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Original Article

Effects of endometrial cancer complicated with HPV infection on HE4 and Th1/Th2 cytokines

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Abstract: Objective: To investigate the effect of human papillomavirus (HPV) infection on human epididymal protein 4 (HE4) and Th1/Th2 cytokines in patients with endometrial cancer (EC). Methods: The clinical data of 121 patients with EC were retrospectively analyzed, among which patients with HPV infection were set as the observation group (n=71), while those without HPV infection were set as the control group (n=50). The serum levels of HE4, Th1 cytokines (IL-2, IL-25, IL-27, IFN-γ), Th2 cytokines (IL-4, IL-6, IL-10) and the proportion/ratio of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$) were compared between the control group and observation group. Results: The serum concentrations of IL-2, IL-25, IL-27 and IFN-γ in the observation group were clearly lower than those in the control group, while the concentrations of serum HE4, IL-4, IL-6 and IL-10 in the observation group were significantly higher than those in the control group. Compared with the control group, the proportion of $CD3^+$ and $CD4^+$ in the peripheral blood of the observation group was lower, the proportion of $CD8^+$ was higher, and the ratio of $CD4^+/CD8^+$ was lower ($P<0.01$ or $P<0.001$). Conclusion: In EC patients complicated with HPV infection, the HE4 level was abnormally elevated, the Th1/Th2 drift to Th2 was more severe, and the degree of inhibition of cellular immune function was more obvious.

Keywords: Human papillomavirus, endometrial cancer, human epididymal protein 4, Th1 cytokines, Th2 cytokines

Introduction

Human papillomavirus (HPV) infection is related to the occurrence and development of tumors, and its pathogenic scope is wide, covering the body's epidermis, breast, and oral cavity [1-3]. Studies have shown that chronic and recurrent infections of high-risk HPV is closely related to the occurrence of gynecological malignant tumors, such as endometrial cancer and cervical cancer [4, 5]. Endometrial cancer (EC), which often occurs in perimenopausal or postmenopausal women, is a common malignant tumor of the reproductive system. As its pathogenesis is very complex, coupled with the relative lack of effective screening methods in the early stage, most patients are only identified in the middle and advanced stages when diagnosed, depriving them of the best treatment timing. Therefore, early and effective gynecological screening for EC, early diagnosis and timely treatment are of great importance to reduce the incidence of EC and improve the prognosis of patients.

As the key factors of signal transmission between cancer cells and the microenvironment, cytokines are mainly involved in mediating the mutual transformation of inflammatory-tumor cells, which can promote the proliferation of tumor cells in the microenvironment of EC. The role of Th1 and Th2, two subtypes of helper T cells (Th), in the immune response of the body is a research hotspot in recent years. Th1 cells can assist cytotoxic T cells to differentiate into effector killer T cells, enhance their killing effect on target cells, and mainly mediating cellular immune response; while Th2 cells assist B cells to proliferate and differentiate into plasma cells, which are mainly involved in the humoral immune response [6]. The relationship between Th1/Th2 drift and the occurrence, development and prognosis of diseases, especially malignant tumors, is a top priority of study [7].

The relationship between human epididymal protein 4 (HE4) and gynecological tumors has well been established, and HE4 was considered as a candidate tumor marker for gynecological tumors such as cervical cancer and ovarian cancer [8]. However, there are few studies on the relationship between HE4 and gynecological tumors combined with HPV, and how HE4 changes in gynecological tumor patients with HPV infection remains poorly understood. This study retrospectively analyzed the changes of HE4 and Th1/Th2 cytokines in EC patients complicated with HPV infection, in order to provide references for the immunodiagnosis and immunotherapy of EC.

