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Original Research

Immune Imbalance in Th1/Th2 and Th17/Treg as a Contributor to Endometrial Polyps: A Prospective Laboratory-Based Study

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Abstract

Background: From an immunological perspective, changes in cytokine levels can shift the immune response, potentially affecting the development of endometrial polyps (EPs). The relationship between the balance of T helper 1 (Th1)/T helper 2 (Th2) and T helper 17 (Th17)/Regulatory T (Treg) cytokines and the pathogenesis of EPs is an area of ongoing research. This study aims to investigate the role of Th1/Th2 and Th17/Treg immune imbalances in the pathogenesis of EPs. **Methods**: A total of 79 patients were included in this prospective laboratory-based study. The experimental group included 20 patients with single EPs, 20 with multiple EPs, and 19 with postmenopausal EPs. The control group included 20 individuals with proliferative endometriums. Immunohistochemical staining was performed to investigate the expression of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ). **Results**: The levels of TNF-α, IFN-γ, IL-17 and transforming growth factor-β (TGF-β) in the single EP group were higher than in the control group ($p_{\text{TNF-}\alpha} = 0.001$, $p_{\text{IFN-}\gamma} < 0.001$, $p_{\text{IL-17}} < 0.001$, $p_{\text{TGF-}\beta} < 0.001$), while the level of IL-6 in the stroma was lower than in the control group ($p_{\text{IL-6}} < 0.001$). The levels of IL-2, IFN-γ, IL-4, IL-17 and TGF-β in the glands of the multiple EPs group were higher than in the glands of the control group ($p_{\text{IL-2}} = 0.041$, $p_{\text{IFN-}\gamma} < 0.001$, $p_{\text{IL-4}} = 0.044$, $p_{\text{IL-17}} < 0.001$, $p_{\text{TGF-}\beta} < 0.001$). The levels of IL-4, IL-6, TNF-α, and IL-17 in the postmenopausal single polyp group were lower than in the total control group ($p_{\text{IL-4}} = 0.020$, $p_{\text{IL-6}} = 0.002$, $p_{\text{TNF-}\alpha} < 0.001$, $p_{\text{IL-17}} = 0.006$), while the levels of IL-2, IFN-γ and TGF-β in the glands were higher than in the control group ($p_{\text{IL-2}} = 0.002$, $p_{\text{IFN-}\gamma} = 0.002$, $p_{\text{TGF-}\beta} = 0.005$). **Conclusion**: Th1/Th2 and Th17/Treg immune imbalances are one of the causes of EPs.

Keywords: endometrial polyps; Th1/Th2; Th17/Treg; cytokines

1. Introduction

Endometrial polyps (EPs) are composed of endometrial glands and fibrotic endometrial stroma with thick-walled blood vessels [1]. They can be single or multiple, with diameters ranging from several millimeters to several centimeters [2]. EPs often occur in 40–50-year-old and postmenopausal women [3]. Up to 82% of EP patients have no clinical symptoms, and a few may show abnormal uterine bleeding [4].

The formation of EPs may be related to the imbalance of estrogen and progesterone receptors, inflammation, oxidative stress, abnormal expression of cytokines, and an imbalance between cell proliferation and apoptosis [5–8]. Evidence suggests that EPs are closely related to estrogen. However, after menopause, despite the decline in ovarian function and the decrease in systemic circulating estrogen levels, the incidence of EPs does not decrease. A study has found that postmenopausal EP is mainly due to the absolute lack of progesterone, which results in the improper functioning of progesterone receptors, thereby promoting the development of EPs [9].

From the perspective of immunology, the change in cytokines may cause the entire immune response to shift in a certain direction, resulting in a completely different outcome. Helper T cells (Th) and regulatory T cells (Treg), both subsets of $CD4^+$ T lymphocytes, play pivotal roles in immune modulation and stimulation [10]. The cytokines secreted by these cells are intimately associated with the processes of inflammation and cellular proliferation. In recent years, with the discovery of Th17 cells, the traditional Th1/Th2 balance model has been further developed into the T helper 1 (Th1)/T helper 2 (Th2) and T helper 17 (Th17)/Regulatory T (Treg) balance model [11]. Under normal circumstances, the number of these subsets of cells maintains a dynamic balance through the mutual regulation of cytokines secreted by them, and participates in the regulation of body immunity. It has been found that the subsets of Th1, Th2, Th17 and Treg cytokines, representative of $CD4^+$ T cells, are related to the development of EPs. However, the relationship between the equilibrium state of Th1/Th2, Th17/Treg and EPs in EPs has not been reported. In this experiment, Th1 representative cy-

tokines such as interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), Th2 representative cytokines interleukin-4 (IL-4), interleukin-6 (IL-6), Th17 representative cytokines interleukin-17 (IL-17), and Treg representative cytokine transforming growth factor-β (TGF-β) were used to detect the cytokines in the tissues of patients with EPs. It is expected to explain the pathogenesis of EPs from the perspective of immunology and provide some theoretical basis for the pathogenesis of EPs.

