

Original Article

Cytokines profiles in cervical mucosa in patients with cervical high-risk human papillomavirus infectionDe-Lai Long^{1,2}, Hua-Lin Song³, Peng-Peng Qu²¹ *Clinical College of Central Gynecology and Obstetrics, Tianjin Medical University, Tianjin, China*² *Department of Gynecological Oncology, Tianjin Central Hospital of Gynecology and Obstetrics, Tianjin, China*³ *Department of Gynecology and Obstetrics, Fuyang People's Hospital, Anhui Province, China***Abstract**

Introduction: The purpose of this study was to detect the expression of local cytokines in cervical mucosa between patients with transient and persistent HR-HPV infection with or without CIN.

Methodology: A total of 150 patients who were diagnosed as HR-HPV infection in Tianjin Central Hospital of Obstetrics and Gynecology from January 2016 to December 2016 were included in this study. The expression levels of 9 cytokines in 150 patients with HR-HPV infection, including interleukin (IL)-1 β , IL-4, IL-6, IL-8, IL-10, IL-12, IL-12p70, IL-21, interferon (IFN)- γ and tumor necrosis factor (TNF)- α , were simultaneously measured by using a multiplex immunoassay. Moreover, HR-HPV genotype was performed by using pyrosequencing. The association between cytokines and HPV genotype was also investigated.

Results: There was a statistically significant difference in IL-1 β level between patients with HPV transient infection and HPV persistent infection ($p = 0.041$). There were statistically significant differences in the levels of IL-1 β , IL-10, IL-21 and TNF- α between patients with low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) ($p = 0.011$, $p = 0.008$, $p = 0.046$ and $p = 0.019$, respectively).

Conclusions: Pro-inflammatory cytokines, IL-1 β and TNF- α , and Th2 type cytokines, IL-10 and IL-21, became stronger in cervical mucosa with the progression of CIN. IL-1 β may be advantageous for HR-HPV persistent infection.

Key words: Cytokine; HR-HPV; immune environment; persistent HPV infection; cervical mucosa.

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Introduction

Persistent high-risk human papillomavirus infection (HR-HPV) infection is an established causal factor for cervical intraepithelial neoplasia (CIN) and cervical cancer [1,2]. There is an annual incidence of 528,000 cases and mortality of 266,000 cases worldwide [3]. There are approximately 5% - 10% women with HR-HPV infection who fail to clear the virus and develop to CIN [4]. Nearly 30% of low grade squamous intraepithelial lesion (LSIL) persists and 10% of LSIL progresses to high grade squamous intraepithelial lesion (HSIL). However, the specific mechanism of this phenomenon is unclear. It is postulated that HPV infection requires a tolerogenic local immune environment, involving avoidance or repression of both the innate and adaptive immune response [5].

The innate immune system contains epithelial barrier, antigen presenting cells (APCs), macrophages and natural killer (NK) cells. Generally speaking, APCs process pathogens and provide pathogen related

molecular patterns (PAMPs) to the pattern recognition receptors (PRR) located on immune cells. It has been reported that PAMPs can combine with PRRs to activate a cascade of NF- κ B pathway, which has the ability to enter nucleus and induces the expression of different genes. Those genes include cytokines, chemokines and others that involve in defending pathogenic invasion [6,7]. However, the function of APCs to recognize L1 protein of HR-HPV is blocked by HPV-infected keratinocytes, which contributes to HPV escape from host immunity and persistent infection.

The dose of response mode is an important part of adaptive immune system. It includes cell-mediated type 1 reactions, characterized by interleukin (IL) - 12 produced by macrophages and dendritic cells, interferon (IFN) - γ produced by NK cells and activated T cells, and proinflammatory cytokines such as IL-6, IL-8, macrophage inflammatory protein (MIP) - 1 α and tumor necrosis factor (TNF). Oncoprotein of HPV

inhibits IFN secretion and promotes production of transforming growth factor (TGF)- β and IL-10, which belongs to cytokines produced by type-2 response and acts as immunosuppressive cytokines [8]. HPV has established immune tolerance and special immune microenvironment for the infection and progression of CIN.

