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UPREGULATION OF FOXP3+ REGULATORY T LYMPHOCYTES AND CD8+ LYMPHOCYTES IN PATIENTS WITH HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS CORRELATED WITH HPV INFECTION

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Modern therapeutic strategies for precancerous cervical intraepithelial neoplasia (CIN) focus on immune-modulatory and cancer vaccination. The local cervical immune status in cervical cancer and CIN could influence HPV infection persistence, progression and carcinogenesis. We analysed the role of FOXP3+ regulatory T lymphocytes, CD4+ and CD8+ T lymphocytes in CIN I, CIN II and CIN III patients with and without HPV infection. Sixty-two patients were enrolled in the study. Each patient underwent a colposcopy-guided cervical biopsy. FOXP3+ lymphocytes and CD4+,CD8+ lymphocytes were detected by immunostaining in tissue samples obtained from a control group (n = 10), patients with CIN I (n = 20), CIN II (n = 14) and CIN III (n = 18) lesions. HPV was assayed by Aptima. The results showed that the numbers of CD4+ T lymphocytes did not differ between patients with CIN I, CIN II, and CIN III. However, patients with CIN II and CIN III had significantly upregulated CD8+T lymphocytes compared to patients with CIN I. In addition, patients with CIN II and CIN III had increased FOXP3 + T lymphocytes compared with patients with CIN I, which was associated with HPV status. Upregulation of FOXP3+ regulatory T lymphocytes and CD8-positive lymphocytes in patients with CIN II and CIN III suggested a pivotal role of T regulatory lymphocytes and CD8+ lymphocytes for counteracting the host immune response in the progression from CIN I to CIN II and CIN III.

Key words: *cervical intraepithelial neoplasia (CIN), lymphocytes, HPV (human papilloma virus).*

INTRODUCTION

Cervical cancer is the fourth most common cancer among women worldwide, with an estimated incidence of 570 000 and with 300 000 deaths worldwide per year. Over the past 40 years, cervical cancer rates have decreased by as much as 65% in several Western countries, where screening programmes have long been established to detect precancerous cervical lesions that can be treated successfully (Bedell *et al*., 2020). In developing countries, due to limited access to effective screening programmes, cervical neoplasia is often identified at an advanced stage with symptoms, resulting in a higher rate of mortality (Torre *et al*., 2012). The majority of cervical cancer cases can histologically be recognised as squamous cell carcinoma (~75% of cases) (Torre *et al*., 2012).

The precursor lesion of cervical cancer is intraepithelial neoplasia grade I–III (CIN I–III), according to the epithelium involved in the dysplasia (Brenton *et al*., 2019). The new WHO classification employs the terms — LSIL (Low-grade Squamous Intraepithelial Lesion) and HSIL (High grade Squamous Intraepithelial Lesion) (Brenton *et al*., 2019). However, previous classifications classified cervical intraepithelial neoplasia (CIN) to three grades – e.g., CIN I, CIN II, and CIN III, based on the degree of dysplasia (Martin *et al*., 2012). CIN I corresponds to LSIL, whereas CIN II and CIN III correspond to HSIL.

Accurate histological grading of CIN lesions is important for clinical management of patients, because CIN lesions are monitored and treated differently. For example, CIN I is usually regarded as benign and no therapy is indicated because it regresses in ~80% of cases (Gurrola-Diaz *et al*., 2008). CIN II and CIN III are regarded as precursors of invasive carcinomas and therapy (conisation or other less invasive procedures) is indicated, as 0.2–4.0% of CIN II and CIN III cases can progress to carcinoma within 12 months Schorge *et al*., 2004; Van Niekerk, *et al*., 2007; Ozaki *et al*., 2011).

However, the group of CIN II patients seem to be heterogenous concerning their prognostic behaviour so that indicated therapy — conisation vs. watchful waiting strategy — is a controversial discussion (Tainio *et al*., 2018).

