



Correlation of serum sex hormone levels with Th1/Th2 balance as well as peripheral CD28, CTLA-4, PD-1 and PD-L1 expression in perimenopausal women

Chun Li¹✉, Ji-Ying Chen¹, An-Lian Zhou²

¹ Gynecology Department, Shenzhen Longhua District Central Hospital in Guangdong Province, Shenzhen 518110, China

² Maternity Care Division, Shenzhen Longhua District Central Hospital in Guangdong Province, Shenzhen 518110, China

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ABSTRACT

Objective: To study the correlation of serum sex hormone levels with Th1/Th2 balance as well as peripheral CD28, CTLA-4, PD-1 and PD-L1 expression in perimenopausal women.

Methods: Perimenopausal women and postmenopausal women who were treated in Gynecology Department of Shenzhen Longhua District Central Hospital between May 2013 and October 2016 were selected as the perimenopausal group and postmenopausal group, and healthy women who received physical examination during the same period were selected as control group. Serum was collected to detect the levels of Th1 and Th2 cytokines as well as endothelial injury marker molecules, and peripheral blood was collected to detect the levels of Th1 and Th2 cells as well as the mRNA expression of CD28, CTLA-4, PD-1 and PD-L1.

Results: The absolute value of Th1 cells in peripheral blood, IFN- γ , IL-2 and ET-1 levels in serum as well as CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of perimenopausal group and postmenopausal group were significantly higher than those of control group while the absolute value of Th2 cells in peripheral blood as well as IL-4, IL-10 and NO levels in serum were significantly lower than those of control group; the absolute value of Th1 cells in peripheral blood, IFN- γ , IL-2 and ET-1 levels in serum as well as CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of postmenopausal group were significantly higher than those of perimenopausal group while the absolute value of Th2 cells in peripheral blood as well as IL-4, IL-10 and NO levels in serum were significantly lower than those of perimenopausal group; peripheral blood Th1 and Th2 cell levels were correlated with ET-1 levels as well as CD28, CTLA-4, PD-1 and PD-L1 mRNA expression. **Conclusion:** Low estrogen levels in perimenopausal women can affect the Th1/Th2 balance and then result in endothelial injury.

1. Introduction

Ovarian function decline and estrogen secretion reduction are the important characteristics of perimenopausal women, and estrogen level decrease will cause the weakening or loss of cardiovascular protective effects of estrogen, and thus increase the risk of cardiovascular disease[1,2]. Perimenopausal and postmenopausal women are the high risk group of clinical cardiovascular events, but it is not yet clear about the specific molecular pathway for the

decrease of estrogen levels in perimenopausal and postmenopausal women to cause the increase of cardiovascular events. CD4+T cells are the important helper T cells in the body that regulate the immune response and inflammatory reaction, the Th1 and Th2 subset function imbalance is considered to be related to the occurrence of coronary heart disease, and the change of Th1 and Th2 cytokines can aggravate the endothelial injury to increase the incidence of coronary heart disease[3,4]. The Th1 and Th2 changes in perimenopausal women are not clear, and the mechanism regulating Th1 and Th2 differentiation is also not elucidated. In the following study, the correlation of serum sex hormone levels with Th1/Th2 balance as well as peripheral costimulatory molecules CD28, CTLA-4, PD-1 and PD-L1 expression in perimenopausal women was analyzed.

✉Corresponding author: Chun Li, Gynecology Department, Shenzhen Longhua District Central Hospital in Guangdong Province, Shenzhen 518110, China.

Tel: 18211557220

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2. Research subjects and research methods

2.1 Research subjects

Perimenopausal women and postmenopausal women who were treated in Shenzhen Longhua District Central Hospital between May 2013 and October 2016 were selected as perimenopausal group and postmenopausal group, perimenopausal women were with irregular menstruation or menopause for less than a year, and postmenopausal women were with menopause for more than a year; healthy women receiving physical examination during the same period were selected as the control group. Perimenopausal group included a total of 54 cases, postmenopausal group included a total of 39 cases, and control group included a total of 60 cases. Informed consent and the consent form were obtained from all included subjects.