Cytokine is an important part of immune microenvironment after HPV infection, and it may influence the outcome of HPV infection. Cytokines may be divided into three categories [9]: (1) Pro-inflammatory cytokines, which act as mediators of innate immunity, include TNF- α , IL-1 (with its two subtypes IL-1 α and IL-1 β), IL-6, MIP and IL-8. (2) Regulatory factors of activation, function and differentiation of lymphocytes, include IL-2, IL-4, IL-21 and TGF- β [10]. (3) Regulatory factors of immune inflammation, which can activate nonspecific effector cells, including TNF- β , IL5, IL-10, IL-12 and IFN- γ .

Therefore, the purpose of this study was to evaluate the relationship between cytokines produced locally in the cervix and the status of HR-HPV infection and the levels of cervical precancerous lesion. Then cytokines which were significantly different were screened out in patients with transient and persistent HR-HPV infection and with different levels of cervical lesion. Moreover, the mechanisms of HR-HPV persistent infection and progression of cervical neoplasia were analyzed to provide immunity theory for blocking HR-HPV persistent infection and progression of cervical lesion.

Methodology

Study population

This study was approved by the Ethics Committee of Tianjin Central Obstetrics and Gynecology Hospital. From January 2016 to December 2016, 150 patients who were diagnosed as HR-HPV infection by Hybrid Capture II (HCII) in Tianjin Central Obstetrics and Gynecology Hospital were enrolled in this cross-sectional study. Exclusion criteria: (1) pregnant or postpartum within the previous 6 months; (2) patients who had history of hysterectomy or invasive cervical procedure; (3) patients who had cancer chemotherapy; (4) patients who had sexual behavior within 3 days; (5) chronic diseases, such as diabetes mellitus, allergy, autoimmune diseases; (6) patients who had vaginal and cervical medication within 1 week; (7) patients who had oral contraceptives and other cervical pathogens infection. All patients were officially informed and gave their written consent. Data on epidemiology were collected by survey.

A total of 36 patients who had twice consecutive negative HR-HPV test after at least one positive HR-HPV infection were defined as transient HR-HPV infection. A total of 40 patients who had more than three times consecutive positive HR-HPV infection were defined as persistent HR-HPV infection. Both patients with transient and persistent HR-HPV infection in this study were diagnosed as having no cervical lesion by biopsy. The other patients who had both HR-HPV infection and cervical lesion were divided into 2 subgroups: LSIL and HSIL groups, including 36 and 38 patients, respectively.

Sample collection

Two kinds of sample were selected in our study, including cervicovaginal lavage (CVL) and cervical exfoliated cells. They were used for testing cytokine and HR-HPV type, respectively. CVL specimens were obtained by washing the cervix with a single 5 mL volume of normal saline and were collected before all other cervical samples. The collected CVL aliquots were centrifuged (3000 rpm) for 10 minutes to separate mucus and cellular components. Cell-free supernatant was separated into a 1.5 mL aliquot and frozen at -80 °C until use. The cervical cells were collected by brush of liquid-based cytology and transferred directly into a special Thin-Prep container (ThinPrep Pap test; Hologic), which were stored at 4 °C until detection.

Assay and Typing of HR-HPV

Thin-Prep samples containing cervical cells were used for DNA extraction using the TIANamp Micro DNA kit (TIANGEN Biotech Co. Ltd., Beijing). Extracted DNA samples were tested for HPV type by polymerase chain reaction (PCR) assay and targeting the L1 region of the viral genome by using PGM1 primers [11]. Amplification of human β -globin gene segment was used as an internal control for DNA quality and cases negative for β -globin were excluded. Then the final PCR production were genotyped using pyrosequencing which was described previously [12]. Each sample was evaluated for the presence of high-risk HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 69). Transient infection samples of positive HPV typing and persistent infection samples of negative HPV typing did not carry on follow-up analysis.

