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Original Article

Value of T lymphocyte subset detection in cervical intraepithelial neoplasia

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Abstract: Objective: This study aimed to explore the value of T lymphocyte subset detection in cervical intraepithelial neoplasia (CIN). Methods: In this retrospective analysis, T lymphocyte subsets in 186 CIN patients were detected. Venous blood T lymphocyte subsets were analyzed in patients with different CIN grades, and Spearman correlation analysis was conducted between CIN grade and T lymphocyte subsets. Results: (1) There were significant differences in the $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ levels before and 1, 2, and 3 months after treatment ($P<0.05$). Furthermore, significant differences were found in $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ between every pair of time points ($P<0.05$). (2) Comparison of human papillomavirus distribution in patients with different CIN grades showed $P<0.05$. (3) The level of T lymphocyte subsets in the venous blood of patients with different CIN grades was compared, and significant differences were found, $P<0.05$. Higher CIN grade was associated with lower levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$, as well as higher level of $CD8^+$. (4) Spearman analysis showed that CIN grade was negatively correlated with the levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ ($P<0.05$) and positively correlated with the level of $CD8^+$ ($P<0.05$). Conclusion: The levels of T lymphocyte subsets were found to be closely associated with the severity of CIN. Therefore, the detection of T lymphocyte subsets in venous blood could be a valuable clinical tool for predicting the presence and degree of CIN.

Keywords: Cervical intraepithelial neoplasia, T lymphocyte subsets, detection

Introduction

Cervical cancer is a common gynaecological malignancy, and epidemiologic analysis has shown that this disease tends to affect younger individuals and has a high mortality [1]. The incidence of cervical cancer in China is 99/100,000, second only to breast cancer, and the mortality rate is 305/100,000 [2]. Development of precancerous lesions to cervical cancer is a gradual process [3]. Cervical intraepithelial neoplasia (CIN), as a type of the significant precancerous lesion of cervical cancer, refers to abnormal proliferative lesions of the cervix that tend to become cancerous but are not diagnosed as carcinoma in situ [4]. At present, the mechanism of CIN is still in an exploratory stage. Studies have found that in the progression of cervical lesions, abnormal cellular immune function and the resulting suppression of the immune system can significantly reduce the recognition and killing ability of mutant epithelial cells, thus promoting the growth and metastasis of malignant cells [5]. T lymphocyte subsets are important factors mediating cellular immune response, that can maintain the body's homeostasis and kill tumour cells [6]. Regulatory T cells inhibit the activation of various immune cells by participating in negative immune regulation [7]. Some studies examining T lymphocyte subsets in peripheral blood in relation to CIN have been confounded by coexisting HR-HPV infection. However, the role of T lymphocyte subsets detection in CIN is still unclear. More reliable studies are needed to explore the relationship between T lymphocyte subsets and CIN. Based on this, this study aimed to investigate the role of T lymphocyte subsets in the progression of

