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Original Article

Relationship between peripheral blood CD3-+ HLA-DR cells and IL-21 levels and ovarian function in patients with premature ovarian failure

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Abstract: Objective: This study aimed to discuss the relationship between peripheral blood (PB) CD3-+ HLA-DR cells and interleukin-21 (IL-21) levels and ovarian function in patients with premature ovarian failure (POF). Methods: Study group (SG) included 53 POF patients and control group (CG) included 53 healthy women of child-bearing age with regular menstruation in the same stage, and the CD3-+ HLA-DR cells, IL-21 levels, six sex hormones and immune cell indices were detected. Pearson correlation coefficient was used to analyze the correlation of CD3-+ HLA-DR cells and IL-21 levels with six sex hormones and immune cell indices. Results: (1) The percentage of CD3-+ HLA-DR cells and IL-21 levels in SG were much higher than those in CG (P<0.05). (2) The levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in SG were much higher than those in CG (P<0.05), but the levels of estradiol (E2), progesterone (P), testosterone (T) and prolactin (PRL) in SG were much lower than those in CG (P<0.05). (3) The levels of CD3+ and CD8+ in SG were much higher than those in CG (P<0.05), but the levels of CD4+ and CD4+/CD8+ in SG were much lower than those in CG (P<0.05). (4) There was a positive correlation between CD3-+ HLA-DR and IL-21 levels (r=0.520, P<0.001). CD3-+ HLA-DR was positively correlated with FSH, LH, CD3+ and CD8+ (P<0.05), and was negatively correlated with E2, P, PRL, CD4+ and CD4+/CD8+ (P<0.05). IL-21 was positively correlated with FSH, LH, CD3+ and CD8+ (P<0.05), and was negatively correlated with E2, P, PRL and CD4+/CD8+ (P<0.05). Conclusion: PB CD3-+ HLA-DR cells and IL-21 levels were highly expressed in POF patients, which was closely related to ovarian function. This implied that the increase of CD3-+ HLA-DR cells and IL-21 levels may be associated with POF.

Keywords: Premature ovarian failure, CD3-+ HLA-DR cells, interleukin-21, ovarian function

Introduction

Premature ovarian failure (POF) refers to the amenorrhea caused by ovarian function failure in women under the age of 40 years, usually complicated with reduction of estrogen, increase of gonadotropin and different degrees of hypoestrogenic symptoms, such as being flush, hot flashes and, hidrosis, *etc*. The incidence of POF is about 1%-3% in women of child-bearing age and POF patients tend to present as younger patients with the global changes of environment and dietary structure [1, 2]. The pathogenesis of POF is still unclear. Most studies have shown that genetic factors, autoimmunity and chemoradiotherapy were major causes of POF and may cause permanent damage to the ovary [3, 4]. POF is a progressive disease without any obvious symptoms in the early stage. Most patients fail to see a doctor before advanced stage, making the treatment more difficult. Therefore, early detection of POF is of great significance to its treatment.

POF has been identified as an autoimmune endocrine disease in most studies [5]. The six sex hormones are the experimental indices for the clinical diagnosis of ovarian function, and the main diagnostic indices include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) [6]. Human leucocyte antigen (HLA) is the product of coding HLA gene complex, showing HLA-DR expression in various lymphocytes. HLA-DR is overexpressed in autoimmune diseases such as autoimmune

thyroiditis and insulin dependent diabetes mellitus [7]. However, it is currently controversial whether the expression of HLA-DR is abnormal in POF. (Interleukin-21) IL-21 is closely related to T cell function and plays a crucial role in acquired and innate immunity, but the expression of IL-21 in POF is rarely reported [8].

This study aimed to detect PB CD3-+ HLA-DR cells and IL-21 levels, six sex hormones and immune cell indices and analyze the correlation of CD3-+ HLA-DR cells and IL-21 levels with the six sex hormones and immune cell indices so as to find the pathogenesis of POF and provide reference for preventative examination, early diagnosis and timely treatment, thus to enhance fertility.