2. Materials and Methods

2.1 Inclusion and Exclusion Criteria

This prospective laboratory-based study was approved by the Ethics Committee of the Changzhou No. 2 People's Hospital (approval number: 202123). A total of 79 patients were included in this study conducted at our hospital from August 2020 to April 2021. The experimental group consisted of 59 patients, including 20 patients in the single polyp group, 20 patients in the multiple polyps group (number of polyps >5), and 19 patients in the postmenopausal single polyp group. The control group included 20 patients without polyps but with abnormal uterine bleeding, pathologically diagnosed as having a "proliferative endometrium". Additionally, we collected endometrial tissue from more than 2 cm away from the single polyp group, referred to as the para-polyp endometrial group (n = 20).

Inclusion criteria: (1) transvaginal color Doppler ultrasonography suggested the possibility of EPs. (2) Patients who signed informed consent and were willing to cooperate with the treatment. (3) Clinical and pathological data of all patients could be collected through inpatient and outpatient information systems, including age, body mass index (BMI), previous EPs operation history, etc.

Exclusion criteria: (1) Pathological type was atypical hyperplasia or endometrial carcinoma. (2) Patients who had received hormone therapy in the past three months, including those with autoimmune, allergic and acute inflammatory diseases, as well as serious internal and surgical diseases. (3) Patients with an intrauterine device (IUD). (4) Patients suffering from uterine leiomyoma, adenomyoma and endometriosis. (5) Patients diagnosed with EPs during pregnancy.

2.2 Standards for Specimen Collection

After routine preoperative preparation, KARL STORZ video hysteroscopy was performed within 3–7 days after menstruation. The procedure is as follows: dilate the uterus with normal saline, uterine pressure is controlled to 12$\sim$14 kPa, intravenous anesthesia, the patients routinely disinfect the vulva, vagina and cervix, and then slowly put the instrument through television monitoring to observe the size and shape of the uterus, cervical canal, uterine horn and endometrium, and to observe the presence of foreign bodies. If there are polyps, hysteroscopic surgery is performed after observing the size, shape and number of polyps. After the operation, a diagnostic uterine curettage was conducted to excise the polyp tissue, which was subsequently preserved in 10% formalin and dispatched for pathological examination.

2.3 Immunohistochemical Detection of Cytokines

The expression levels of Th1 (TNF-α, IFN-γ, IL-2)/Th2 (IL-4, IL-6) and Th17 (IL-17) cells in the pathological tissues of EPs were detected using immunohistochemistry and compared with the control group.

Cytokine density was analyzed by evaluating tissue segments at a magnification of $\times$10, identifying six areas with high cytokine marker accumulation (brown). These areas were then counted at a magnification of $\times$40, and the mean positive cell value was determined for each segment. The sections stained by immunohistochemistry were examined by 2 blinded pathologists.

The antibodies were from Boster Biological Technology Co., Ltd., Wuhan, Hubei, China: Rabbit anti-human IL2 Antibody (1:400; Cat# BA1644-1); Rabbit anti-human IL-4 Monoclonal antibody (1:400; Cat# 66142-1-Ig); Rabbit anti-human IL-6 polyclonal antibody (1:100; Cat# 66146-1-Ig); Mouse anti-human IL-17 monoclonal antibody (1:40; Cat# 66148-1-Ig); Rabbit anti-human TNF-α polyclonal antibody (1:500; Cat# 60291-1-Ig); Rabbit anti-human IFN-γ polyclonal antibody (1:300; Cat# A00393-3); Rabbit anti-human TGF-β polyclonal antibody (1:600; Cat# BA0290). Histochemical kit DAB chromogenic agent (Cat# G1211, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China); MaXvision secondary antibody detection kit (Cat# KIT-5005; Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, Fujian, China); Pv-9000 secondary antibody detection kit (Cat# PV-9000; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China); Bond Polymer Refine Detection Leica Biosystems Newcastle Ltd. (Newcastle, Tyne and Wear, UK), the above reagents and instruments are provided by the department of Pathology.

2.4 Statistical Analysis

All statistical analyses were performed using SPSS 25.0 software (version 25.0; IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variances was tested using Levene's test. For continuous variables with normal distribution, analysis was performed using an independent-samples *t* test, and the data were expressed as mean $\pm$ standard deviation (mean $\pm$ s). For continuous variables with non-normal distribution, a nonparametric test with 2 independent samples (Mann-Whitney U test) was used for analysis, and the data were expressed as median (*P*25, *P*75). The qualitative data were described by the number of cases or percentage and assessed using Pearson's chi-square test or Fishers exact test. $p < 0.05$ was considered statistically significant.