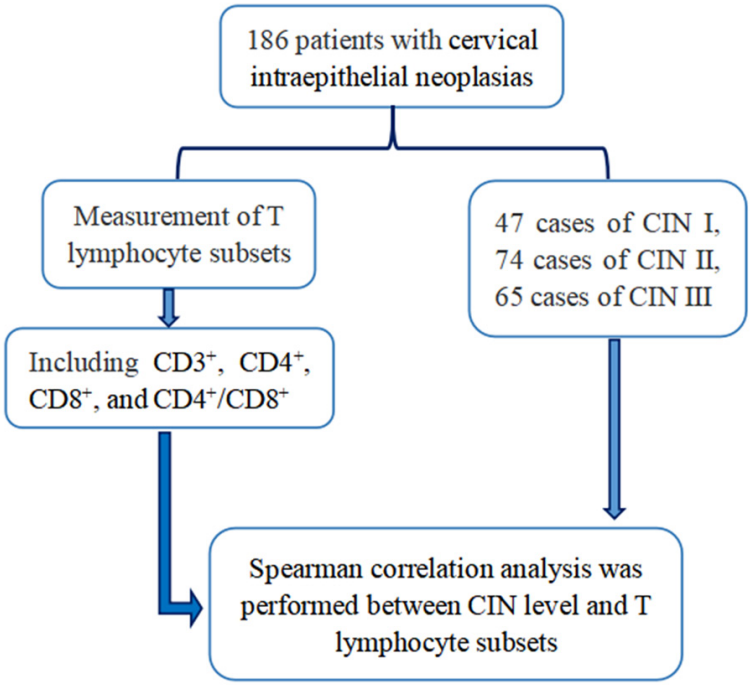

Figure 1. Flowchart of this study. T lymphocyte subsets of 186 CIN patients were detected. Venous blood T lymphocyte subsets of patients with different CIN grades were analysed, and Spearman correlation analysis was conducted between CIN grade and T lymphocyte subsets. Note: CIN: cervical intraepithelial neoplasia.

CIN by detecting the levels of T lymphocyte subsets in venous blood.

Materials and methods

Patients source

In this retrospective analysis, 186 CIN patients admitted to People's Hospital Affiliated of Quanzhou Medical College from February 2019 to December 2022 were selected as study subjects.

Patients were eligible if they met the CIN-related diagnostic criteria in the Pathological Diagnosis of Cervical Cancer and Precancerous Lesions [8], after cervical histopathological examination, were 18 years old or more, were married, had normal cognitive function, and were routinely treated by western medicine (recombinant α-2b vaginal effervescent capsules).

Patients were excluded if they had comorbid infectious diseases, autoimmune diseases, mental diseases, or malignant tumours other than CIN, received immunosuppressants or corticosteroids within 3 months before admission, underwent radiotherapy or chemotherapy, tested positive for human immunodeficiency (HIV), had organic lesions of the heart, liver, kidney, or other vital organs, or were pregnant or breastfeeding.

Another 50 healthy people of comparable age were selected as a control group. This study was approved by the Medical Ethics Committee of People's Hospital Affiliated of Quanzhou Medical College. The flowchart of this study is shown in **Figure 1**.

Cervical tissue biopsy

Cervical tissue of patients were biopsied under colposcope. Forceps were used to extract the suspicious lesions according to the acetic acid and iodine test results. (1) The patients were instructed to assume the bladder lithotomy position and expose the cervix, vagina, and vaginal dome with a sterile speculum. (2) A sterile cotton ball was used to wipe the cervical mucus or blood, and a sterile sampling brush was inserted 2 cm into the cervical tube. The cotton swab was rotated for 5 times and held for a few seconds. (3) The collected samples were put into a test tube filled with normal saline. (4) The cervix was swabbed with a cotton ball dipped in 5% acetic acid stain for 1 min, followed by a 2-minute waiting period to observe presence of any areas that turned vinegar white. (5) The cervix was wiped with a cotton ball dipped in iodophor to observe whether there was staining. (6) The uncoloured part of the tissue was taken by forceps, fixed in 10% formalin, and sent for inspection. Cervical biopsy was graded by CIN grades I, II, and III according to the condition [9].

Treatment methods

Conventional western medical treatment was carried out, using 1 recombinant α-2b vaginal effervescent capsule (S20050075) inserted into the vagina, starting on the 3rd day after the end of menstruation. This was administered

every night before bed for 10 days, with a total treatment course of 3 months.

Detection of T lymphocyte subsets in venous blood

In each patient, 10 mL of fasting venous blood was collected in the morning and stored at -80°C for testing. (1) Blood samples were treated with heparin sodium and added to the bottom of a 5 mL flow tube, and 10 μL of CD3, CD4, and CD8 each were added. (2) After mixing, the mixture was placed at room temperature and kept away from light for 20 min. (3) Red blood cell lysate was added and left for 10 min. (4) The solution was centrifuged at 1500 rpm for 10 min to retain the supernatant. (5) PBS (2 mL) was added to the up-clear liquid for centrifugal washing. (6) Finally, 0.5 mL PBS was used for buffering. The number and proportion of $CD3^+$, $CD4^+$, and $CD8^+$ subgroups in the cell suspension were determined by the Thermo Fisher Attune NxT flow cytometer and corresponding reagents.