Materials and methods

General material

In total, 53 POF patients were included in the study group (SG). Inclusion criteria: This study included ① patients under the age of 40 years with an amenorrhea period of over 6 months; ② patients with FSH level >40 UI/L and E2 level >73.2 pmol/L (examination interval was1 month); and ③ patients not taking any hormone drugs within the past 3 months. Exclusion criteria: This study excluded ① patients with tumors or endocrine diseases; and ② patients suffering from POF due to genetic and iatrogenic factors. The control group (CG) included 53 healthy women of child-bearing age with regular menstruation in the same stage. This study was conducted with the permission of the Medical Ethics Committee of our hospital. All patients signed an informed consent.

Methods

① Five ml of peripheral venous blood was collected from POF patients at the time of admission as well as the healthy women of child-bearing age on the day of physical examination at admission, and PB lymphocytes were separated by centrifugation at 3,000 rpm for 5 min. Lymphocytes were incubated through homologous monoclonal antibodies of FITC-anti-CD3 and PE-conjugated anti-HLA-DR. Attune NxT flow cytometry (FCM) purchased from Thermo Fisher Scientific (China) Co., Ltd. was used to detect the expression of CD3-+ HLA-DR cells and the levels of CD3+, CD4+ and CD8+ in T-lymphocyte subsets, and then the ratio of CD4+/CD8+ was calculated. ② Five ml of peripheral venous blood was collected from POF patients at the time of admission as well asthe healthy women of child-bearing age on the day of physical examination at admission, and the serum was separated by centrifugation at 3,000 rpm for 5 min. Radioimmunoassay was used to detect IL-21 levels and the kit was provided by Beijing Furui Runze Biotechnology Co., Ltd. Cobas e601 automatic electrochemical luminescence immunoanalyzer purchased from Beijing Marin Medical Equipment Co., Ltd. was used to detect the levels of FSH, LH, E2, progesterone (P), testosterone (T) and prolactin (PRL).

Observation targets

The two groups were compared in percentage of CD3-+ HLA-DR cells, IL-21 levels, sex hormones (FSH, LH, E2, P, T and PRL) and immune cell indices (CD3+, CD4+, CD8+ and CD4+/CD8+ in T-lymphocyte subsets) and analyzed for the correlation of CD3-+ HLA-DR and IL-21 with the six sex hormones and immune cell indices.

Statistical analysis

SPSS 22.0 was used for data analysis. The measurement data in conformity with normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared through independent-samples t test. Pearson correlation coefficient was used for correlation analysis. $P<0.05$ was considered to be statistically significant.

Results

General data

In SG, patients were aged between 24-36 years, with an average age of (30.67±5.92) years; their weight was between 40-78 kg, with the average weight of (57.76±12.78) kg; the course of disease was 2-9 years, with the average course of disease at (5.46±1.79) years; there were 48 married women and 5 unmarried women. In CG, patients were aged 26-39 years, with an average age of (31.59±5.57) years; their weight was between 42-74 kg, with the average weight of (55.29±10.55) kg; the course of disease was 2-8 years, with

Table 1. Comparison of the general data between two groups

Group	Case	Age (years)	Weight (kg)	Course of disease (years)	Marital status	
					Married	Unmarried
SG	53	30.67±5.92	57.76±12.78	5.46±1.79	48 (90.57)	5 (9.43)
CG	53	31.59±5.57	55.29±10.55	5.51±1.69	46 (86.79)	7 (13.21)
t		0.824	1.085	0.148		
P		0.412	0.28	0.883		

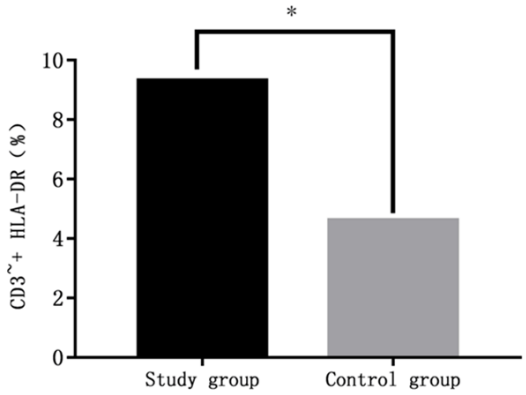

Figure 1. Comparison on percentage of CD3-+ HLA-DR cells between two groups. The percentage of CD3-+ HLA-DR cells in SG was much higher than that in CG (*P<0.05*).