Cytokine Measurement

The levels of 9 cytokines (IL-1 β , IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-21, IFN- γ , and TNF- α) were simultaneously measured by using multiplex

Table 1. Concentrations of 9 cytokines in cervical mucosa of 150 patients.

| Cytokines | Low detectable limit (pg/mL) | Upper detectable limit (pg/mL) | Samples within detectable limits (n) | Samples out of detectable limits (n) | Percentage of samples within detectable limits (%) |
|--|------------------------------|--------------------------------|--------------------------------------|--------------------------------------|--|
| Pro-inflammatory cytokines | | | | | |
| IL-1β | 0.72 | 3730 | 146 | 4 | 97.3 |
| TNF-α | 0.76 | 3088 | 100 | 50 | 66.7 |
| IL-8 | 1.79 | 2470 | 135 | 15 | 90.0 |
| Regulatory factors of lymphocytes | | | | | |
| IL-2 | 1.95 | 3878 | 88 | 62 | 58.7 |
| IL-4 | 3.77 | 12238 | 41 | 109 | 27.3 |
| IL-21 | 0.74 | 1045 | 90 | 60 | 60.0 |
| Regulatory factors of immune inflammation | | | | | |
| IL-12 | 1.01 | 3758 | 75 | 75 | 50.0 |
| IFN-γ | 1.20 | 3536 | 80 | 70 | 53.3 |
| IL-10 | 2.49 | 9056 | 113 | 37 | 75.3 |

immunoassay and tested using MilliPlex MAP Human Cytokine/Chemokine immunoassay kits (Millipore corporation, Billerica, MA). A standard curve was made according to the manufacturer’s instructions. Briefly, samples (25 μL per well) were incubated with antibody-conjugated microspheres in 96-well filter-membrane assay plates with agitation overnight. Plates were then washed with buffer provided in the assay kits and vacuum filtration. Besides, analyte-bound beads were incubated with a biotinylated detection antibody cocktail and finally with streptavidin-phycoerythrin. After additional wash steps and resuspension of beads in instrument sheath fluid, plates were run on Luminex 100 instrument (Luminex, Austin, TX). Regression curves (5-parameter logistic) were fit, and unknown concentrations were determined in pg/mL by using MiraiBio MasterPlex QT version 2.5 analysis software (Hitachi Solutions America, Ltd., South San Francisco, CA).

Statistical Analysis

Statistical analysis was performed using software SPSS 22.0 (International Business Machines, corp., Armonk, NY, USA). The independent sample t test (parametric) was used for analysis if there were differences in the mean value of 9 cytokines between

two groups. *p* < 0.05 was considered to be significantly different in all analyses.

Results

General information of patients

The average age of the 150 patients was (48.81 ± 3.67) years. There were 31 menopausal women in this study. There was no significant difference in age among the four groups (*p* > 0.05). All patients had HR-HPV (HC-2) test report and cervical biopsy report.

Cytokine detection using a multiplex immunoassay

In order to investigate cytokines which participate in cervical clearance or HR-HPV infection and the process of cervical lesion, the concentrations of 9 cytokines (IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-21, IFN-γ, TNF-α) in cervical mucosa were detected in our study. The results were shown in Table 1. There were significant expression levels in IL-1β, IL-8, IL-10, IL-21, TNF-α, IL-2, IL-12 and IFN-γ in cervical mucosa. Compared with those genes, IL-4 expression was comparative lower.