2.2 Research methods

2.2.1 Serum sample collection and detection methods

5 mL of peripheral blood was collected from the perimenopausal group and the postmenopausal group before hormone replacement therapy, 5 mL of peripheral blood was collected from the control group during physical examination, the blood was let stand at room temperature and then centrifuged to separate serum, and enzyme-linked immunosorbent assay kits were used to determine IFN- γ , IL-2, IL-4 and IL-10 levels.

2.2.2 Peripheral blood sample collection and detection methods

5 mL of EDTA anticoagulant peripheral blood was collected from perimenopausal group and postmenopausal group before hormone replacement therapy, 5 mL of EDTA anticoagulant peripheral blood was collected from the control group during physical examination, and the peripheral blood was divided into two and tested in accordance with the following ways: (1) used to incubate CD4, IFN- γ and IL-4 monoclonal antibodies, then added red blood cell lysis buffer, continued to be incubated and then washed with PBS twice, and finally determining the Th1 and Th2 cell count in flow cytometer; (2) added in lymphocyte separation medium and centrifuged to absorb the mononuclear cells suspended in the middle and then extract RNA, and using fluorescence quantitative PCR kit to detect CD28, CTLA-4, PD-1 and PD-L1 mRNA expression.

2.3 Statistical methods

SPSS 22.0 software was used to input and analyze data, serum indexes and peripheral blood indexes were compared by variance analysis and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1 Peripheral blood Th1 and Th2 cell function

Analysis of the absolute value of peripheral blood Th1 and Th2 cells among three groups of women was as follows: the absolute value of Th1 cells in peripheral blood of perimenopausal group and postmenopausal group were significantly higher than that of control group while the absolute value of Th2 cells were significantly lower than that of control group; the absolute value of Th1 cells in peripheral blood of postmenopausal group was significantly higher than that of perimenopausal group while the absolute value of Th2 cells was significantly lower than that of perimenopausal group. Differences in pair-wise comparison of the absolute value of Th1 and Th2 cells in peripheral blood were statistically significant among three groups of women ($P < 0.05$). The data were shown in Table 1.

Analysis of serum Th1 cytokines IFN- γ and IL-2 as well as Th2 cytokines IL-4 and IL-10 levels among three groups of women was as follows: serum IFN- γ and IL-2 levels of perimenopausal group and postmenopausal group were significantly higher than those of control group while IL-4 and IL-10 levels were significantly lower than those of control group; serum IFN- γ and IL-2 levels of postmenopausal group were significantly higher than those of perimenopausal group while IL-4 and IL-10 levels were significantly lower than those of perimenopausal group. Differences in pair-wise comparison of serum IFN- γ , IL-2, IL-4 and IL-10 levels were statistically significant among three groups of women ($P < 0.05$). The data were shown in Table 2.

Table 1.

Comparison of absolute value of cells in peripheral blood among three groups of women (106/L).

Groups	<i>n</i>	Th1	Th2
Control group	60	12.77±2.25	3.29±0.56
Perimenopausal group	54	16.51±2.95 ^a	2.14±0.37 ^a
Postmenopausal group	39	20.21±3.76 ^{ab}	1.75±0.26 ^{ab}

^a: compared with control group, $P < 0.05$; ^b: compared with perimenopausal group, $P < 0.05$.

Table 2.

Comparison of serum Th1 and Th2 cytokine levels among three groups of women (ng/L).

Groups	<i>n</i>	Th1 cytokines		Th2 cytokines	
		IFN- γ	IL-2	IL-4	IL-10
Control group	60	8.95±1.14	9.55±1.04	18.47±3.16	32.56±5.62
Perimenopausal group	54	20.17±2.69 ^a	18.32±2.36 ^a	12.31±2.25 ^a	21.57±3.57 ^a
Postmenopausal group	39	33.26±5.51 ^{ab}	35.21±5.62 ^{ab}	7.75±0.93 ^{ab}	13.25±1.95 ^{ab}

^a: compared with control group, $P < 0.05$; ^b: compared with perimenopausal group, $P < 0.05$.