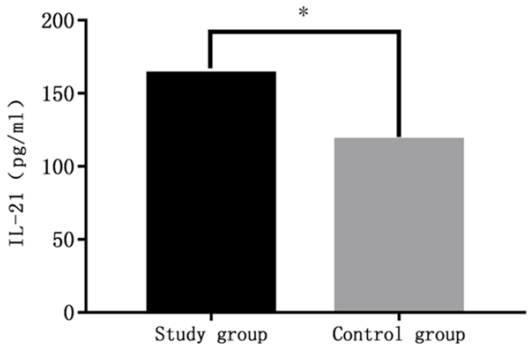

Figure 2. Comparison of IL-21 level between two groups. The IL-21 level of SG was much higher than that of CG (*P<0.05*).

the average course of disease of (5.51±1.69) years; there were 46 married women and 7 unmarried women. There was no difference in terms of baseline data between the two groups (*P*>0.05) (**Table 1**).

CD3-+ HLA-DR cells and IL-21 levels

The percentage of CD3-+ HLA-DR cells was (9.31±4.62) in SG, much higher than that of (4.61±2.48) in CG (*P*<0.05). The IL-21 level of SG was (163.45±37.53) pg/ml, much higher than that of (118.07±17.58) pg/ml of CG (*P*<0.05), as shown in **Figures 1**, **2**.

Levels of six sex hormones

The levels of FSH and LH were respectively (84.44±27.64) IU/L and (47.32±15.13) IU/L in SG, much higher than those of (7.01±1.38) IU/L and (5.34±1.19) IU/L in CG (*P*<0.05). The levels of E2, P, T and PRL were respectively (64.61±20.84) pmol/L, (4.19±1.33) pmol/L, (1.09±0.28) pmol/L and (133.24±38.16) ng/L in SG, much lower than those of (161.37±50.98) pmol/L, (161.37±50.98) pmol/L, (16.61±5.05) pmol/L and (252.47±63.84) ng/L in CG (*P*<0.05), as shown in **Table 2**.

Levels of immune cell indices

The levels of CD3+ and CD8+ were respectively (80.65±8.72)% and (38.58±4.88)% in SG, much higher than those of (68.33±8.47)% and (25.54±4.72)% in CG (*P*<0.05). The levels of CD4+ and CD4+/CD8+ were respectively (30.36±3.07)% and (0.77±0.23)% in SG, much lower than those of (38.96±5.28)% and (1.57±0.27)% in CG (*P*<0.05), as shown in **Table 3**.

Correlation of CD3-+ HLA-DR and IL-21 with the six sex hormones and immune cell indices

There was a positive correlation between CD3-+ HLA-DR and IL-21 (*r*=0.520, *P*<0.001). CD3-+ HLA-DR was positively correlated with FSH, LH, CD3+ and CD8+ (*P*<0.05), and was negatively correlated with E2, P, PRL, CD4+ and CD4+/CD8+ (*P*<0.05) (**Table 4** and **Figures 3-5**). IL-21 was positively correlated with FSH, LH, CD3+ and CD8+ (*P*<0.05), and was negatively correlated with E2, P, PRL and CD4+/CD8+ (*P*<0.05), as shown in **Table 4** and **Figures 6-9**.

Table 2. Comparison on levels of 6 sex hormones between two groups ($\bar{x} \pm s$)

Group	Number of cases	FSH (IU/L)	LH (IU/L)	E2 (pmol/L)	P (pmol/L)	T (pmol/L)	PRL (ng/L)
SG	53	84.44±27.64	47.32±15.13	64.61±20.84	4.19±1.33	1.09±0.28	133.24±38.16
CG	53	7.01±1.38	5.34±1.19	161.37±50.98	16.61±5.05	1.85±0.62	252.47±63.84
t value		20.369	20.137	12.79	17.314	8.133	11.671
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3. Comparison on levels of immune cell indices between two groups ($\bar{x} \pm s$)