Furthermore, the levels of each cytokine in each group were shown in Table 2. Due to the low relevance ratio (< 60%), IL-2, IL-4, IL-12 and IFN-γ were excluded in analysis. There were significant differences in the levels of IL-1β between transient HR-HPV

Table 2. The levels of IL-1β, IL-8, IL-10, IL-21 and TNF-α in the four groups. Data were expressed as means ± SD. **p* < 0.05 vs the transient HR-HPV infection group, and #*p* < 0.05 vs the LSIL group.

| Cytokines | Transient HR-HPV infection | Persistent HR-HPV infection | LSIL | HSIL |
|-----------|----------------------------|-----------------------------|-----------------|-----------------|
| IL-1β | 8.78 ± 7.12 | 16.47 ± 3.34* | 17.39 ± 14.26 | 99.84 ± 141.17# |
| IL-8 | 432.91 ± 359.66 | 448.87 ± 396.44 | 370.14 ± 272.11 | 503.40 ± 449.72 |
| IL-10 | 10.62 ± 4.60 | 14.62 ± 12.09 | 7.34 ± 2.93 | 13.82 ± 11.15# |
| IL-21 | 19.85 ± 33.36 | 10.42 ± 15.06 | 4.27 ± 2.59 | 18.74 ± 28.36# |
| TNF-α | 13.35 ± 17.11 | 15.70 ± 16.06 | 6.30 ± 6.04 | 14.71 ± 11.81# |

infection group and persistent HR-HPV infection group ($p = 0.041$) (Figure 1). Moreover, there were obvious differences in the levels of IL-1 β , IL-10, IL-21 and TNF- α between LSIL group and HSIL group ($p = 0.011$, $p = 0.008$, $p = 0.046$ and $p = 0.019$, respectively) (Figure 1).

The mean values of cytokines of three categories in transient HR-HPV infection group, persistent HR-HPV infection group, LSIL group and HSIL group were shown in Figure 2. There was notable difference in the levels of cytokines of various category in the four groups ($p > 0.05$). The levels of pro-inflammatory cytokines were significantly increased in comparison those of regulatory factors of lymphocytes and regulatory factors of immune inflammation in the four groups, respectively ($p > 0.05$). However, there were not statistically significant differences between regulatory factors of lymphocytes and regulatory factors of immune inflammation in the four groups ($p < 0.05$, respectively). Similarly, there were not statistically significant differences in pro-inflammatory cytokines, regulatory factors of lymphocytes and regulatory factors of immune inflammation among the four groups ($p < 0.05$, respectively).

The prevalence of 15 types HR-HPV infection

All specimens of patients with persistent HR-HPV infection and patients with transient HR-HPV infection

were positive. A total of 10 out of 15 HR-HPV genotypes were detected in our study (Table 3). These 10 kinds of HPV were included in the detection range of HCII. In 114 patients with persistent HR-HPV infection, 94 infected with one type of HR-HPV, 16 infected with two types of HR-HPV and 4 infected with three types of HR-HPV.

Figure 2. The mean values of cytokines of different categories in four groups.