Persistent infection with HPV is a known aetiological factor in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer (Nguen *et al*., 2005; Guzman an-Olea *et al*., 2012). Among the factors that influence the probability of a cervical HPV infection becoming persistent, cell-mediated immunity of an individual is considered an important mechanism in protection against the virus and elimination of virus-infected cells (Nguen *et al*., 2005; Guzman an-Olea *et al*., 2012; Molina *et al*., 2020).

Tumours possess a variety of cell membrane-bound antigens, recognised as non-self by the immune system, which stimulates a cytotoxic immune response characterised by CD4, CD8, antigen-presenting cells, and infiltration of other inflammatory cells (Davoodzahed *et al*., 2017; Ostroumov *et al*., 2018; Shibata *et al*., 2019). These infiltrating cells are recognised as a response by immune surveillance mechanisms designed to inhibit tumour growth and spread (Davoodzahed *et al*., 2017; Camila *et al*., 2018; Das *et al*., 2018; Ostroumov *et al*., 2018; Shibata *et al*., 2019).

Tumour infiltrating lymphocytes (TILs), mainly CD4 and CD8 T cells, have been extensively described in anti-tumour immunity (Loddenkemper *et al*., 2009; Kojima *et al*., 2013; Molina *et al*., 2020). T-regulatory lymphocytes suppress the immune response and commonly express FOXP3, CD4, and CD25 (Litwin *et al*., 2021). T-regulatory lymphocytes can be detected in tissue by double immunohistochemistry with CD4 and CD25 biomarkers, and by FOXP3 immunohistochemistry. Intracellular expression of FOXP3 is currently considered the most specific marker for T regulatory lymphocytes (Kojima *et al*., 2013; Maskey *et al*., 2019; Litwin *et al*., 2021). T regulatory (Treg) cells are important in peripheral immunological tolerance, down-regulation of persistent inflammation and prevention of autoimmune reactions by inhibition of other T-cell responses (Loddenkemper *et al*., 2009; Kojima *et al*., 2013; Maskey *et al*., 2019; Molina *et al*., 2020).

The extent and composition of infiltrating immune cells in cervical tissue affects the natural history of HPV infections, because the virus must evade both innate and adaptive immune responses to establish a productive and persistent infection (Maskey *et al*., 2019; Litwin *et al*., 2021).

The role of T regulatory lymphocytes, CD4+ and CD8+ T lymphocytes in cervical intraepithelial neoplasia has been outlined (Loddenkemper *et al*., 2009; Kojima *et al*., 2013; Das *et al*., 2018; Litwin *et al*., 2021). T-cell infiltrates are predominant as the grade of the lesion progresses into more advanced lesions (Maskey *et al*., 2019). FoxP3 meta-analysis suggested that increased presence of regulatory T cells is associated with more severe disease (Litwin *et al*., 2021). However the precise interplay of T regulatory lymphocytes, CD4+ and CD8+ T lymphocytes in HPV-positive and HPV-negative patients with different CIN grades (CIN I, CIN II, and CIN III) is still debated.

We analysed the role of FOXP3-positive regulatory T lymphocytes, CD4-positive and CD8-positive T lymphocytes in CIN I, CIN II, and CIN III patients with and without HPV infection.

MATERIALS AND METHODS

Patients. Sixty-two patients aged 18–46 years referred to the Department of Gynecology at Rīga East University Hospital were enrolled.

The study conformed to the Declaration of Helsinki. The protocol was approved by the Committee of Ethics, Institute of Experimental and Clinical Medicine, University of Latvia (Rīga, Latvia). All patients signed informed consent prior to enrolment.

Exclusion criteria were previous treatment for cervical disease (including loop electrosurgical excision procedure (LEEP), cold-knife conisation, cryotherapy, LASER therapy, or hysterectomy, prior chemotherapy or radiation treatment for cervical neoplasia, pregnancy, HIV infection and inability to give informed consent.

There were 20 cases of CIN I, 14 cases of CIN II, and 18 cases of CIN III. All patients underwent a colposcopy-guided cervical biopsy. The control group consisted of ten patients who underwent biopsy after cytology testing suspicious for dysplasia, but histologically the CIN diagnosis has not been confirmed. The patients from the control group were HPV-negative and did not histologically and clinically show signs of acute or chronic cervicitis.