Polymerase Chain Reaction (PCR) was used for detecting human papillomavirus (HPV) genes and genotypes, including high-risk-HPV (HR-HPV) 16, 31, 33, 52, and 58, and low-risk-HPV (LR-HPV) 6, 11, and 43. HPV DNA load detection methods: The cervical brush was inserted into the cervix and rotated for 3 turns in a sterile environment, then carefully removed to avoid touching any other surface. The cervical brush was placed in a clean tube to collect the shed cells inside and outside the cervix, and then the collected shed cells were timely tested for HR-HPV. DNA extraction and PCR amplification standards were carried out per the instructions (PCR fluorescence method). Detection was by the DA7600 PCR amplification instrument, and the PCR amplification instrument and HR-HPV nucleic acid quantitative detection kits were purchased from Beijing Lepu Medical Technology Co., LTD.

Observation indicators

The levels of venous blood T lymphocyte subsets (including $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$) of patients with different CIN grades were compared, and Pearson correlation analysis was conducted between CIN grade and T lymphocyte subsets.

Statistical approach

The data in this study were statistically analysed using SPSS 23.0. Measured data were described by mean ± standard deviation. Comparison between two groups was performed by t-test, and comparison among three or more groups was performed by F test. Counted data were described by case number (percentage) and processed by the Chi-square test. Spearman correlation analysis was used to analyse the correlation of data. The test level was α=0.05.

Results

Baseline data and HPV distribution of patients with different CIN grades

Among the 186 cases of CIN, 47 cases were at CIN grade I, 74 cases at CIN grade II, and 65 cases at CIN grade III. There were 153 patients with HR-HPV (82.25%) and 33 patients with LR-HPV (17.74%). A significant difference was found in the comparison of HPV distribution of different CIN grade patients, $P<0.05$. The proportion of HR-HPV was higher in patients at CIN grade II and CIN grade III than in those at CIN grade I, as shown in **Table 1**.

Dynamic changes in T lymphocyte subsets during treatment

There were significant differences in the $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ levels before and 1, 2, and 3 months after treatment ($P<0.05$). Furthermore, significant differences were found in $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ between every pair of time points ($P<0.05$) (**Tables 2**, **3**).

Venous blood T lymphocyte subsets in patients with different HPV types

The $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ levels were compared between patients with LR-HPV and HR-HPV, but the *P* value was greater than 0.05, as shown in **Table 4**.

Venous blood T lymphocyte subsets in patients with different CIN grades

The levels of T lymphocyte subsets in the venous blood were found to be different in

Table 1. Baseline data and HPV distribution of patients with different CIN grades

CIN grade	Age ($\bar{x}$±s, years)	BMI ($\bar{x}$±s, kg/m^2)	HPV types [n (%)]	
			HR HPV	LR HPV
grade I (n=47)	43.16±8.25	22.55±2.15	30 (63.83)	17 (36.17)
grade II (n=74)	44.04±7.68	22.48±1.86	68 (91.89)	6 (8.11)
grade III (n=65)	42.84±7.53	22.82±1.83	55 (84.62)	10 (15.38)
F/x^2	0.442	0.576	15.890	
P	0.644	0.563	<0.001	

Note: BMI: Body Mass Index, HPV: human papillomavirus, HR HPV: high-risk-HPV, LR HPV: low-risk-HPV, CIN: cervical intraepithelial neoplasia.

Table 2. Dynamic changes of T lymphocyte subsets during treatment

Time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
pre-treatment	46.80±8.70	23.69±7.10	23.57±6.97	1.16±0.71
1 month after treatment	51.36±7.14	25.27±6.86	20.04±5.58	1.38±0.58
2 months after treatment	58.26±5.57	30.28±5.58	19.33±5.47	1.72±0.67
3 months after treatment	61.25±5.52	35.16±4.13	18.16±2.08	1.96±0.32
F	127.558	143.306	7.625	53.316
P	<0.001	<0.001	0.001	<0.001

Table 3. Post hoc pairwise Bonferroni test significance (during treatment)

Time of treatment	$CD3^+$ (%)			$CD4^+$ (%)			$CD8^+$ (%)			$CD4^+/CD8^+$		
	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months
pre-treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	<0.001	<0.001	<0.001	0.017	<0.001
1 month	-	<0.001	<0.001	-	<0.001	<0.001	-	<0.001	<0.001		0.015	<0.001
2 months	-	-	<0.001	-	-	<0.001	-	-	<0.001			0.009

Table 4. Venous blood T lymphocyte subsets in patients with different HPV types (x±s)

HPV type	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
HR-HPV (n=153)	46.46±8.56	23.27±6.79	23.97±7.09	1.12±0.70
LR-HPV (n=33)	48.33±9.14	25.61±8.10	21.73±6.07	1.34±0.72
t	1.125	1.733	1.686	1.629
P	0.262	0.085	0.094	0.105

Note: HPV: human papillomavirus, HR HPV: high-risk-HPV, LR HPV: low-risk-HPV.