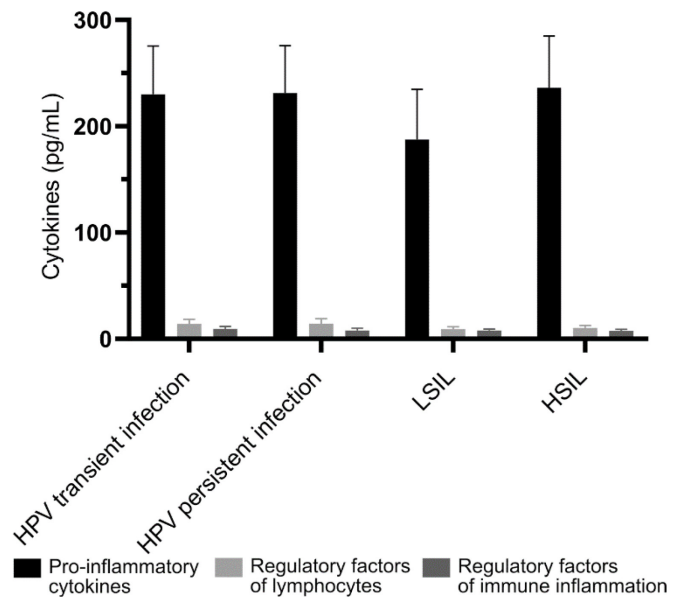
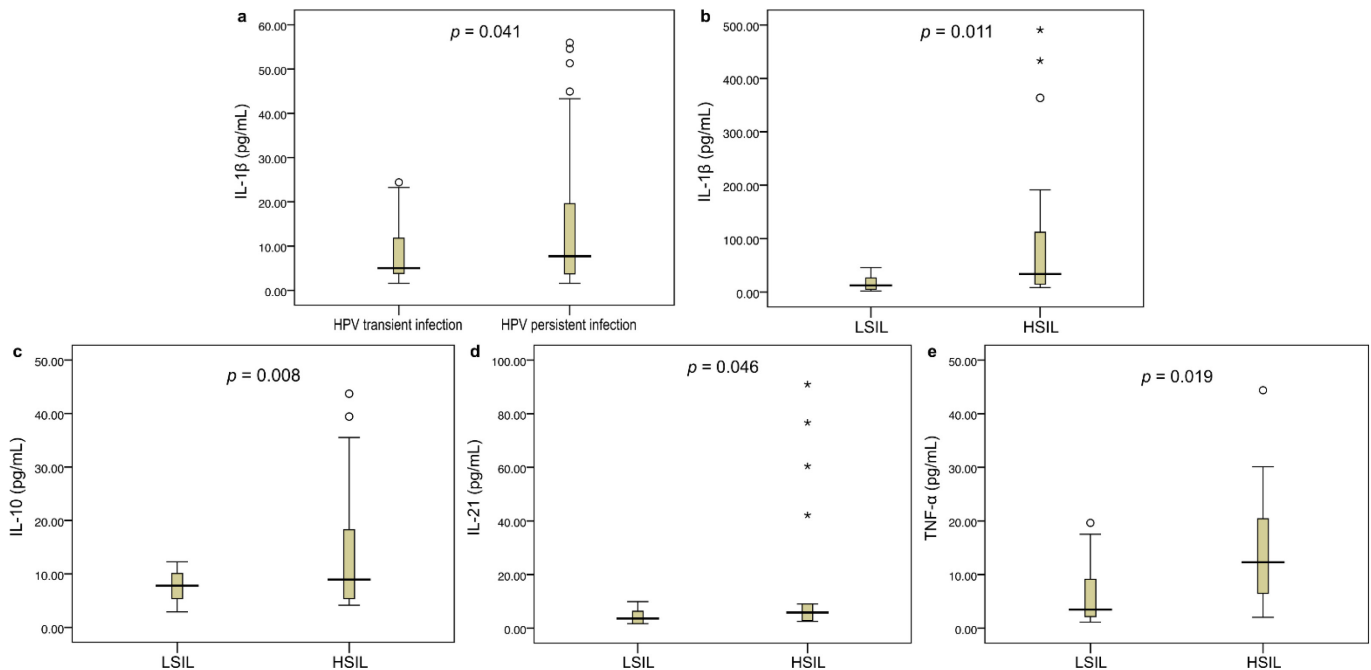


Figure 1. The levels of IL-1 β , IL-8, IL-10, IL-21, TNF- α in cervical mucosa. (a) The levels of IL-1 β in transient HPV infection group and persistent HPV infection group. (b) The levels of IL-1 β in LSIL group and HSIL group. (c) The levels of IL-10 in LSIL group and HSIL group. (D) The levels of IL-21 in LSIL group and HSIL group. (e) The levels of TNF- α in LSIL group and HSIL group. Data were shown as box-and-whisker plots (medians and 25th-75th percentiles).



There were no significant differences in the levels of various cytokines in different HR-HPV types (the data was not shown). Similarly, no significant differences were found in the levels of cytokines between HR-HPV-16 and other HR-HPV types (the data was not shown).

Discussion

In this cross-sectional study of women with HR-HPV infection, we analyzed the levels of 9 cytokines in cervical mucosa between patients with transient HR-HPV infection and persistent HR-HPV infection, and between patients with LSIL and HSIL. In this study, increased levels of IL-8, IL-1β, IL-10, IL-21 and TNF-α were associated with the progression of cervical precancerous lesions. It suggested that various types of immune cells secreted multiple cytokines to participate in cervical HR-HPV infection and progression of cervical lesion broadly.