3.2 CD28, CTLA-4, PD-1 and PD-L1 expression in peripheral blood mononuclear cells

Analysis of CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells among three groups of women was as follows: CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of perimenopausal group and postmenopausal group were significantly higher than those of control group; CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of postmenopausal group were significantly higher than those of perimenopausal group. Differences in pair-wise comparison of CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells were statistically significant among three groups of women ($P < 0.05$). The data were shown in Table 3.

Table 3.

Comparison of CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells among three groups of women.

Groups	n	CD28	CTLA-4	PD-1	PD-L1
Control group	60	1.05±0.17	1.02±0.15	0.98±0.12	1.08±0.20
Perimenopausal group	54	1.89±0.24 ^a	2.05±0.33 ^a	1.72±0.21 ^a	1.94±0.27 ^a
Postmenopausal group	39	3.02±0.48 ^{ab}	2.87±0.41 ^{ab}	3.25±0.49 ^{ab}	3.15±0.46 ^{ab}

^a: compared with control group, $P < 0.05$; ^b: compared with perimenopausal group, $P < 0.05$.

3.3 Serum endothelial injury marker levels of three groups of women

Analysis of serum endothelial injury markers ET-1 (ng/L) and NO (μmol/L) levels among three groups of women was as follows: serum ET-1 levels of perimenopausal group and postmenopausal group were significantly higher than that of control group while NO levels were significantly lower than that of control group; serum ET-1 level of postmenopausal group was significantly higher than that of perimenopausal group while NO level was significantly lower than that of perimenopausal group. Differences in pair-wise comparison of serum ET-1 and NO levels were statistically significant among three groups of women ($P < 0.05$). The data were shown in Table 4.

Table 4.

Comparison of serum endothelial injury markers among three groups of women.

Groups	n	ET-1	NO
Control group	60	28.75±5.12	113.26±22.51
Perimenopausal group	54	42.11±7.62 ^a	75.26±9.35 ^a
Postmenopausal group	39	55.96±8.21 ^{ab}	52.31±7.38 ^{ab}

^a: compared with control group, $P < 0.05$; ^b: compared with perimenopausal group, $P < 0.05$.

3.4 Pearson correlation analysis results

CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of perimenopausal group were positively correlated with peripheral blood Th1 cell level, and r value was 0.672, 0.591, 0.704 and 0.625 respectively; they were negatively correlated with peripheral blood Th2 cell level, and r value was -0.661, -0.731, -0.538 and -0.597 respectively.

Peripheral blood Th1 cell level of perimenopausal group was positively correlated with serum ET-1 level and negatively correlated with serum NO level, and r value was 0.772 and -0.627 respectively; Th2 level was negatively correlated with serum ET-1 level and positively correlated with serum NO level, and r value was -0.619 and 0.645 respectively.

4. Discussion

Perimenopausal women and postmenopausal women are the high-risk group of cardiovascular diseases, and menopause is also regarded as an independent risk factor for increased cardiovascular risk[5,6]. In perimenopausal and postmenopausal stage, ovarian function declining and estrogen secretion reducing can make the cardiovascular system lose the protection from estrogen, and then increase the risk of cardiovascular diseases. However, it is not yet clear at present about the molecular pathways of estrogen to reduce the increased risk of cardiovascular disease. Inflammation is an important pathological change throughout each link of cardiovascular disease, and the disorders of Th1 and Th2 subsets as well as the Th1/Th2 balance shifting to Th1 will start inflammation and cause the occurrence of cardiovascular disease[7,8]. In the study, analysis of the balance of Th1/Th2 cells in peripheral blood showed that the absolute value of Th1 cells in peripheral blood of perimenopausal group and postmenopausal group were significantly higher than that of control group while the absolute value of Th2 cells were significantly lower than that of control group; the absolute value of Th1 cells in peripheral blood of postmenopausal group was significantly higher than that of perimenopausal group while the absolute value of Th2 cells was significantly lower than that of perimenopausal group. This means that as the estrogen levels decrease in perimenopausal and postmenopausal women, the balance of Th1/Th2 cells changes, the number of Th1 cells increases, and the number of Th2 cells decreases.