Table 5. Venous blood T lymphocyte subsets in patients with different CIN grades ($\bar{x}$±s)

CIN grade	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
grade I (n=47)	52.64±7.44	30.28±6.07	18.86±5.52	1.74±0.63
grade II (n=74)	48.67±7.50[a]	22.54±6.07[a]	22.55±5.96[a]	1.14±0.77[a]
grade III (n=65)	40.43±6.57[a,b]	20.22±5.54[a,b]	28.13±6.21[a,b]	0.76±0.31[a,b]
F	43.730	42.110	34.99	43.730
P	<0.001	<0.001	<0.001	<0.001

Note: [a]stands for comparison with CIN grade I, [b]stands for comparison with CIN grade II, both *P*<0.05. CIN: cervical intraepithelial neoplasia.

patients with different CIN grades, *P*<0.05. Higher CIN grade was associated with lower levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$, as well as a higher level of $CD8^+$. See **Tables 5** and **6**.

Correlation between CIN grade and T lymphocyte subsets

Spearman analysis showed that CIN grade was negatively correlated with the levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ (*P*<0.05) and positively correlated with the level of $CD8^+$ (*P*<0.05). See **Table 7** and **Figure 2**.

Table 6. Post hoc pairwise Bonferroni test significance (different CIN grades)

CIN grade	$CD3^+$ (%)		$CD4^+$ (%)		$CD8^+$ (%)		$CD4^+$ (%)	
	CIN grade II	CIN grade III	CIN grade II	CIN grade III	CIN grade II	CIN grade III	CIN grade II	CIN grade III
grade I	0.011	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001
grade II	-	<0.001	-	0.048	-	<0.001	-	0.001

Note: CIN: cervical intraepithelial neoplasia.

Table 7. Correlation between CIN grade and T lymphocyte subsets

T lymphocyte subset	CIN grade	
	r	*P*
$CD3^+$	-0.566	<0.001
$CD4^+$	-0.500	<0.001
$CD8^+$	0.531	<0.001
$CD4^+/CD8^+$	-0.646	<0.001

Note: CIN: cervical intraepithelial neoplasia.

Discussion

CIN is one of the risk factors for cervical cancer. Early screening can facilitate timely detection and intervention of lesions, which helps prevent cervical cancer [10]. Colposcopy combined with cervical histopathologic biopsy, membrane-based thin layer cytology, and second-generation gene hybridization capture technology is commonly used for the clinical detection of CIN and further assessment of the risk of cervical cancer [11]. But these methods are not suitable for everyone, and the cost of testing can be prohibitive for many individuals. Relevant studies have shown that low immune function often accompanies cervical lesions [12]. Therefore, exploring the role of immune T-lymphocyte subsets in CIN is necessary.

This study found that the proportion of HR-HPV was higher in patients at CIN grade II and III than that in patients at CIN grade I, suggesting that the degree of lesions in HR-HPV patients was worse. Further research found no significant difference in the levels of $CD3^+$, $CD4^+$, $CD8^+$, or $CD4^+/CD8^+$ between LR-HPV patients and HR-HPV patients, suggesting that no significant impact on immune function was found regardless of whether patients were infected with HR-HPV. This differed from the results of a previous study [13]. This inconsistency may be related to the inclusion and exclusion criteria [14], or the sample size of this study was small enough to highlight the relationship between HPV type and T lymphocyte subsets. Different inclusion and exclusion criteria may result in the inclusion of different types of patients in the study, possibly leading to variations in the results of relevant indicators, and introducing some degree of deviation in the results between the two studies.