HR-HPV infection is an essential factor in inducing cervical lesion. Once HPV infected cervical epithelial cells, the immune response of host involved in the removal of HPV. The monocytes and macrophages, which constitute the host innate immunity, are activated and result in raised levels of IL-1β that favor to maintain HPV infection [9]. With the assistance of pro-inflammatory cytokine, HPV may occur innate immune escape.

In cervical precancerous lesion with HR-HPV infection, the number of macrophages are increased, CD4⁺ and CD8⁺ T cells gather in the cervix and secrete magnanimous pleiotropic cytokines, such as IL-1β, IL-10, IL-21 and TNF-α. IL-1β and TNF-α are typical representative of pro-inflammatory cytokines [13]. IL-10 and IL-21 belong to Th2 type cytokines, and the low detection of Th1 type cytokines may imply the correlation of shift of Th1/Th2 and lesion progression.

Many studies [9,14,15] showed that high levels of IL-1β and TNF-α related to the occurrence and development of cervical lesions. Both normal cervical and lesion cells can produce IL-1β and TNF-α [16], and they in turn stimulate lesion cell proliferation and even cancelation. With the binding of IL-1β and TNF-α with their respective receptors, p38 MAPK and NF-κB signaling pathway can be activated. Followed by production of chemokines, vascular growth factor and

tumor growth factor provide favorable immune microenvironment for the survival of tumor cells and progression of lesion. Additionally, the circulating levels of TNF-α were increased with the increased clinical stage progress, and expression levels of KI-67, Bcl-2 and p53 in stage Iβ-IIa of cervical carcinoma were positively correlated with the levels of TNF-α [17].

As a potent immunosuppressive factor, IL-10 plays an extremely essential function in the course of HR-HPV infection and cervical precancerous condition. High expression of IL-10 in HPV persist infection may be beneficial to the immunoescape of HPV [15]. And, it can promote the occurrence and development of tumor cells by activating STA3 pathway and play the role of immune suppression [16], which can explain our results of IL-10. Besides, in response to (lipopolysaccharide) LPS, the levels of IL-10 were up-regulated accompanied with the decreased levels of IL-12 [18], which inhibited the anti-proliferative effect of IL-12 [19].

In addition to IL-10, IL-21 is also a kind of Th2 type cytokine. IL-21 is a pleiotropy cytokine, which be secreted by NKT cells, CD4⁺ and CD8⁺ T cells, and IL-21 in turn enhances the cytotoxicity of them. It can activate conventional dendritic cells and then induces IL-1β expression. In addition, it can promote M2 to M1 transition of the tumor-associated macrophages, followed by increased levels of IL-10 and chemokines [20]. In view of its wide range of influence on immune cells and cytokines, it may play a central role in immune response. At present, there are few studies on IL-21 in cervical area. Research showed that combination of IL-21 and IL-2 could availablely stimulate peripheral blood mononuclear cells with cytotoxicity against SiHa cells, which may be the mechanism owing to down-regulated Treg and Th17 cell differentiation [21].

Contrast with regulatory factors of lymphocytes and regulatory factors of immune inflammation, the expression of pro-inflammatory cytokines was higher in the four groups. Pro-inflammatory cytokines may occupy an important position in cervical immune micro-environment [16], and have profound effect on persistent HR-HPV infection and cervical lesion. Certain study showed that pro-inflammatory cytokines were highly expressed in persistent HPV infection,

Table 3. The incidence of HR-HPV types in the patients with LSIL and HSIL but without cervical lesion.

| HR-HPV type | 16 | 18 | 31 | 33 | 39 | 51 | 52 | 56 | 58 | 68 |
|--------------------|----|----|----|----|----|----|----|----|----|----|
| No cervical lesion | 9 | 14 | 3 | 2 | 2 | 1 | 7 | 3 | 3 | 2 |
| LSIL | 13 | 10 | 3 | 1 | 1 | - | 4 | 2 | 6 | 1 |
| HSIL | 23 | 12 | - | 1 | - | - | 1 | 5 | 8 | - |

which then caused the negative regulation of p53 and promoted proliferation of tumor cells and formation of blood-vessels [22]. Although there was no statistical difference in the levels of pro-inflammatory cytokines in the four groups, their roles cannot be ignored.

