ± 3.1$ as TNF-α positive. Both parameters showed significant differences. **Conclusion:** This study may raise the suspicion of possible role of HPV6 in pathogenesis of ovarian tumors and the benefit of immunological markers in progression of the disease which need further works to prove this role.

KEY WORDS: Human papillomavirus-6, Interleukin-6, Ovarian tumor, Paclitaxel, Tumor necrosis factor-alpha

INTRODUCTION

Cancer is a malignant form of disease resulting from uncontrolled cell division. The resulting proliferating cells have the ability to invade other tissues through direct cell migration or through the vascular system.[1,2] In general terms, carcinogenesis represents a complex, multistep process. During the past 30 years, it has become exceedingly apparent that several viruses play significant roles in the multistage development of human neoplasms; in fact, approximately 15–20% of cancers are associated with viral infections. Oncogenic viruses can contribute to different steps of the carcinogenic process, and the association of a virus with a given cancer can be anywhere from 15% to 100%.[3]

Over 90% of ovarian cancer is described as epithelial in origin. Epithelial ovarian cancer is the most lethal cancer of all the gynecological cancers in females. It is characterized by late and unspecific onset of symptoms and thus the presence of disseminated disease at the time of diagnosis.[4] The World Health Organization classifies ovarian tumors according to their most probable cell of origin and histomorphological features.[5] Recently, the roles of infections during carcinogenesis in several types of oncological diseases were comprehensively summarized.[6,7] Cancer arises from one single cell. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a precancerous lesion to malignant tumors.[8] The human papillomavirus (HPV) has been described as an "equal opportunity" pathogen as much a part of the human condition as sexuality itself, up to 80% of sexually active people will acquire an HPV infection of some type at 1 time in their life. Inflammation has been shown to play

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many roles in ovarian cancer tumor growth, with the pro-inflammatory cytokine interleukin-6 (IL-6) having been established as a key immunoregulatory cytokine.[9]

MATERIALS AND METHODS

Twenty-one patients with different types of malignant epithelial tumor included in this study after taking informed consent to participate in the study. Serum samples from patients were collected from patients diagnosed as epithelial ovarian tumor from general and private hospital in Al-Najaf. All the patients had provided their medical history and had undergone clinical and ultrasound examination. The average age of this group was 45.8 years and the range was 10–80 years. The blood sample was dispensed in a plain tube and left for 20 min at room temperature (20–25°C) for clotting. Then, it was centrifuged at 3000 rpm for 10 min to collect serum. The serum was divided into aliquots (0.3 ml) and stored in the freezer (−20°C) until use. Enzyme linked immunosorbent assay for IL-6 and tumor necrosis factor α (TNF α) detection. Two enzyme-linked immunosorbent assays (ELISA) sandwich type were used, one for IL-6 detection and the second for TNF-α detection. The instructions of the procedure for each kit were followed, as it was recommended by the manufacturer.

RESULTS

The study showed that ovarian cancer usually occurred in patients aged more than 50 years 15 (71%) and 41–50 years 4 (19%), while the percentage

was 1 (4.7%) in the age groups of 20–30 years and 31–40 years. Ovarian cancer patients lived in urban areas 12 (57%), while in rural areas 9 (42%). Ovarian cancer was more common among smoker women 13 (61%), compared with non-smoker women 8 (38%). Patient with history of other tumors 4 (19%), while the percentage of patients have no history of other tumors 17 (80%) [Table 1]. High percentage of patient with ovarian cancer were in stage III. Compared with non-smoker women 8 (38%). Patient with history of other tumors 4 (19%), while the percentage of patients have no history of other tumors 17 (80%). High percentage of patient with ovarian cancer in stage III [Table 2].

Six cases of 9 (Stage III) malignant surface epithelial tumors were HPV positive which is statistically significant. Two cases of 6 (Stage IV) malignant surface epithelial tumors were HPV positive. While showed single case out of 2 (Stage I), and single case out 4 (Stage II) malignant surface epithelial tumors were positive HPV expression.

The distribution of TNF-α and IL-6 expression in ovarian tumors in HPV-positive and negative cases is summarized in Tables 3 and 4. A total of ovarian carcinomas were categorized as TNF-α negative 5.7 ± 1.6 and 28.3 ± 3.1 as TNF-α positive. Both parameters showed significant differences $P < 0.005$, and similarly, IL-6 is significantly higher in ovarian tumor which is positive for HPV6.