Tissue processing, histology, immunohistochemistry. Formalin-fixed paraffin embedded tissue was cut in 3-μm-thick sections. The sections were stained with H&E for histopathologic examination. For immunohistochemistry, antigen retrieval was achieved by treatment in a domestic microwave for 30 minutes in EDTA buffer pH = 9.0. Sections were incubated in 3.0% H_2O_2/PBS to quench endogenous peroxidase activity, and then blocked with protein block (Dako). The slides were then incubated 1 hour at room temperature with primary antibodies against the following antigens: CD4 (mouse monoclonal DAKO, Denmark, IR649, ready to use), CD8 (mouse monoclonal, DAKO, Denmark, IR623, ready to use) and mouse monoclonal FOXP3 antibody (AbCam, ab 20034, 236A/E7). The EnVision kit was used for visualisation of bonding of primary antibodies. 3'3-diaminobenzidine-tetrahydrochloride (DAB) was applied as chromogen (seven minutes) Sections were counterstained in haematoxylin (two minutes). For a positive control, tissue of human palatine tonsils was used. Negative controls were performed by omitting the primary antibody.

To evaluate the immunopositive cells, at least ten high-powered fields (magnification × 400) were assessed both in epithelium and stroma. Cytoplasmic immunoreactivity for CD4 and CD8-positive lymphocytes and nuclear immunoreactivity for FOXP-3-positive lymphocytes were semi-quantitated by consensus of two pathologists (SI and TZ) who were blinded to the clinicopathological data. The results were expressed as cells per square millimeter.

HPV testing. A recent study in the UK demonstrated that Aptima mRNA assay versus a DNA assay would almost certainly yield cost savings and reduce unnecessary testing and procedures, benefiting the National Health Service and women in the Cervical Screening Programme (Weston *et al*., 2020). Therefore, the Aptima HPV assay was also used in our study. All patients underwent HPV testing from cervical smears. The Aptima HPV assay is an *in vitro* nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk types of human papillomavirus (HPV) in cervical specimens. The high-risk HPV types detected by the assay include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The Aptima HPV assay does not discriminate between the 14 high-risk types (Weston *et al*., 2020).

Statistical analysis. Group data are expressed as mean ± SD for morphological data and median (range) for clinical data. The D'Agostino-Pearson omnibus test was used for the assessment of normality. Differences between groups (patients' age) were analysed using one-way analysis of variance (ANOVA), Chi-squared test (the numbers of positive cases) and Kruskal–Wallis test with Dunns post test for morphological data. OR and 95% CI were calculated using cross-tabulations to describe the association of potential risk factors with the rate of lymphocyte infiltration. A $p < 0.05$ was considered statistically significant. Data was analysed using SPSS software, version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics. There were no differences in patient average age, menarche, coitarche, cycle days, numbers of partus and smoking status between the groups.

HPV in patients with CIN was significantly more frequently detected compared to the control group.

HPV was found in 65 % of cases in patients with CIN I, in 71% in patients with CIN II and in 83% in patients with CIN III.

The number of HPV positive cases was significantly higher in patients with CIN III compared to patients with CIN I ($p = 0.035$). Table 1 demonstrated patient characteristics.

Number of CD4+ cells/mm^2. The results showed that the number of CD4+ cells/mm^2 did not differ between the patients with CIN I, CIN II, and CIN III (Fig. 1). However, the number of CD4+ cells/mm^2 was significantly higher in patients with CIN I, CIN II and CIN III compared to the control group ($p < 0.05$). The number of CD4+ cells/mm^2 was not significantly different between HPV-positive and HPV-negative patients. There were no differences in patient average age, menarche, coitarche, cycle days, parity, smoking status and the number of CD4+ cells/mm2 between the groups.