Materials and methods

General information

The clinical data of 121 patients with EC treated in The First Affiliated Hospital of Xi'an Jiaotong University from October 2017 to August 2019 were analyzed retrospectively. Among them, EC patients without HPV infection were set as the control group (n=50), while those complicated with HPV infection were set as the observation group (n=71). The general information of patients in the two groups is shown in **Table 1**. This study was reviewed and approved by the Medical Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University. Inclusion criteria: (1) Patients between 28-70 years old; (2) Patients with primary EC; (3) Patients diagnosed by histopathology; (4) Patients receiving antitumor treatment for the first time; (5) Patients whose HPV infection type was mainly high-risk HPV16, HPV18, HPV52 or HPV58; (6) Patients who had not received immunosuppressive therapy within 2 months before enrollment; (7) Patients who cooperated to complete various examinations. Exclusion criteria: (1) Patients with other malignant tumors; (2) Patients with liver and renal dysfunction; (3) Patients with autoimmune diseases; (4) Patients that halfway withdrew or had incomplete data; (5) Patients with expected survival time of less than 3 months; (6) Patients who were pregnant or lactating. This study was conducted with the approval of the Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University and with the consent of the patient's family.

Methods

Determination of serum HE4 content: 4 mL of fasting venous blood was collected from the patient, centrifuged at 2,500 r/min for 10 min (the centrifuge was purchased from Jidi Instrument Co., Ltd., Guangzhou, China), and the supernatant was separated and stored. Electrochemiluminescence method [9] (the kit was purchased from Tellgen Corporation, Shanghai, China) was employed to detect serum HE4 levels.

Detection of Th1/Th2 cytokine [10]: Serum Th1/Th2 cytokine levels were detected by enzyme-linked immunosorbent assay (ELISA) (the ELISA kit was purchased from Sigma-Aldrich, Germany). Th1 cytokines included interleukin-2 (IL-2), IL-25, IL-27 and interferon-γ (IFN-γ); and Th2 cytokines included secretion of IL-4, IL-6 and IL-10.

Measurement of the proportion of T lymphocyte subsets [11]: 100 μL of pre-extracted anticoagulant blood was successively pipetted and put into three tubes, where PreCP labeled mouse anti-human CD3, FITC labeled mouse anti-human CD4 and PE labeled mouse anti-human CD8 (Becton, Dickinson and Company, USA) with 10 μL of each were added respectively for immunolabeling reactions. Meanwhile, the Isotype controls, that is, PreCP-labeled mouse IgG, FITC-labeled mouse IgG and PE-labeled mouse IgG (Becton, Dickinson and Company, USA), were added, mixed and incubated at room temperature for 30 min away from light. Then approximately 4 mL red blood cell lysate was added, and incubated at room temperature for 30 min before centrifugation. After that, they were rinsed twice with PBS and added to 2 mL prepared 4% paraformaldehyde solution (solvent PBS) (the paraformaldehyde was purchased from Sigma-Aldrich, USA, Art. No.: 58127-5G). The proportion of T lymphocyte subsets in the peripheral blood, including $CD3^+$, $CD4^+$ and $CD8^+$, was detected by flow cytometry (Beckman coulter, USA, Model No: DxFLEX), and the ratio of $CD4^+/CD8^+$ was calculated.

Statistical processing

The statistical analysis was performed by SPSS 19.0. The measurement data were expressed as ($\bar{x} \pm sd$), the independent t-test was used for

Table 1. General clinical data

Group	Observation group (n=71)	Control group (n=50)	t/χ^2	P
Age (year)	48.9±6.4	49.2±5.6	0.267	0.79
BMI (kg/m^2)	22.17±2.85	22.57±3.10	0.733	0.465
Pathological type			0.498	0.48
Adenocarcinoma	66	48		
Non-adenocarcinoma	5	2		
Clinical staging			0.776	0.678
Stage I	21	12		
Stage II	28	19		
Stage III	22	19		
Lymph node metastasis			1.064	0.302
Yes	38	22		
No	33	28		
Myometrial infiltration depth			0.26	0.61
≤1/2	35	27		
>1/2	36	23		

Note: BMI: body mass index.

Table 2. Comparison of HE4 levels ($\bar{x}$ ± sd)

Group	HE4 (pmol/L)
Observation group (n=71)	193.47±24.30
Control group (n=50)	136.44±17.89
t/χ^2	14.112
P	<0.001

Note: HE4: human epididymal protein 4.