Group	Number of cases	CD3+ (%)	CD4+ (%)	CD8+ (%)	CD4+/CD8+
SG	53	80.65±8.72	30.36±3.07	38.58±4.88	0.77±0.23
CG	53	68.33±8.47	38.96±5.28	25.54±4.72	1.57±0.27
t value		7.378	10.251	13.983	16.421
P value		<0.001	<0.001	<0.001	<0.001

Table 4. Correlation of CD3-+ HLA-DR and IL-21 with 6 sex hormones and immune cell indices

Index	CD3-+ HLA-DR (%)		IL-21 (pg/ml)	
	r	P	R	P
FSH (U/L)	0.579	<0.001	0.632	<0.001
LH (U/L)	0.542	<0.001	0.620	<0.001
E2 (pmol/L)	-0.290	0.003	-0.311	<0.001
P (pmol/L)	-0.406	<0.001	-0.423	<0.001
T (pmol/L)	-0.158	0.105	-0.116	0.237
PRL (ng/L)	-0.295	0.002	-0.245	0.011
CD3+ (%)	0.552	<0.001	0.693	<0.001
CD4+ (%)	-0.213	0.028	-0.151	0.121
CD8+ (%)	0.597	<0.001	0.788	<0.001
CD4+/CD8+	-0.333	<0.001	-0.294	0.002

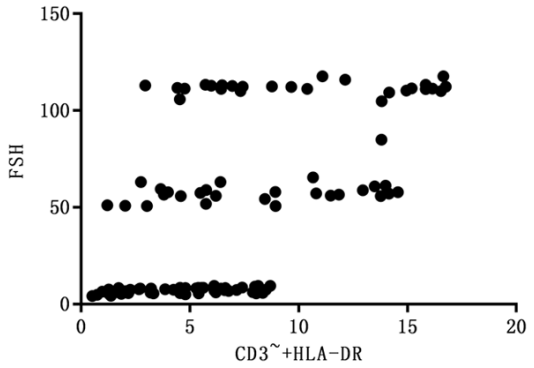

Figure 3. Correlation of CD3~+ HLA-DR and FSH.

Discussion

The pathogenesis of POF is that the number of ovarian follicles in the ovary decreased gradually under the influence of the internal and external environment and the formed oocytes are involved in a faster process of atresia. As a result, large numbers of ovarian follicles are consumed prematurely and the secretion of progestational hormone and estrogen are reduced. Then, the inhibiting effect on FSH and LH is released after acting on hypophysis, which increases the levels of FSH and LH in the serum [8-10]. Most studies have shown that the immune system affects ovarian function by directly or indirectly acting on the generation of ovarian follicles and the atresia of oocytes [11, 12]. Studies have indicated that about 20% of POF cases are caused by autoimmune damage. This means that the autoimmune system is unable to identify ovarian tissues, so it will attack the target cells in the ovary and damage the ovarian function, eventually leading to ovarian failure [13]. Patients with POF presented with luteolysis of the

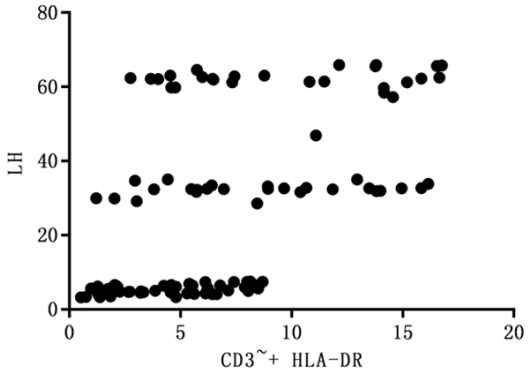

Figure 4. Correlation of CD3~+ HLA-DR and LH.

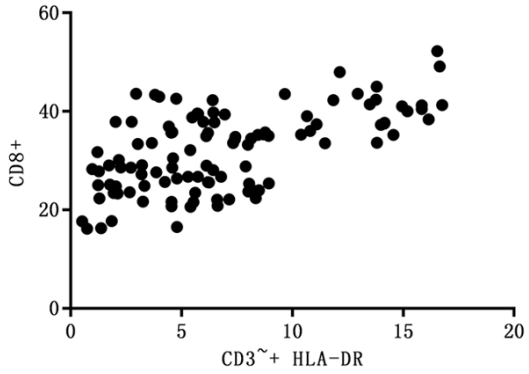

Figure 5. Correlation of CD3~+ HLA-DR and CD8+.