In the process of CD4+T cell differentiation to Th1 and Th2 cell subsets, the functions of different cell subsets are different, and they can synthesize and secrete different types of cytokines[9,10]. The cytokines secreted by Th1 cells include IFN- γ , IL-2, TNF- α and other pro-inflammatory factors, and they can mediate cellular immune response and inflammatory response; Th2 cytokines mainly secrete IL-4, IL-10 and other anti-inflammatory factors, which can restrain the differentiation of Th1 cells and the inflammatory response mediated by them, and can also promote the humoral immune response. In order to further clarify the change of Th1 and Th2 cell function in perimenopausal women, serum levels of Th1 and Th2 cytokines were analyzed in the study, and the result showed that serum IFN- γ and IL-2 levels of perimenopausal group and postmenopausal group were significantly higher than those of control group while IL-4 and IL-10 levels were significantly lower than those of control group; serum IFN- γ and IL-2 levels of postmenopausal group were significantly higher than those of perimenopausal group while IL-4 and IL-10 levels were significantly lower than those of perimenopausal group. This means that as the

level of estrogen decreases in perimenopausal and postmenopausal women, the Th1 cytokine secretion increases and the Th2 cytokine secretion decreases.

The differentiation process of Th1 cells and Th2 cells in perimenopausal and postmenopausal women is adjusted by a variety of costimulatory molecules such as CD28, CTLA-4, PD-1 and PD-L1. CD28 is an important positive costimulatory molecule on CD4+T cell surface[11], and CTLA-4, PD-1 and PD-L1 are the important negative costimulatory molecules on Treg cell surface[12,13]. In the study, detection of above costimulatory molecules in peripheral blood of perimenopausal women showed that CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of perimenopausal group and postmenopausal group were significantly higher than those of control group; CD28, CTLA-4, PD-1 and PD-L1 mRNA expression in peripheral blood mononuclear cells of postmenopausal group were significantly higher than those of perimenopausal group. This means that both positive costimulatory molecules and negative costimulatory molecules show the trend of high expression in perimenopausal and postmenopausal women and are positively correlated with the content of Th1 cells while negatively correlated with the content of Th2 cells. The analysis shows that the high expression of positive costimulatory molecule CD28 can directly promote the differentiation of Th1 and inhibit the differentiation of Th2; the high expression of negative costimulatory molecules CTLA-4, PD-1 and PD-L1 can restrain the differentiation of the Treg and weaken the Treg inhibition on Th1 differentiation so as to indirectly promote the differentiation of Th1 and inhibit the differentiation of Th2.

Endothelial injury is one of the ways for inflammatory response to increase the risk of cardiovascular diseases. Normal endothelial function is of great significance for the maintenance of the vascular intima integrity, and endothelial injury will destroy the integrity of the vascular intima, increase the inflammatory cell gathering in local area and promote foam cell and fatty streak deposition to result in arterial atheromatous plaque formation and increase the risk of cardiovascular diseases. ET-1 and NO are the common indexes to reflect endothelial function, ET-1 is an active peptide with vasoconstrictive effect, and it is massively secreted in the process of endothelial damage[14,15]; NO is a gas molecule with vasodilator effect, and its secretion declines in the process of endothelial injury[16,17]. In order to define the effect of Th1/Th2 balance change on endothelial function in perimenopausal women, the serum endothelial injury molecule levels in perimenopausal and postmenopausal women as well as their correlation with Th1/Th2 balance change were analyzed in the study, and the results showed that serum ET-1 levels of perimenopausal group and postmenopausal group were significantly higher than that of control group while NO levels were significantly lower than that of control group; serum ET-1 level of postmenopausal group was significantly higher than that of perimenopausal group while NO level was significantly lower than that of perimenopausal group; peripheral blood Th1 cell level was positively correlated with serum ET-1 level and negatively correlated with serum NO level while Th2 level was negatively correlated with serum ET-1 level and positively correlated with serum NO level. This means that the Th1/Th2 imbalance and the inflammation activation in perimenopausal women can cause endothelial injury, and then increase the risk of cardiovascular diseases.

Based on above discussion, it is believed that the decrease of estrogen levels in perimenopausal women will affect the expression of costimulatory molecules such as CD28, CTLA-4, PD-1 and PD-L1 to cause Th1/Th2 balance shifting to Th1, then lead to endothelial injury and increase the risk of cardiovascular diseases.

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