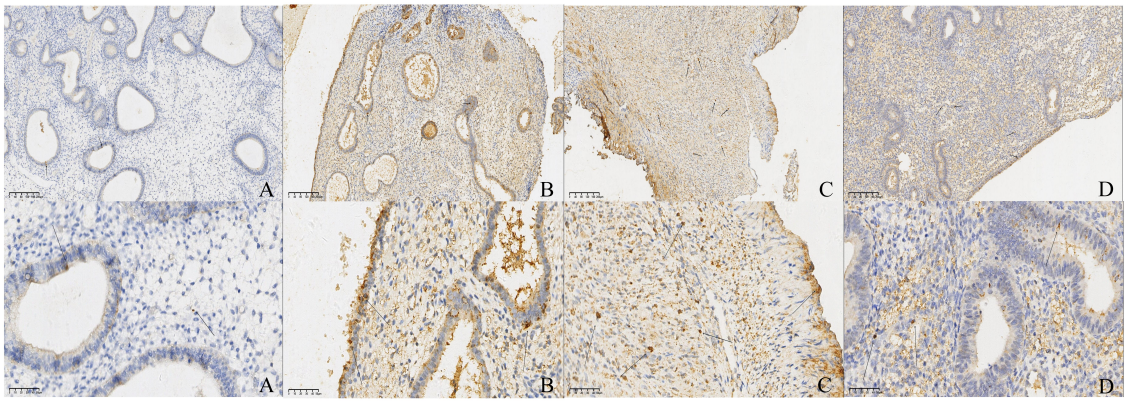

Fig. 1. The number of positive cells for IL-2 (black arrow) in the gland was significantly higher in B and C than in group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. IL-2, interleukin-2; EPs, endometrial polyps. Scale bar: 200 μm (line 1); Scale bar: 50 μm (line 2).

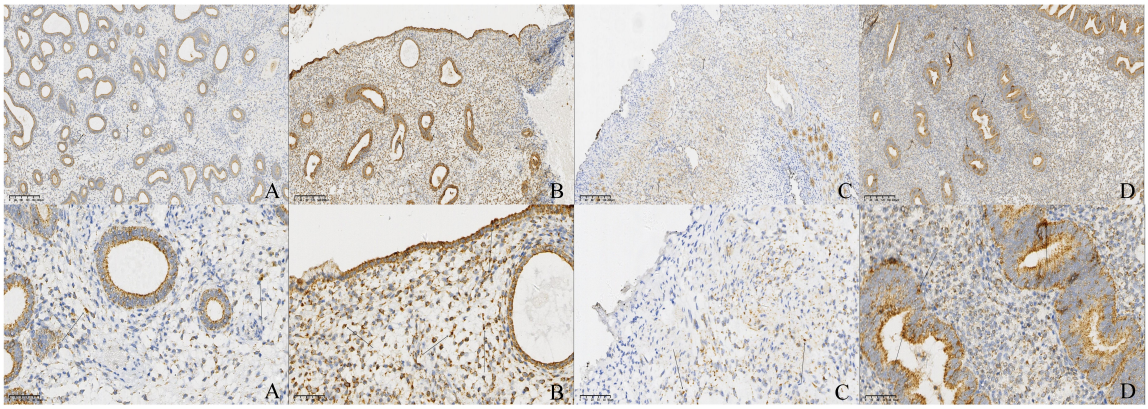

Fig. 2. The number of positive cells for IL-4 (black arrow) in the stroma was lower in group C than in group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. IL-4, interleukin-4. Scale bar: 200 μm (line 1); Scale bar: 50 μm (line 2).

3. Results

3.1 Expression and Distribution of Th1, Th2, Treg and Th17 Cytokines in EPs Tissues

In the first phase of the study, EPs were evaluated using a magnification of $\times$10 to analyze the density of cytokines, and six cytokine markers (IL-2, IL-4, IL-6, IL-17, TNF-α, IFN-γ) with significant accumulation regions identified. Subsequently, the expression differences of cytokine markers in the tissue were examined at a magnification of $\times$40. The positive cells appeared brown and were located in the cytoplasm. All six cytokines were expressed in the tissues of EPs (Figs. 1,2,3,4,5,6).

3.2 Comparison of Th1, Th2, Treg and Th17 Cytokines in EPs Tissue

In the comparison between the single polyp group and the control group: the total number of TNF-α, IFN-γ, IL-17, and TGF-β positive cells in the epithelium tissue of the single polyp group was significantly higher than that in the control group ($p_{\text{TNF-}\alpha} = 0.001$, $p_{\text{IFN-}\gamma} < 0.001$, $p_{\text{IL-17}} < 0.001$, $p_{\text{TGF-}\beta} < 0.001$), while the number of IL-6 positive cells in the stroma was significantly lower than in the control group ($p_{\text{IL-6}} < 0.001$).