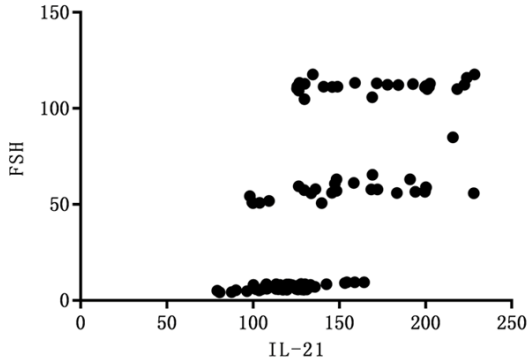

Figure 6. Correlation of IL-21 and FSH.

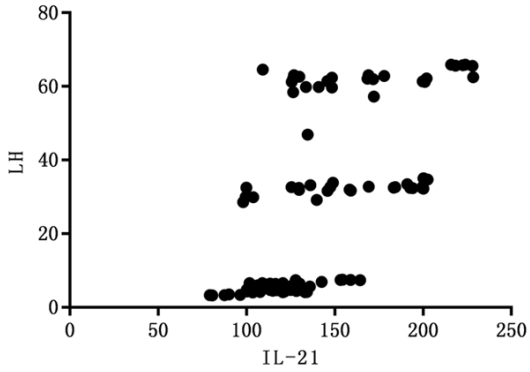

Figure 7. Correlation of IL-21 and LH.

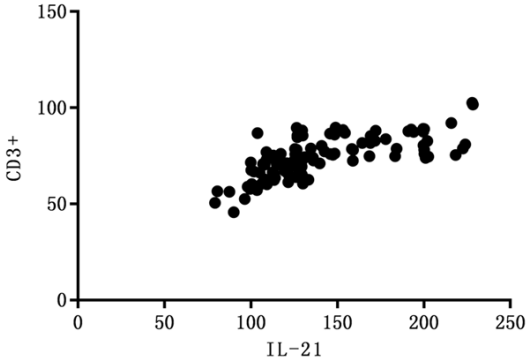

Figure 8. Correlation of IL-21 and CD3+.

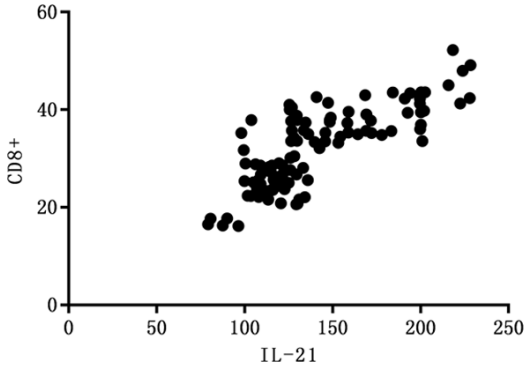

Figure 9. Correlation of IL-21 and CD8+.

ovary, decrease of follicular theca cells and granulosa cells, infiltration of plasmacytes and interstitial lymphocytes, and decrease or disappearance of oocytes. POF is fundamentally different from normal menopause.

The biological function of HLA is mainly to bind and express antigen peptide so that it can be identified by T cells, leading to an immune response [14]. HLA-DR is an antigen of major histocompatibility complex II (MHC II). It is expressed on the cell surface of lymphoid tissues by the coding of DR subregion in HLA Class II on chromosome 6. The cells of HLA Class II are not expressed in autoimmune diseases, but the molecules of Class II can be induced to expression. Some studies have shown that autoimmune diseases such as multiple sclerosis are associated with HLA-DR expression [15, 16]. Human cluster of differentiation (CD) codes from 1 to 166. Thereinto, the CD3 molecule is a marker on the surface of T lymphocytes. So the expression of CD3-+ HLA-DR cells can be detected with FCM. The antigens are presented to lymphocytes through antigen-presenting cells when lymphocytes are stimulated by antigens, thereby inducing an immune response [17]. HLA-DR has an antigen presenting function, regardless of gender, race, age, *etc*. Therefore, the expression of HLA-DR in lymphocytes can be used as an important index to evaluate immune function. This study showed that the percent-

age of CD3-+ HLA-DR cells in SG was much higher than that in CG ($P<0.05$), implying that CD3-+ HLA-DR cells may be involved in the immune mechanism of POF.