This study compared the T lymphocyte subsets in the venous blood of patients with different CIN grades and found significant differences in the T lymphocyte subsets in between patients with different CIN grades. Higher CIN grade was found to be associated with lower levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$, and higher level of $CD8^+$. These results indicated that as CIN lesions progress, there is a gradual decline in the immune function among patients. There was an abnormal expression of T lymphocyte subsets in venous blood, and this abnormality was gradually intensified with the development of the disease. This may be because CIN damages the body's immune function and causes the expression imbalance of T lymphocyte subsets, which is consistent with the results of related studies [15]. T lymphocyte subsets are the primary effector cells in cervical local cellular immunity. Under normal physiologic conditions, T lymphocyte subsets in venous blood remain relatively stable. However, when the body produces CIN lesions, immune function is impaired, leading to abnormal expression of T lymphocyte groups in venous blood. $CD3^+$ refers to T lymphocytes, which can represent the immune function state of human cells [16]. $CD4^+$ and $CD8^+$ are significant players in the T-cell-mediated anti-tumour immune response. $CD4^+$ is the helper/induction of T lymphocytes, leading to the immune response. It is mainly responsible for antigen transfer and can differentiate and produce antibodies with the assistance of B cells [17]. $CD8^+$ belongs to inhibitory T cells and cytotoxic T cells and can play an immunosuppressive role in immune response [18]. $CD4^+$ and $CD8^+$ cells can induce and

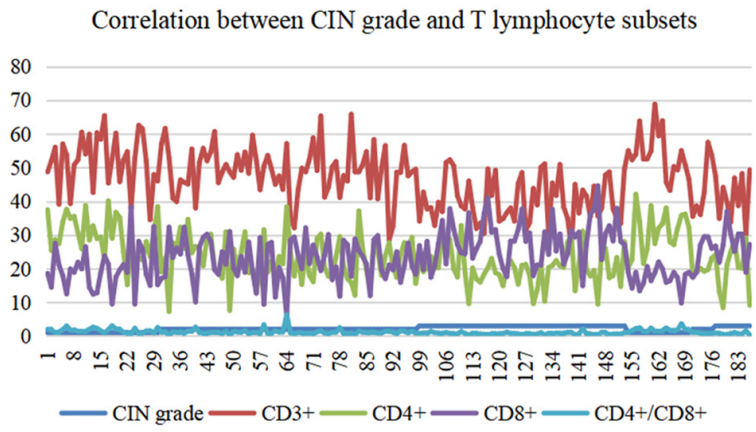

Figure 2. Correlation between CIN grade and T lymphocyte subsets. Note: CIN: cervical intraepithelial neoplasia.

restrict each other to regulate immune function and maintain the stability of the local immune environment. As the severity of CIN lesion increases, the immune system is more severely impaired, leading to more pronounced abnormalities in T lymphocyte subsets such as $CD3^+$, $CD4^+$, $CD8^+$, and the ratio $CD4^+/CD8^+$. Therefore, clinical attention should be paid to the immune function. Clinicians can take targeted intervention measures according to the immune function status of CIN patients to delay the malignant degeneration of CIN.

In this study, Spearman correlation analysis found that CIN grade was negatively associated with the levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ and positively correlated with $CD8^+$. This suggested that venous blood T lymphocyte subsets were closely related to CIN lesions. T lymphocytes maintain immune stability and regulate the immune response through contact between cells, competitive inhibition, and secretion of inhibitory cytokines. In addition, T lymphocytes can directly kill target cells, inhibit T cell activation, and enhance the ability of the immune system to identify and destroy tumour cells, ultimately improving the body's resistance to tumour cells [19, 20]. An abnormal decrease in $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ levels and an abnormal increase of $CD8^+$ indicate immune dysfunction or immunosuppression of T lymphocytes, which could lead to immune escape of tumour cells, promote proliferation, differentiation, invasion, and metastasis of malignant tumour cells, and thus worsen the degree of CIN lesions [21, 22]. CIN grade was closely related to T lymphocyte subsets, suggesting that clinical detection of T lymphocyte subsets is beneficial to evaluate the severity of CIN lesions.

In summary, the degree of CIN lesions is closely related to T lymphocyte subsets. The combined determination of T lymphocyte subsets in venous blood has particular clinical value for predicting CIN. However, the sample size of this study was relatively small, and this was a single-centre study. Therefore, we suggest to conduct a multi-centre study in the future to further explore the role of T lymphocyte subsets in the progression of CIN.

Disclosure of conflict of interest

None.

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