In the comparison between the Multiple polyps group and the control group: the total number of IL-2, TNF-α, IFN-γ, IL-4, IL-17 and TGF-β positive cells in the EPs tissue of the multiple polyps group was significantly higher than in the control group ($p_{\text{IL-2}} = 0.041$, $p_{\text{TNF-}\alpha} = 0.046$, $p_{\text{IFN-}\gamma} < 0.001$, $p_{\text{IL-4}} = 0.044$, $p_{\text{IL-17}} < 0.001$, $p_{\text{TGF-}\beta} < 0.001$).

In the comparison between the postmenopausal single polyp group and the control group: the total number of IL-4, IL-6, TNF-α and IL-17 positive cells in the postmenopausal single polyp was significantly lower than in the control group ($p_{\text{IL-4}} = 0.020$, $p_{\text{IL-6}} = 0.002$, $p_{\text{TNF-}\alpha} < 0.001$, $p_{\text{IL-17}} = 0.006$), while the number of IFN-γ and TGF-β positive cells in the gland was significantly higher than in the control group ($p_{\text{IL-2}} = 0.002$, $p_{\text{IFN-}\gamma} = 0.002$, $p_{\text{TGF-}\beta} = 0.005$) (Table 1).

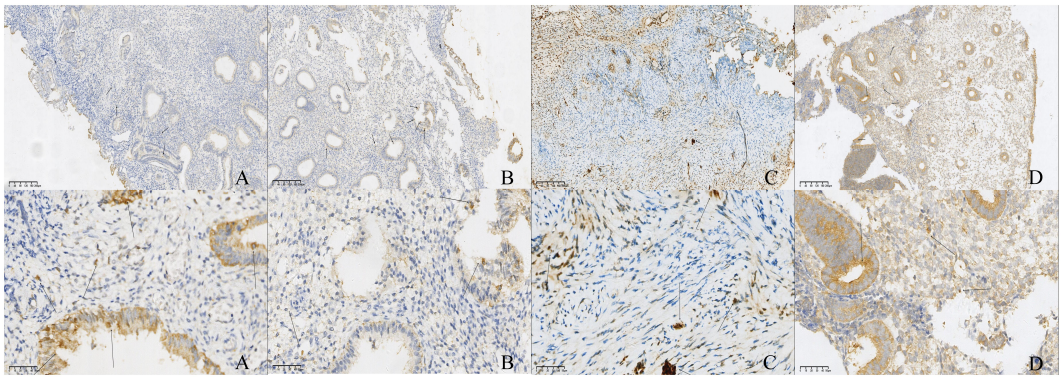

Fig. 3. The number of positive cells for IL-6 (black arrow) in the stroma and gland was significantly lower in groups A, B and C than in group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. IL-6, interleukin-6. Scale bar: 200 μm (line 1); Scale bar: 50 μm (line 2).

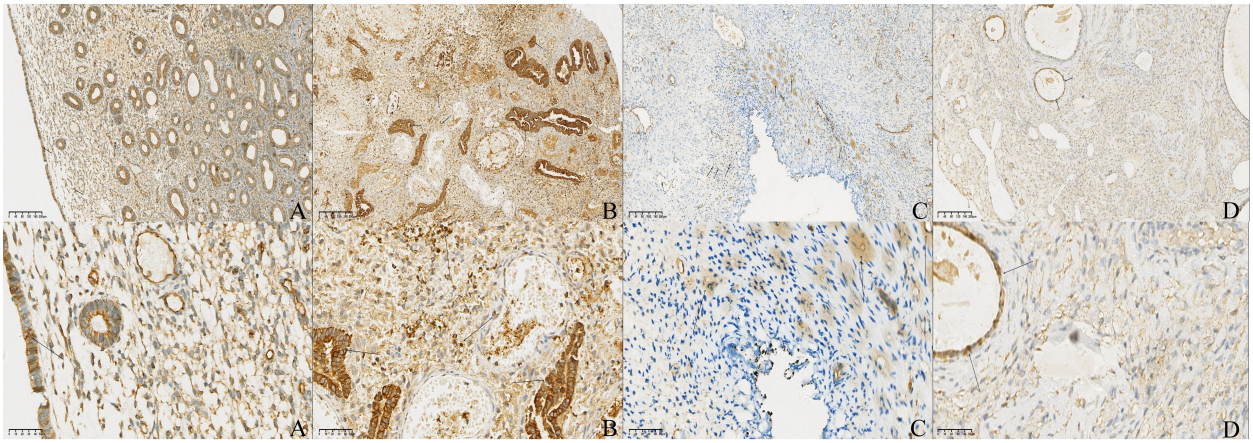

Fig. 4. The number of positive cells for IL-17 (black arrow) in the epithelium and gland was significantly different in group A, B than in group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. IL-17, interleukin-17. Scale bar: 200 μm (line 1); Scale bar: 50 μm (line 2).