As a cross-sectional study, there are some limitations in our study. Firstly, participants in our research had HR-HPV infection before, but we failed to get the samples of which microenvironment can regard as base-line level and dynamic variation process. It may reduce convincing of the results. Secondly, the essence of our samples for cytokines detection, CVLs, was dilution, which may limit measurement of low-level cytokines. Diverse approaches have been employed in studies of cervical immune markers, including ophthalmic sponges, aspirates and CVLs [15]. Moreover, the reason why we used CVLs for detection of immunoglobulin in this study was that we did it in our previous study. Contrasted with ophthalmic sponges, CVLs indeed processed a low detection rate and concentration [23]. Lastly, there were no explicit definitions of cervical HR-HPV transient and persistent infection at present.

Nowadays, there are multiple means of test on cytokines and HPV typing. On the term of cytokines, both mRNA level of cervical tissue cytokine and peripheral blood mononuclear cells of patients with cervical HPV infection could be used for test cytokines. Whereas, the mRNA level may not reflect the protein level and systemic immune response does not represent. Therefore, detection of cervical local protein levels is appropriate for local immunity cytokine test. On the term of HPV typing, new technology emerges endlessly, such as HCII and Combas 100 based on DNA detection, Aptima targeting HPV mRNA from the E6/E7 oncogenes, and so on. Pyrosequencing, a new technology targeting HPV L1 capsid protein, processes equal sensibility and specificity compared with HCII.

In conclusion, the increased local levels of pro-inflammatory cytokine (IL-1 β) was associated with the decreased possibility of clearance in our study. The levels of IL-8, IL-1 β , IL-10, IL-21 and TNF- α were found to be lower in patients with LSIL than those in patients with HSIL. Cytokines may reflect cervical mucosa immune response against HR-HPV, and they can be used as biological markers for the HR-HPV infection and prognosis of CIN. However, further researches with follow-up visits and large numbers of subjects were requisite for exact mechanism.