Number of CD8+ cells/mm^2. Patients with CIN I, CIN II, and CIN III had significantly higher CD8+ cells/mm^2 upregulation compared to patients in the control group (12 (8–21) vs. 6 (2–10) cells/mm^2, $p < 0.0001$; 15 (6–32) vs. 6

Table 1. Patient characteristics

Patients characteristics	Number of subjects	Age, median (range)	HPV positive (cases, %)	Menarche, years	Cycle, days	Coitarche, years	Partus, number	Smoking history, number of patients
Control group	10	31 (19–57)	1 (10%)	14 (10–17)	28 (21–33)	19 (16–25)	2 (1–5)	2
CIN I	20	27 (21–42)	13 (65%)*	14 (10–16)	26 (21–30)	17 (16–22)	1 (1–3)	4
CIN II	14	29 (20–47)	10 (71%)*	14 (10–17)	27 (22–32)	18 (16–23)	2 (1–4)	3
CIN III	18	28 (20–43)	15 (83%)*; **	13 (10–17)	26 (21–30)	16 (14–20)	2 (1–4)	5

CIN I-III- cervical intraepithelial neoplasia grade I–III

*$p < 0.05$ compared to control group; **$p = 0.03$ compared to control group, one-way analysis of variance (ANOVA).

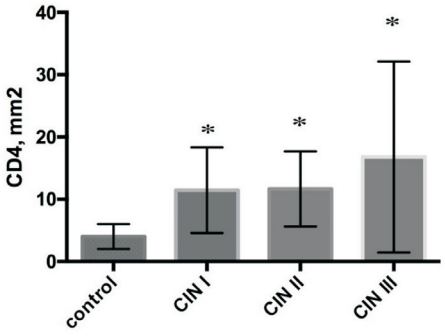

Fig. 1. The number of CD4+ cells/mm^2 tissue in the control group and patients with CIN I, CIN II, and CIN III. * $p < 0.05$ vs. control group. Kruskal–Wallis test with Dunns post test.

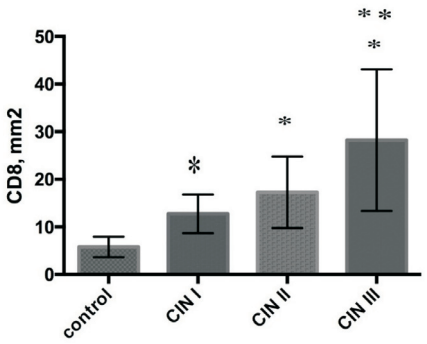

Fig. 2. The number of CD8+ cells/mm^2 tissue in in the control group and patients with CIN I, CIN II, and CIN III. * $p < 0.0001$ compared to control group; ** $p = 0.0005$ compared CIN I and CIN II; $p = 0.02$ compared to CIN II and CIN III. Kruskal–Wallis test with Dunns post test.

(2–10) cells/mm^2, $p < 0.0001$; and 28 (12–58) vs. 6 (2–10) cells/mm^2, $p < 0.0001$, respectively, Fig. 2).

In addition, patients with CIN III had higher number of CD8+ cells/mm^2 compared to patients with CIN I and CIN II (28 (12–58) vs. 12 (8–21) cells/mm^2, $p = 0.0005$ and 28 (12–58) vs. 15 (6–32) cells/mm^2, respectively, $p = 0.026$. When all patients were analysed together, there was a significant correlation between the HPV positivity and the number of CD8+ cells ($p = 0.003$).

There were no differences in patient average age, menarche, coitarche, cycle days, numbers of partus, smoking status and numbers CD8+ cells/mm^2 between the study groups.

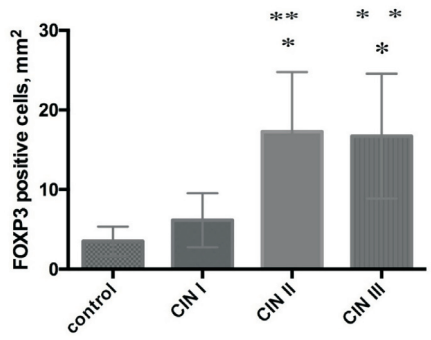

Fig. 3. The number of of FOXP3 +T lymphocytes/mm^2 tissue in the control group and patients with CIN I, CIN II, and CIN III. * $p < 0.0001$ compared CIN II vs control group; ** $p = 0.0002$ compared CIN III vs. control group; ** $p < 0.0001$, compared CIN I vs CIN II. Kruskal–Wallis test with Dunns post test.