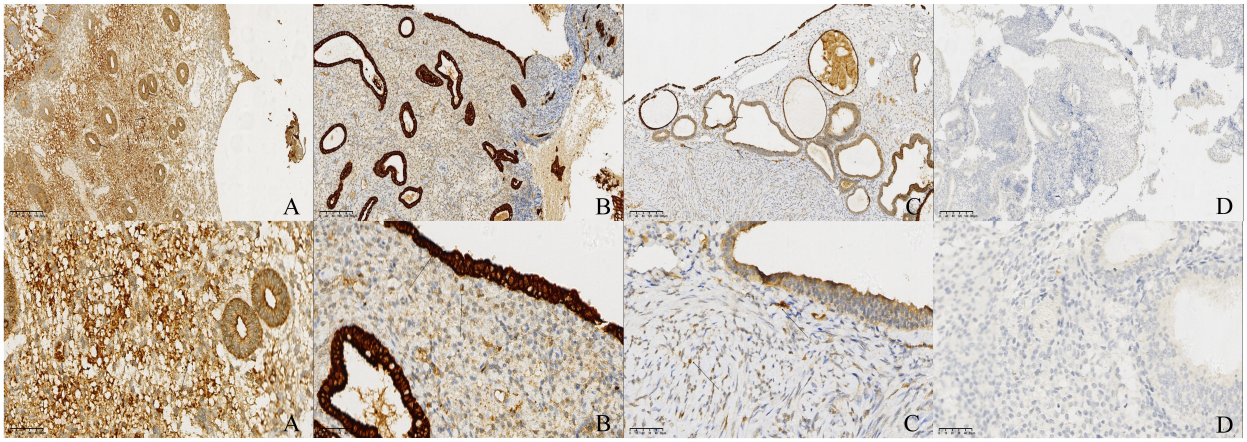

Fig. 5. The number of positive cells for TNF-α (black arrow) in the stroma and gland was significantly higher in groups A and B than in group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. TNF-α, tumor necrosis factor-α. Scale bar: 200 μm (line 1); Scale bar: 50 μm (line 2).

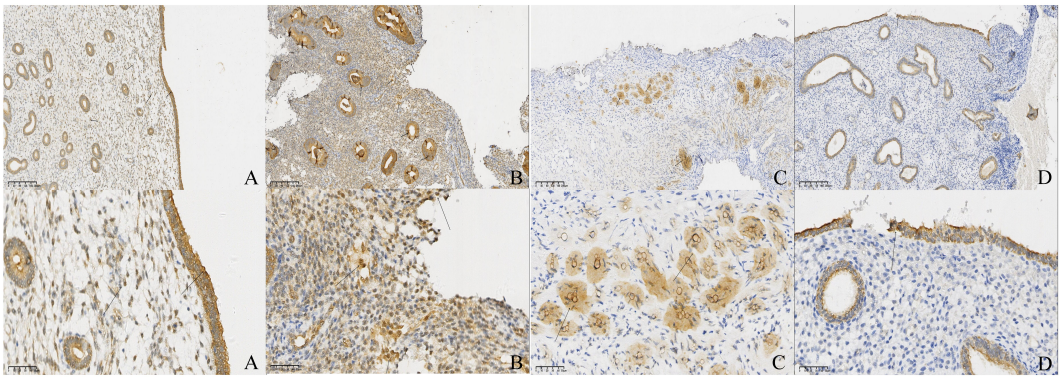

Fig. 6. The number of positive cells for IFN-γ (black arrow) in the gland was significantly higher in group B than in group D. However, the number of positive cells in the epithelium of Group A showed no significant difference compared with Group D. (A) Single EP; (B) multiple EPs; (C) postmenopausal EP; (D) Control group. IFN-γ, interferon-gamma. Scale bar: 200 µm (line 1); Scale bar: 50 µm (line 2).

3.3 Comparison of Cytokine Expression Between the Single Polyp Group and the Multiple Polyps Group, and Between the Single Polyp Group and the Postmenopausal Single Polyps

The total number of cells positive for IL-17 ($p_{\text{IL-17}} < 0.001$) was significantly higher in the single group than in the multiple group. The number of cells positive for TGF-β ($p_{\text{TGF-}\beta} < 0.001$) in total was significantly lower in the single group than in the multiple polyp group (Fig. 7A). The total number of cells positive for IL-2 ($p_{\text{IL-2}} < 0.001$), IL-4 ($p_{\text{IL-4}} < 0.04$), IL-6 ($p_{\text{IL-6}} < 0.03$) was significantly lower in the single group than in the multiple polyp group, respectively (Fig. 7B).