DISCUSSION

Ovarian cancer is a major cause of morbidity and mortality among the gynecological malignances, ovarian carcinoma is difficult to diagnose and it is usually discovered only in its advanced stages, also may relate with chronic breast bacterial infection and abortion.[10,11] This study revealed that most patients with ovarian cancer were in the age group above 50 years. Ovarian cancer distribution by age in Misan, Iraq, in 2017, was 30% of patients aged 60–70 years, 22% of patients aged 40–50 years, and 16% of patients aged 50–60 years.[12] In the United Kingdom, it was 70.6% of women aged 75–79 years.[13] While in Egypt and Jordan, most patients were below the age of 50 years.[14]

Regarding the place of residence, the majority of patients were from urban areas (about 57%) and 9 (42%) patients came from rural areas. In Iraq,

Table 1: Demographics and history of patients

Status	Characteristic	Number of patients	Percentage
Age/years	20–30	1	4.76
	31–40	1	4.76
	41–50	4	19.04
	>50	15	71.42*
Smoking	Smoker	13	61.9
	Non-smoker	8	38.1
Residence	Rural	9	42.86
	Urban	12	57.14
History of other tumors	+ve	4	19.05
	−ve	17	80.95
Stage of tumor	I	2	9.52
	II	4	19.05
	III	9	42.86*
	IV	6	28.57

*$P<0.005$

Table 2: Association of HPV6 with epithelial ovarian tumor according to the stage of tumor

Status	Stage I		Stage II		Stage III		Stage IV		Total	
	n	%	n	%	n	%	n	%	n	%
HPV6 +ve	1	50.0	1	25.0	6	66.66*	2	33.33	10	47.61
HPV6 −ve	1	50.0	3	75.0	3	33.33	4	66.66	11	52.38
Total	2	9.5	4	19.04	9	42.85	6	28.57	21	100

*$P<0.005$, −ve (negative), +ve (positive), HPV: Human papillomavirus

Table 3: Relationship between interleukin 6 and tumor necrosis factor with HPV6

Immunological marker	HPV6 +ve	HPV6 −ve
IL 6	22.1±2.3	7.4±1.2
TNF-α	28.3±3.1	5.7±1.6

$P<0.005$, −ve (negative), +ve (positive), HPV6: Human papillomavirus

Table 4: Immunological markers after treatment with paclitaxel drug

Immunological marker	HPV6 +ve	HPV6 −ve
IL 6	19.2±1.8	6.9±1.4
TNF-α	27.2.1±2.1	5.2±1.3

$P<0.005$, −ve (negative), +ve (positive), IL: Interleukin 6, TNF-α: Tumor necrosis factor-α

patients typically lived in urban areas: 1.5 times more frequently than in rural areas;[14] in Iran, the rates of female reproductive cancers were significantly higher among residents of cities than villages.[15]

Our results showed that ovarian cancer patients have no history of tumor more than whom have a history of other tumors as familial hereditary ovarian cancer. While the family history is a risk factor in the predisposition to ovarian cancer in first-degree relatives (mother, daughter, sister).

The largest percentage of our patients presented in a late stage: Stage III was noted in 9 (42%) and Stage IV in 6 (28%) of patients. For all patients in this study, the typical presentation was late; Stages III and IV were seen in 70% of the cases. Similar results, with 78% of Stages III or IV cases, have also been reported by Alghamdi, 2014.[14] Another study done at 2014 found that Stages III and IV accounted for only 56.2% of their cases.[16,17] In Alexandria, typical presentation was late; Stage III was the initial presentation in 48 patients (41.3%) and Stage IV in 44 patients (37.9%), which add up to the total of 79.2% of cases.[18] Most of the patients in Egypt (84.3%) presented with advanced Stages III and IV, whereas only 15.7% of patients presented with Stages I and II. While in England, the percentage of Stage III was 31.1% and Stage IV was 18.1%, whereas Stage I was noted in 30.6% and Stage II in 5% of cases.[11] Level of IL-6 is high in HPV positive compared to negative cases as shown in Table 3. This result is consistence with Ren *et al*., 2013, who found that fibroblast senescence promotes cervical cancer development through high-risk HPV E6-activated IL-6/STAT3 signaling in tumor microenvironment.[19-21] In the current study, TNF is high when compare HPV-positive cases to negative cases and this may be explained as the potential roles of TNF-α as an important and pleiotropic cytokine that plays a critical role in immune regulation through prominent anti-inflammatory and immunoregulatory activities, its genetic variants may affect the host immune system and HPV infection and, subsequently,

the HPV-associated cancer development.[22] This in agreement with Gaiotti *et al*. who found that TNF-alpha inhibits the growth of normal cervical keratinocytes but stimulates proliferation of human papillomavirus (HPV)-immortalized and cervical carcinoma-derived cell lines when mitogens such as epidermal growth factor or serum are depleted.[23]

CONCLUSION

This study may raise the suspicion of possible role of HPV6 in pathogenesis of ovarian tumors and the benefit of immunological markers in progression of the disease which need further works to prove this role and studies also required to assess other types of HPV.

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