FOXP3 T lymphocytes. The number of FOXP3+ regulatory T lymphocytes did not significantly differ between patients with CIN I and the control group. Hovewer, in patients with CIN II and CIN III, FOXP3+ regulatory T lymphocytes were significantly more upregulated in patients compared to the control group (respectively, 15 (6–32) vs. 3 (2–7) cells/mm^2, $p < 0.0001$; and 17 (2–23) vs. 3 (2–7) cells/mm^2, $p = 0.0002$, respectively, Fig. 3).

However, the number of FOXP3+ regulatory T lymphocytes did not differ in patients with CIN II and CIN III.

There were no differences in patient average age, menarche, coitarche, cycle days, numbers of partus, smoking status, and numbers of FOXP3+ regulatory T lymphocytes between the groups.

When all patients were analysed together, there was a significant correlation between HPV positivity and the number of FOXP3+ regulatory T lymphocytes (p = 0.02).

Figure 4 demonstrates a representative microphotograph of CD4, CD8 and FOXP3+ regulatory T lymphocytes in a patient with CIN.

DISCUSSION

Cervical cancer is the fourth most common cancer in women worldwide (Bedell *et al.,* 2020). Prevention of

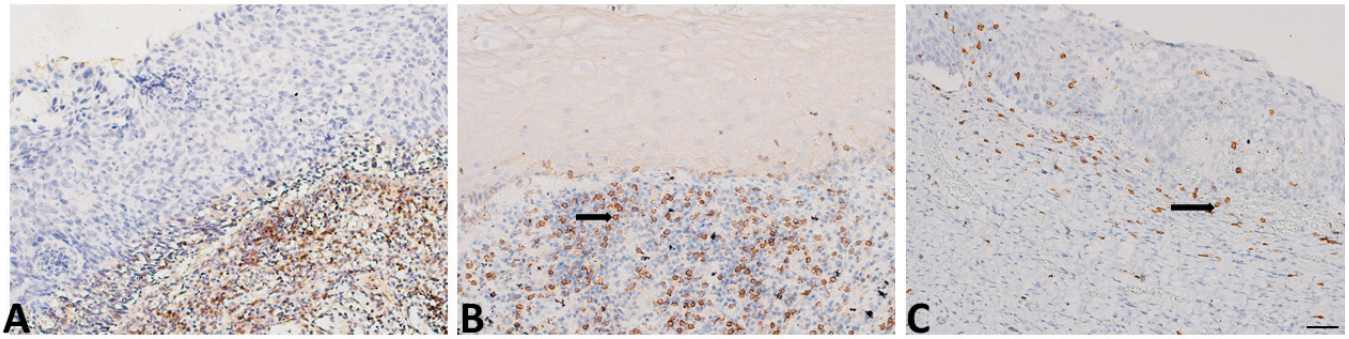

Fig. 4. Representative photomicrograph of CD4+ lymphocytes (A), CD8+ lymphocytes (B) and FOXP3 T regulatory lymphocytes (C) in patients with CIN. Immunohistochemical staining method, magnification × 200, scale bar-50 μm. The CD4+, CD8+, and FOXP3 T lymphocytes were mostly distributed in stromal tissue, some cells were observed in epithelium. Arrows indicate immunopositive cells.

HPV-related cervical cancer relies on costly HPV vaccines and repeated cervical screenings with PAP smears (Zheng *et al*., 2018).

Only a small proportion of women infected with HPV develop cervical cancer and the risk of progression to malignancy is very high with persistent HPV infection. Host immune response seems to play a role in eliminating the viral infection and preventing cancer progression (Molina *et al*., 2020). We have characterised here the subpopulations of T lymphocytes that infiltrate various grades of cervical neoplasia in immunocompetent women and could be involved in the regression or progression of dysplastic lesions.