The total number of positive cells for IL-17 ($p_{\text{IL-17}} < 0.001$) and TGF-β ($p_{\text{TGF-}\beta} < 0.001$) was significantly higher in the single group than in the postmenopausal group, respectively (Fig. 7C). The total number of TNF-α ($p_{\text{TNF-}\alpha} < 0.001$), IFN-$\gamma$ ($p_{\text{IFN-}\gamma} < 0.001$), IL-4 ($p_{\text{IL-4}} = 0.002$) and IL-6 ($p_{\text{IL-6}} = 0.003$) positive cells in EPs tissue of single polyp group was significantly higher than in the postmenopausal group (Fig. 7D).

4. Discussion

In this study, we found that: (1) In the tissue of patients with single and multiple Eps, the balance of Th1/Th2 and Th17/Treg subsets of $CD4^+$ T cells shifted to Th1 and Th17, which may be an important reason for the occurrence of EPs in the single polyp group. (2) Compared with the multiple polyps group, the cellular immune balance Th17/Treg of EPs in the single polyp group shifted more significantly to Th17, indicating that the shift of Th17/Treg balance to Th17 was not more pronounced with the increase in the number of polyps. (3) Different from premenopausal single and multiple EPs, in the postmenopausal EP patients, the balance of Th1/Th2 and Th17/Treg subsets of $CD4^+$ T cells shifts to Th1 and Treg, which may be an important cause of postmenopausal EP.

Th1 cells primarily secrete IL-2, TNF-α, and IFN-γ, guiding both the innate and acquired immune systems to produce cytokines and antibodies, activate macrophages, and promote cellular immunity, which is linked to inflammation and tissue damage [12]. In this study, we found that there was a high expression of Th1 cytokines in EPs tissues of single, multiple and postmenopausal single polyps, which was consistent with most scholars' studies. TNF-α is highly expressed in EPs, and the expression of TNF-α and IL-6 is negatively correlated with the expression of progesterone receptor. It is analyzed that the decrease in progesterone receptors weakens the inhibitory effect of progesterone on estrogen receptors, resulting in a sustained high level of estrogen receptors, resulting in polyps [13]. In EP patients with infertility, the value of TNF-α in uterine lavage fluid and serum before surgery were significantly higher than in the infertility patients without EPs in the middle stage of endometrial proliferation. The concentrations of TNF-α in uterine lavage fluid and serum in patients with EPs after surgery were significantly lower than those before surgery ($p < 0.05$) [14]. The levels of IFN-γ in serum and endometrium were significantly higher than those in infertile patients without EPs [15]. Based on this, we can speculate that the high expression of Th1 cytokines mediates inflammation and immune injury, leading to the occurrence of EPs.

Th2 cells stimulate B cells to produce antibodies, promote eosinophil and mast cell differentiation, inhibit immune inflammation and reduce tissue damage. In this study, we found that there was a low expression of Th2 cytokines in both single and postmenopausal EP patients, which was consistent with another study: during the proliferative phase of the endometrium, the contents of IL-6 and IL-10 in uterine lavage fluid of EPs patients increased significantly [16]. Based on this, we can speculate that the low expression of Th2 cytokines and insufficient immune protection lead to the occurrence of EPs.

Table 1. Comparison of Th1, Th2, Treg and Th17 cytokines in EP tissues.