As a member of IL-2 cytokine family, IL-21 is secreted by activated T follicular helper cells, CD4+ T cells, and Th17 cells. It has a receptor IL21R in normal lymph nodes, the thymus and other lymphocytes, mainly including B cells, T cells, monocytes and natural killer (NK) cells [18, 19]. IL-21 binds to its receptor IL21R, and acts on dendritic cells, lymphocyte subsets and monocytes. Some studies have shown that IL-21 plays a role in the pathogenesis of autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis, and alleviates the disease by blocking the IL-21 signaling pathway [20, 21]. As shown in this study, the IL-21 level of SG was much higher than that of CG, which implied that IL-21 may be involved in the immune mechanism of POF. Pearson analysis further indicated that there was a positive correlation between CD3-+ HLA-DR and IL-21. So the increased percentage of CD3-+ HLA-DR cells and IL-21 level plays a role on the immune mechanism of POF.

Sex hormones play a crucial role in the immune system. The increase of FSH levels is the most common hormonal change in hypoovarianism [22, 23]. It is generally believed that FSH >40 IU/L means that the ovarian follicles have been depleted. High expression of FSH and LH indicates complete ovarian failure [24, 25]. This study indicated that the levels of FSH and LH in SG were much higher than those in CG, but the levels of E2, P, T and PRL in SG were much lower than those in CG, which suggested complete ovarian failure of POF patients. Pearson analysis further showed that CD3-+ HLA-DR and IL-21 were positively correlated with FSH and LH, and negatively correlated with E2, P and PRL. Results indicated that the increase of CD3-+ HLA-DR and IL-21 was closely associated with ovarian failure.

CD4+/CD8+ is an important index of immune regulation. The range of <1.4 or >2.0 indicates cellular immune dysfunction. The levels of immune cells were also detected in this study, and the results showed that the levels of CD3+ and CD8+ in SG were much higher than those in CG, but the levels of CD4+ and CD4+/CD8+ in SG were much lower than those in CG, which implied that T cells mediated the immune injury of POF patients. The reason may be that the imbalance of killer cells, suppressor cells and helper cells in T cell subsets can lead to immune suppression and cellular immune dysfunction [26, 27]. The enhancement and differentiation of CD8+ T cells induces an immune response, leading to apoptosis and accelerated ovarian atresia [28, 29]. Pearson analysis further showed that CD3-+ HLA-DR and IL-21 were positively correlated with CD3+ and CD8+ and negatively correlated with CD4+/CD8+; and CD3-+ HLA-DR was negatively correlated with CD4+. Results indicated that the increase of CD3-+ HLA-DR and IL-21 may be related to immune dysfunction.

In conclusion, PB CD3-+ HLA-DR cells and IL-21 levels were highly expressed in POF patients, and there was a positive correlation between CD3-+ HLA-DR and IL-21. This implied that CD3-+ HLA-DR and IL-21 may participate in the immune mechanism of POF together. Such sex hormones as FSH and LH were highly expressed in POF patients. CD3-+ HLA-DR and IL-21 were positively correlated with FSH and LH. This implied that the increase of CD3-+ HLA-DR and IL-21 was associated with ovarian function failure. CD4+/CD8+ was minimally expressed in POF patients, with a ratio of <1.4. CD3-+ HLA-DR and IL-2 were negatively correlated with CD4+/CD8+. This implied that the increase of CD3-+ HLA-DR and IL-21 may be related to immune dysfunction. This study showed that the increase of CD3-+ HLA-DR cells and IL-21 level may be related to POF.

Disclosure of conflict of interest

None.

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