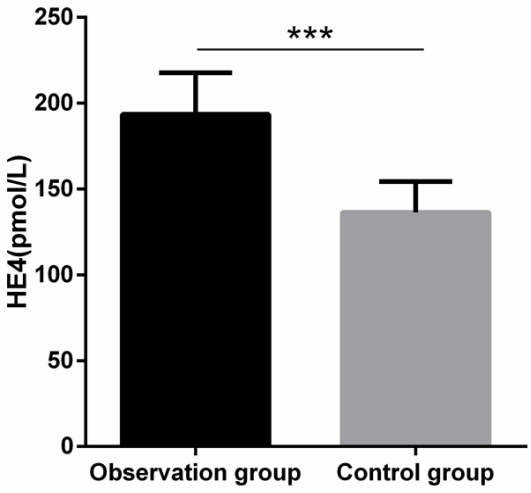

Figure 1. Comparison of HE4 levels between the two groups. HE4: human epididymal protein 4. Compared with control group, ***P<0.001.

the comparison between the two groups. While the counting data were described in the form of (%), and the inter-group comparison was conducted by the χ^2 test. P<0.05 indicated a statistically significant difference.

Results

Comparison of general clinical data

There were no significant differences between the two groups in age, BMI, pathological type (adenocarcinoma and non-adenocarcinoma, and adenocarcinoma with squamous metaplasia, clear cell carcinoma and serous papillary adenocarcinoma were all classified as non-adenocarcinoma in this study), clinical staging (stage I, II, III), lymph node metastasis and myometrial infiltration depth (P>0.05) (**Table 1**).

Comparison of HE4 levels

The serum HE4 level in the observation group (193.47±24.30) pmol/L was significantly higher than that in the control group (136.44±17.89) pmol/L), (P<0.001, **Table 2** and **Figure 1**).

Comparison of Th1 cytokines

Compared with the control group, the serum concentrations of IL-2, IL-25, IL-27 and IFN-γ in the observation group were significantly reduced (all P<0.05, **Table 3**).

Comparison of Th2 cytokines

The serum concentrations of IL-4, IL-6 and IL-10 in the control group were significantly reduced

Table 3. Comparison of Th1 cytokines ($\bar{x}$ ± sd)

Group	IL-2 (pg/mL)	IL-25 (pg/mL)	IL-27 (pg/mL)	IFN-γ (pg/mL)
Observation group (n=71)	28.55±5.49	14.95±2.29	19.83±3.10	20.86±4.29
Control group (n=50)	30.74±4.38	16.02±2.90	21.10±3.28	23.02±5.50
t/χ^2	2.343	2.265	2.166	2.425
P	0.021	0.025	0.032	0.017

Note: Th1: helper T cell 1; IL-2: interleukin-2; IL-25: interleukin-25; IL-27: interleukin-27; IFN-γ: interferon-γ.

Table 4. Comparison of Th2 cytokines ($\bar{x}$ ± sd)

Group	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Observation group (n=71)	64.03±5.11	37.86±4.69	38.77±4.43
Control group (n=50)	61.47±6.24	34.30±5.10	36.50±4.20
t/χ^2	2.475	3.965	2.835
P	0.015	<0.001	0.005

Note: Th2: helper T cell 2; IL-4: interleukin-4; IL-6: interleukin-6; IL-10: interleukin-10.

Table 5. Comparison of T cell subsets in peripheral blood ($\bar{x}$ ± sd)

Group	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Observation group (n=71)	50.48±5.55	22.28±4.18	32.10±3.98	0.67±0.16
Control group (n=50)	53.29±5.79	24.86±4.27	29.97±4.57	0.82±0.13
t/χ^2	2.694	3.314	2.726	5.476
P	0.008	0.001	0.007	<0.001

compared with the observation group (all P< 0.05, **Table 4**).

Comparison of T cell subsets in peripheral blood

Compared with the control group, the proportion of $CD3^+$ and $CD4^+$ in peripheral blood of patients in the observation group was lower, the proportion of $CD8^+$ was higher, and the ratio of $CD4^+/CD8^+$ was lower (all P<0.01, **Table 5**).