Group	Cytokine	Cytokine expression in different locations (Immunohistochemical count, per 40× field)			
		Epithelium	Stroma	Gland	Total
Control group (n = 20)					
	IL-17	30.12 ± 14.88	69.00 (48.25, 128.25)	53.00 (44.75, 66.62)	48.0 (30.50, 70.00)
	IL-2	34.58 ± 13.73	118.25 (74.75, 186.00)	42.75 (19.25, 58.50)	44.75 (26.88, 74.38)
	IL-4	43.92 ± 14.20	189.25 ± 79.51	54.95 ± 24.74	55.00 (43.00, 165.88)
	IL-6	53.50 ± 12.31	292.18 ± 71.75	82.50 (62.12, 94.88)	82.50 (57.75, 265.25)
	TNF-α	47.00 (43.75, 52.62)	84.25 (38.75, 153.25)	84.33 ± 37.86	62.75 (44.75, 96.88)
	IFN-γ	33.75 (24.38, 42.75)	81.25 (44.00, 129.38)	44.25 (22.50, 53.25)	44.00 (24.50, 63.75)
	TGF-β	22.15 ± 11.11	48.00 (16.50, 79.00)	36.50 (15.75, 47.25)	28.25 (16.25, 48.25)
Single polyp group vs. Control group (n = 20)					
	IL-17	50.80 ± 8.08***	255.18 ± 65.70***	138.00 (124.12, 150.88)***	136.50 (54.75, 208.50)***
	IL-2	37.71 ± 19.12	39.50 (10.00, 168.75)	42.00 (16.00, 69.25)	39.50 (15.50, 64.00)
	IL-4	40.60 ± 9.80	166.75 ± 63.32	64.72 ± 21.92	61.75 (43.88, 109.12)
	IL-6	45.62 ± 16.40	188.40 ± 72.04***	78.42 ± 31.41	77.75 (49.38, 157.50)
	TNF-α	51.25 (47.12, 69.75)	239.25 (112.50, 260.38)**	120.90 ± 42.71**	104.50 (58.38, 150.12)**
	IFN-γ	58.33 ± 24.22***	216.75 ± 86.58***	92.45 ± 47.88***	89.25 (51.62, 173.38)***
	TGF-β	44.95 ± 14.31***	125.92 ± 53.98***	72.65 ± 19.99***	69.75 (46.38, 97.12)***
Multiple polyps group vs. Control group (n = 20)					
	IL-17	47.08 ± 14.68***	182.18 ± 71.07***	97.40 ± 24.82***	95.75 (60.38, 134.62)***
	IL-2	41.27 ± 10.39	141.57 ± 58.87	51.75 (38.88, 79.75)*	56.25 (39.50, 119.62)*
	IL-4	49.15 ± 11.94	196.35 ± 56.48	80.45 ± 22.08**	75.25 (54.25, 158.5)*
	IL-6	46.05 ± 17.94	247.32 ± 99.75	103.88 ± 51.30	103.25 (50.62, 193.00)
	TNF-α	57.75 (47.38, 63.12)	209.50 (34.75, 258.25)	105.20 ± 35.52	89.75 (50.25, 142.00)*
	IFN-γ	53.60 ± 21.78*	240.25 (148.88, 265.62)**	101.30 ± 40.83***	81.50 (56.38, 169.38)***
	TGF-β	42.00 (33.50, 49.25)***	149.95 ± 84.86***	90.38 ± 31.01***	81.25 (42.75, 127.12)***
Postmenopausal single polyps vs. Control group (n = 19)					
	IL-17	25.79 ± 10.58	33.00 (26.50, 72.50)**	53.00 (32.50, 60.50)	34.00 (23.00, 59.00)**
	IL-2	35.32 ± 9.58	148.08 ± 62.13	60.39 ± 19.14**	57.50 (37.50, 108.50)
	IL-4	29.92 ± 6.51***	84.76 ± 44.00***	51.82 ± 11.46	48.00 (31.00, 68.00)*
	IL-6	31.37 ± 14.85***	140.58 ± 98.74***	79.42 ± 46.17	58.50 (31.00, 111.50)**
	TNF-α	30.61 ± 7.74***	44.00 (31.25, 80.50)*	46.50 (42.50, 52.00)**	41.00 (29.50, 48.00)***
	IFN-γ	31.11 ± 10.43	50.97 ± 29.48	72.37 ± 27.44**	49.50 (29.00, 70.50)
	TGF-β	24.76 ± 7.59	17.00 (11.25, 38.00)*	54.82 ± 24.86**	29.50 (17.00, 47.00)

Data indicates the number of positive cells. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. TGF-β, transforming growth factor-β.

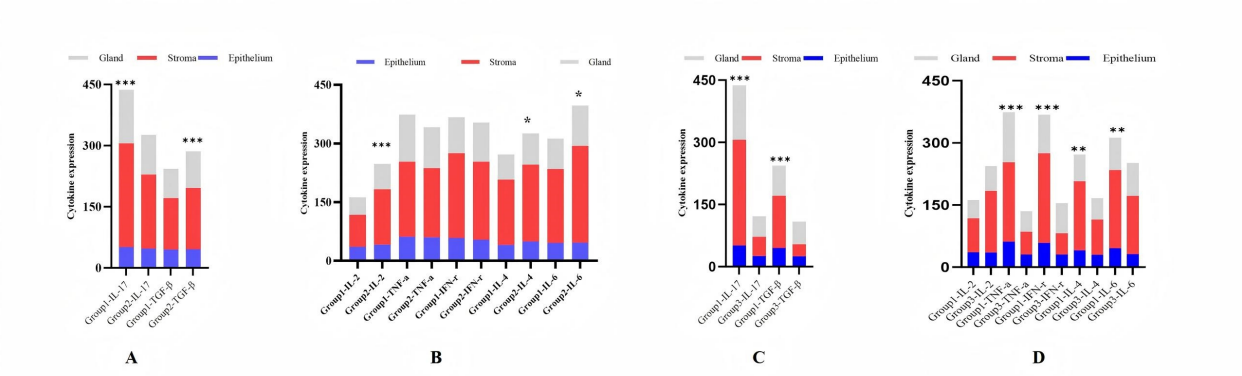

Fig. 7. Comparison of Th1/Th2 and Th17/Treg Cytokine Expression Levels in Polyp Tissues among the Three Experimental Groups. (A) Comparison of Th17/Treg Cytokine Expression in Polyp Tissues between Group 1 and Group 2. (B) Comparison of Th1/Th2 Cytokine Expression in Polyp Tissues between Group 1 and Group 2. (C) Comparison of Th17/Treg Cytokine Expression in Polyp Tissues between Group 1 and Group 3. (D) Comparison of Th1/Th2 Cytokine Expression in Polyp Tissues between Group 1 and Group 3. Group 1: single group; Group 2: multiple group; Group 3: postmenopausal group. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Annotation: Since the postmenopausal group only selected individuals with single polyps, in order to ensure the rigor of the experiment (by controlling the single variable: different numbers of polyps), the two groups were not compared. Th17, T helper 17; Treg, Regulatory T; Th1, T helper 1; Th2, T helper 2.