In normal uterine cervix, CD4+ and CD8+ T cells are mostly found in subepithelial tissue compared to the epithelial compartment, and the proportion of the T-cell population is similar in both compartments (Monnier-Benoit *et al*., 2006). This profile is modulated by female hormonal status (Trifonova *et al*., 2014).

Cervical CD4+ and CD8+ T-cell infiltrates undergo alterations during infectious and neoplastic processes. In cervical tissue, the main trigger is HPV infection (Molina *et al*., 2020). In HPV-infected cervical tissue, persistent occurrence of HPV infection initiates cellular transformation, stimulating CD4+ and CD8+ T cells and mediating an immune response. When the immune response is incapable of completely clearing the tumour cells during the elimination phase, it promotes the generation of tumour cell variants with decreased immunogenicity (Molina *et al*., 2020).

Assessing stromal CD4+ and CD8+ cells can be a useful prognostic indicator of regression or persistence of CIN (Ovestad *et al.,* 2010).

In addition, it has been shown that T-cell infiltrates were predominant as the grade of the lesion progressed into more advanced lesions (Maskey *et al*., 2019).

Our study showed that the numbers of CD4 T-lymphocytes did not differ between patients with CIN I, CIN II, and CIN III, their numbers were higher than in the control group. The current study showed that patients with CIN III had significant CD8 T-lymphocytes upregulation compared to patients with CIN II and CIN I.

Our results are in line with previous findings (Maskey *et al*., 2019) and extend them by demonstrating that infiltrating CD8+ lymphocytes are upregulated in CIN III compared to CIN II and CIN I and that they can contribute to the progression of invasive carcinoma.

The number of CD8+ lymphocytes infiltrating cervical tumour mass was found to be greater than that of CD4+ lymphocytes in most of the cases, which corresponds to our finding where CD8+ were higher than CD4+TILs in severe dysplastic cases (Maskey *et al*., 2019; Bedell *et al*., 2020; Weston *et al*., 2020).

However, some studies showed that that CD8+ T cells are more abundant than CD4+ in both the stroma and epithelium, regardless of CIN grade, although the difference was not found to be statistically significant (Bedoya *et al*., 2013). However, other study showed that CD8+ T cells increase in number in cervical stroma in patients with CIN III and CIN II compared to CIN I (Brito *et al*., 2021).

The role of T regulatory lymphocytes has been studied in different malignant tumours (Shang *et al*., 2015).

Recent studies showed that T regulatory cells might be a potential biomarker to stratify cervical cancer patients and evaluate therapeutic efficacies in personalised immuno-oncology studies (Yang *et al*., 2020).

A recent meta-analysis study demonstrated increased T regulatory cell number in stromal tissue in patients with CIN III and CIN II cervical cancer (Litwin *et al*., 2021). However, the differences between T regulatory lymphocytes in normal cervical tissue and between CIN II and CIN III are still under investigation.

It has been demonstrated that HPV-derived lesions (CIN and cervical cancer) have a significantly higher number of infiltrating lymphocytes and FOXP3+ Tregs compared to three other common tumour entities — colon carcinoma, skin melanoma, and bronchial carcinoma (Loddenkemper *et al*., 2009). Lower ratios of CD25+/Foxp3+ cells in the stroma have been found in case of CIN regression (Ovestad *et al*., 2010).

A recent study showed that total FoxP3 expression (epithelium and dysplasia-connected stroma) was higher in CIN II and CIN III compared to CIN I which is consistent with the current study (Vattai *et al*., 2021).

Our results support previous evidence and extend this by demonstrating the concomitant increase of CD8 + cells and FOXP3+ cells, but not CD4+ cells, in patients with CIN III and CIN II compared to CIN I.

It could be suggested that HPV can promote the T-cell responses in the patients with CIN. In addition, previous studies showed that cytotoxic CD8 T-cell infiltrates appear to be principal effectors in eliminating HPV infected pre-neoplastic cervical epithelial cells and severe dysplastic cells, and are orchestrated by T-reg responses (Maskey *et al*., 2019; Litwin *et al*., 2021). Furthermore, our study showed that the number of FOXP3 T regulatory lymphocytes was significantly higher in patients with HSIL, compared to patients with LSIL. This may suggest that an increased activation of T regs leads to the progression of cervical neoplasia. However, the fact that HPV infection is necessary for the development of SIL and cervical carcinoma should be taken into account since various viruses have been shown to drive Treg expansion (Maskey *et al*., 2019; Bedell *et al*., 2020; Weston *et al*., 2020; Litwin *et al*., 2021). Indeed, we found a strong association between HPV infection and the numbers of T regulatory lymphocytes in patients with HSIL.