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References

- Nichols AC, Palma DA, Chow W, Tan S, Rajakumar C, Rizzo G, Fung K, Kwan K, Wehrli B, Winquist E, Koropatnick J, Mymryk JS, Yoo J, Barrett JW (2013) High frequency of activating PIK3CA mutations in human papillomavirus-positive oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg* 139: 617-622.
- Yuan CH, Filippova M, Duerksen-Hughes P (2012) Modulation of apoptotic pathways by human papillomaviruses (HPV): mechanisms and implications for therapy. *Viruses* 4: 3831-3850.
- Coleman HN, Greenfield WW, Stratton SL, Vaughn R, Kieber A, Moerman-Herzog AM, Spencer HJ, Hitt WC, Quick CM, Hutchins LF, Mackintosh SG, Edmondson RD, Erickson SW, Nakagawa M (2016) Human papillomavirus type 16 viral load is decreased following a therapeutic vaccination. *Cancer Immunol Immunotherapy* 65: 563-573.
- Stanley MA (2012) Epithelial Cell Responses to Infection with Human Papillomavirus. *Clin Microbiol Rev* 25: 215-222.
- Kaul R, Hirbod T (2010) Genital epithelial cells: foot soldiers or fashion leaders? *J Leukoc Biol* 88: 427-429.
- Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev* 22: 240-273.
- Friedman R, Hughes AL (2002) Molecular evolution of the NF- κ B signaling system. *Immunogenetics* 53: 964-974.
- Iwata T, Fujii T, Morii K, Saito M, Sugiyama J, Nishio H, Morisada T, Tanaka K, Yaguchi T, Kawakami Y, Aoki D (2015) Cytokine profile in cervical mucosa of Japanese patients with cervical intraepithelial neoplasia. *Int J Clin Oncol* 20: 126-133.
- Daniilidis A, Koutsos J, Oikonomou Z, Nasioutziki M, Hatziparadisi K, Tantanasis T (2016) Cytokines of cervical mucosa and human papilloma virus infection of the cervix: A Descriptive Study. *Acta Cytol* 60: 58-64.
- Spolski R, Leonard WJ (2014) Interleukin-21: a double-edged sword with therapeutic potential. *Nat Rev Drug Discov* 13: 379-395.
- Broutian TR, He X, Gillison ML (2011) Automated high throughput DNA isolation for detection of human papillomavirus in oral rinse samples. *J Clin Virol* 50: 270-275.
- Chen X, Wallin K, Duan M, Gharizadeh B, Zheng B, Qu P (2015) Prevalence and genotype distribution of cervical human papillomavirus (HPV) among women in urban Tianjin, China. *J Med Virol* 87: 1966-1972.
- Otani S, Fujii T, Kukimoto I, Tsukamoto T, Ichikawa R, Nishio E, Iwata A (2019). Cytokine expression profiles in cervical mucus from patients with cervical cancer and its precursor lesions. *Cytokine* 120: 210-219.
- Behbakht K, Friedman J, Heimler I, Aroutcheva A, Simoes J, Faro S (2002) Role of the vaginal microbiological ecosystem and cytokine profile in the promotion of cervical dysplasia: a case-control study. *Infect Dis Obstet Gynecol* 10: 181-186.
- Scott ME, Shvetsov YB, Thompson PJ, Hernandez BY, Zhu X, Wilkens LR, Killeen J, Vo DD, Moscicki AB, Goodman MT

- (2013) Cervical cytokines and clearance of incident human papillomavirus infection: Hawaii HPV cohort study. *Int J Cancer* 133: 1187-1196.
16. Fernandes JV, DE Medeiros Fernandes TA, DE Azevedo JC, Cobucci RN, DE Carvalho MG, Andrade VS, DE Araújo JM (2015) Link between chronic inflammation and human papillomavirus- induced carcinogenesis (Review). *Oncol Lett* 9: 1015-1026.
 17. Gening TP, Antoneeva II, Abakumova TV, Peskov AB, Sidorenko EG, Gening SO, Dolgova DR (2014) Dynamics of proinflammatory cytokine levels and their role in the development of local and systemic effects during progressing cervical cancer. *Bull Exp Biol Med* 157: 776-780.
 18. Demoulin SA, Somja J, Duray A, Guénin S, Roncarati P, Delvenne PO, Herfs MF, Hubert PM (2015) Cervical (pre)neoplastic microenvironment promotes the emergence of tolerogenic dendritic cells via RANKL secretion. *Oncol Immunology* 4: e1008334.
 19. Vicari AP, Trinchieri G (2004) Interleukin-10 in viral diseases and cancer: exiting the labyrinth? *Immunol Rev* 202: 223-236.
 20. Leonard WJ, Wan CK (2016) IL-21 Signaling in Immunity. *F1000 Res* 5: 224.
 21. Tian Y, Yuan C, Ma D, Zhang Y, Liu Y, Zhang W, Hou F, Cui B (2011) IL-21 and IL-12 inhibit differentiation of treg and TH17 cells and enhance cytotoxicity of peripheral blood mononuclear cells in patients with cervical cancer. *Int J Gynecol Cancer* 21: 1672-1678.
 22. Hudson JD, Shoaibi MA, Maestro R, Carnero A, Hannon GJ, Beach DH (1999) A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J Exp Med* 190: 1375-1382.
 23. Lieberman JA, Moscicki AB, Sumerel JL, Ma Y, Scott ME (2008) Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates. *Clin Vaccine Immunol* 15: 49-54.

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