Under normal circumstances, Th1 and Th2 cells are in dynamic balance and interact with negative feedback regulation. In normal reproductive women, the balance of Th1/Th2 cells moves to Th2 [17]. The results showed that the expression levels of Th1 cytokines IL-2, IFN-γ and TNF-α in the peripheral blood of patients with EPs were significantly increased, while the expression levels of Th2 cytokines IL-4 and IL-6 were decreased ($p < 0.05$). The expression of IL-10 was slightly lower, but there was no significant difference ($p > 0.05$). The above results confirmed that the occurrence of EPs is related to the balance/shift of Th1/Th2 cells to Th1. Reversing the imbalance of Th1/Th2 can prevent the postoperative recurrence of EPs. Changing the expression of cytokines in the uterine cavity of patients with EPs can shift the balance of Th1/Th2 cells towards Th2, reducing the local inflammatory reaction. The content of INF-γ significantly decreases, while the content of IL-6 significantly increases. This can prevent the recurrence of EPs.

Th17 cells are a new class of $CD4^+$ T cell subsets that can secrete IL-17 at high levels and participate in inflammatory responses and autoimmune diseases [18]. In this study, we found that IL-17 was highly expressed in the epithelium, stroma and glands of Eps in both the single and multiple polyps group. Some studies have found that the Th17 response is up-regulated in the peripheral blood of patients with EPs, leading to increased secretion of many pro-inflammatory factors, such as IFN-γ, TNF-α, IL-17, and IL-6 [19,20]. This supports our conclusion that the high expression of Th17-type cytokines in the polyps of premenopausal EP patients promotes inflammatory responses, thereby contributing to the occurrence of premenopausal EPs.

Treg cells secrete TGF-β, which participates in the regulation of immunosuppression. TGF-β may play an important regulatory role in the proliferation and fibrosis of intimal stroma and the formation and development of thick-walled vessels in EPs [5,21]. In this study, we found that there was a high expression of TGF-β in the epithelium, stroma and glands of EPs in both the single and multiple polyps group. This is consistent with other findings that the expression of TGF-β1 in stroma cells of EPs is significantly higher than in the normal endometrium, whether in the proliferative phase or secretory phase. The increased expression of TGF-β1 in EP tissue can increase the risk of postoperative uterine adhesion due to the overexpression of TGF-β1, which increases the production of extracellular matrix and inhibits the growth of epithelial cells [22]. Therefore, we conclude that the high expression of TGF-β1 in premenopausal polyps promotes interstitial hyperplasia, fibrosis and the formation and development of thick-walled blood vessels. However, the immunosuppressive effect is relatively insufficient, thus contributing to the occurrence of premenopausal EPs.

This study had several limitations. First, the sample size collected for the study is limited; except for 19 cases of postmenopausal single polyps, there were only 20 cases in each of the other groups. However, it meets the minimum sample size required for statistical analysis. In addition, this study only involved experimental analysis of specimens collected from surgeries of EP patients. As the research is still ongoing, we did not choose to include peripheral blood for further validation analysis. Furthermore, the expression of cytokines in this study was assessed using immunohistochemistry, rather than the more commonly used and more precise flow cytometry. Finally, this study was limited to

finding the differential expression of inflammatory factors in EPs and cannot directly prove a causal relationship.

5. Conclusion

To sum up, the regulation of the uterine environment depends not only on the balance of Th1/Th2 cells but also on the balance of Th17/Treg cells. In the tissues of patients with single and multiple EPs, the balance of CD4+ T cell subsets Th1/Th2 and Th17/Treg shifts towards Th1 and Th17. Compared with the multiple group, the cellular immune Th17/Treg balance of EPs in the single group shifted more significantly to Th17, indicating that the shift of Th17/Treg balance to Th17 was not more obvious with the increase of the number of polyps. In the tissues of postmenopausal EPs patients, the balance of $CD4^+$ T cell subsets Th1/Th2 and Th17/Treg shifts towards Th1 and Treg expression, and the specific mechanism needs to be further studied.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

HY and SL designed the research study. HY and LB performed the research. SL and LB provided help and advice on the immunohistochemistry. HY analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Changzhou No. 2 People's Hospital (approval number: 202123).

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Conflict of Interest

The authors declare no conflict of interest.

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