Discussion

HPV is a recognized tumor-related virus. After infecting the body, HPV can activate the cellular immune response and the humoral immune response system in the body; among which the former can clear the foreign HPV, while the latter can prevent the re-invasion of this subtype of HPV into the body [12]. Many studies have pointed out that HPV infection is related to the incidence of cervical cancer and EC to some extent [13, 14], among which high-risk HPVI6 and HPV18 infection are the primary cause of cervical cancer, while HPV52 and HPV58 infection are the most likely to cause EC [15]. In addition, HPV16 also has a strong ability to infiltrate and adhere to endometrial cells, and its positive expression can promote a large amount of endometrial cell proliferation and inhibit cell apoptosis. Therefore, this study mainly selected patients with high-risk HPV16, HPV18, HPV52 and HPV58 infections as the key research participants.

Th1 and Th2 cells restrict each other, and are in a dynamic equilibrium state under normal circumstances, but once the balance is broken, it will trigger autoimmune diseases, infectious diseases and tumors [16, 17]. If Th1/Th2 drifts to predominant Th1, IL-2 and TNF-β will have a synergistic effect, resulting in the breakage of DNA in tumor cells, which in turn leads to apoptosis of cancer cells. While when Th1/Th2 drifts to predominant Th2, IL-10 can promote the overexpression of Th2 cytokines while inhibiting the synthesis and secretion of Th1 cytokines, which hinders the immune function of the body. After excluding the influence of pathological type, clinical staging and lymph node metastasis of EC in the two groups, the serum concentrations of IL-2, IL-25, IL-27 and IFN-γ in the observation group were found to be significantly lower compared with the control group, while the serum concentrations of IL-4, IL-6 and

IL-10 were significantly increased over those in the control group, indicating that Th1/Th2 drifting towards Th2 was more serious in patients with HPV infection. What's more, in the present study, the proportion of $CD3^+$, $CD4^+$ and the ratio of $CD4^+/CD8^+$ in peripheral blood were lower in the observation group, with a higher proportion of $CD8^+$, suggesting that the cellular immune function of patients with EC complicated with HPV infection was significantly inhibited. Cicchini L et al. also revealed that HPV reduced the immune function by down-regulating CXCL14, and the decline of immune function was one of the risk factors of persistent HPV infection, which accorded with our study [18].

HE4 belongs to the family of lactic acid protein domain proteins and is the core epitope protein expressed in human epididymal epithelial cells. It is expressed in female endometrial glands, intracervical glands and oviduct epithelium, but its expression level is exceedingly low in benign tumors. Angioli R et al. suggested that serum HE4 level could serve as an early diagnostic indicator for EC [19]. Presl J et al. believed that HE4 could be used as a marker to predict the clinical stage of EC, intrauterine tumor diffusion and muscle infiltration [20]. Similar results were obtained in the study of Prueksaritand N et al. [21]. According to L Minář et al., serum HE4 levels in patients with EC were significantly increased over those with benign endometrial tumors, and HE4 was considered to be a predictive indicator of EC risk and a reference indicator for treatment plans [22]. Capriglione S et al. also confirmed that serum HE4 level was positively correlated with tumor stage in patients with EC [23], pointing out that early detection of serum HE4 level was conducive to early detection of EC, and believed that preoperative screening of serum HE4 level could provide a reference for the prognosis of patients. Back to our study, higher HE4 levels were detected in both groups, and the HE4 level in the control group was lower than in the observation group, indicating that HE4 has important clinical significance for the diagnosis of EC, and may also be related to the immune function of patients.

However, this study only compared the changes of serum HE4, Th1/Th2 and peripheral blood T cell subsets in EC patients without HPV infection to those with HPV infection, but did not study the changes of the above factors in EC with HPV infection after anti-HPV treatment. A comparative study is still needed in our follow-up research, so as to more clearly illustrate the role and significance of HE4, Th1/Th2 in the occurrence and development of EC complicated with HPV infection.

In summary, the HE4 level of EC patients complicated with HPV infection is abnormally increased, the Th1/Th2 ratio drift to Th2 is more serious, and the degree of inhibition of cellular immune function is more clear.

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Disclosure of conflict of interest

None.

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