CIN II is characterised as a HSIL lesion that requires surgical treatment; however, growing evidence shows that the group of CIN II patients is very heterogenous concerning progression or regression of disease and about 50% of CIN II lesions have a potential of a regressive course (Tainio *et al*., 2018). It has been shown that the prevalence of cervical tolerogenic T cells is correlated inversely with spontaneous regression of CIN (Kojima *et al*., 2013).

In addition it is of particular importance by which manner the infiltration of CD4, CD8 T lymphocytes, and T regulatory lymphocytes in the tissue correspond to the probability of regression of CIN, which could be one potential limitation of the study, which should be addressed in future research.

A limitation of the current study is that it was a retrospective study that included only patients from one centre. A multicentric study should be aimed for as a study design for future investigations. At the same time, the strength of the study is its demonstration of the CD4+, CD8+, and FOXP3+ cell distribution in cervical tissue of both control group and CIN I, CIN II, and CIN III.

CONCLUSIONS

To conclude, our study demonstrated that patients with CIN III had increased numbers of CD8+ cells compared to patients with CIN II and CIN I, and increased FOXP3+ cell number compared to CIN I.

Up-regulation of T regulatory lymphocytes and CD8-positive T lymphocytes suggested the pivotal role for counteracting the host immune response for the progression from CIN I to CIN III, which correlated with HPV status. Prime targets for new immune-based non-invasive therapies for the HSIL treatment could be beneficial. FOXP3+ and CD8+ lymphocytes routine immunohistochemical tissue detection could be beneficial for the stratification of CIN progression/regression.

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PALIELINĀTS FOXP3+ T REGULATORU LIMFOCĪTU UN CD8+ LIMFOCĪTU SKAITS SAISTĪTS AR HPV INFEKCIJU PACIENTĒM AR SMAGĀS PAKĀPES CERVIKĀLU INTRAEPITELIĀLU NEOPLĀZIJU

T regulatorie limfocīti pieder pie CD4+ T limfocītu subpopulācijas, kura nomāc autoreaktīvās imūnās šūnas, tādējādi novēršot autoimunitāti. Mūsu darba mērķis bija novērtēt T regulatoru limfocītu, CD4+ un CD8+ T limfocītus pacientēm ar CIN I, CIN II un CIN III atkarībā no HPV infekcijas klātbūtnes. Pētījumā tika iekļautas 62 pacientes, kurām tika veikta dzemdes kakla biopsija kolposkopijas laikā. Tika veikta imūnhistoķīmiskā izmeklēšana, izmantojot CD4, CD8 un FOXP3 antivielas kontroles grupas pacientēm (n = 10), pacientēm ar CIN I (n = 20), CIN II (n = 14) un CIN III (n = 18). HPV novērtēšana tika veikta, izmantojot *Aptima* HPV kitu. Pētījumā iegūtie rezultāti parādīja, ka CD4+ T limfocītu skaits pacientiem ar CIN I, CIN II un CIN III būtiski neatšķiras. Tomēr pacientēm ar CIN II un CIN III CD8+ T limfocītu skaits bija būtiski palielināts salīdzinājumā ar pacientēm ar CIN I. Turklāt, pacientēm ar CIN II un CIN III tika novērots palielināts FOXP3+ T regulatoru limfocītu skaits salīdzinājumā ar pacientēm ar CIN I, kas korelē ar HPV infekcijas klātnūtni. Pētījumā iegūtie rezultāti parādīja būtisku CD8+ T limfocītu un T regulatoru limfocītu nozīmi dzemdes kakla displāzijas progresijai no vieglās uz smagās pakāpes displāziju.