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Original Article

A correlation analysis of Th1/Th2 cells in preeclampsia patients

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Abstract: This study aims to detect Th1, Th2 cytokine expression and the Th1/Th2 balance in patients with pregnancy-induced hypertension (PIH) and to analyze their correlation with PIH. PIH patients were divided into gestation hypertension, mild eclampsia, and severe eclampsia groups. Healthy pregnant women were recruited as a control group. Flow cytometry was used to measure the level of Th1 and Th2 cells. ELISA was used to test the levels of Th1 cytokines IL-2, IFN-γ, and Th2 cytokine IL-4, and the relationships between the cytokine level and mean artery pressure (MAP) and 24h urea protein were analyzed. The PIH disease groups showed significantly increased levels of IL-2 and IFN-γ but a reduced level of IL-4 compared to the control group ($P<0.05$). Of note, patients with severe eclampsia presented significantly higher levels of IL-2 and IFN-γ but lower levels of IL-4 compared to the gestation hypertension and mild eclampsia groups ($P<0.05$). In severe eclampsia patients, we also found significantly higher IFN-γ/IL-4 and IL-2/IL-4 ratios ($P<0.05$). These two ratios were positively correlated with MAP or 24 h urea protein in the disease group ($P<0.01$). Our data demonstrated that in PIH patients, the Th1 cytokine level was elevated but the Th2 cytokine level was down-regulated, resulting in an imbalance of Th1/Th2 cells towards a Th1 type immune response.

Keywords: Pregnancy induced hypertension, Th1, Th2, Th1/Th2

Introduction

Pregnancy-induced hypertension (PIH) is a syndrome affecting pregnant women. Currently, the pathogenesis of PIH has not been fully revealed. In the clinic, PIH affects multiple systems, so it has severe impacts on both maternal and fetal health [1]. Previous studies have shown that PIH was mainly caused by vessel spasms and the activation of the coagulation system, which thus caused a rapid hypo-perfusion of multiple organs and a series of syndromes, such as PIH, hypertension, proteinuria, and edema [2-4]. Some patients may develop eclampsia, seizures, coagulation dysfunction, cerebral hemorrhage, brain edema, liver/kidney failure, or placental premature.

In the CD4+ cell population, helper T cells (Th1) generally secrete various cytokines to exert synergistic effects with Th2-secrted cytokines in the normal humoral and cell immune response during pregnancy. PIH may aggravate the systemic vascular endothelial dysfunction and abnormal coagulation function. Current investigations on PIH pathogenesis mainly include immune theory and the activation-induced injury of vascular endothelial cells [6, 7]. Certain important inflammatory cytokines induce the body inflammatory response, among which, interleukin-2 (IL-2) is a critical cytokine in the immune response, while IL-4, as an endogenous anti-inflammatory cytokine, exerts an important protective function [8]. IL-4 also inhibits Th1 immune cell activation via inducing Th2 immune cell differentiation and therefore acts as an upstream factor during Th1/Th2 balance [9]. Th1 and Th2 immune cells secrete several cytokines to induce the body cell immune and humoral immune response [10, 11].

In this study, we aimed to detect the expressions of Th1/Th2 cells and their cytokines IL-2, IFN-γ and IL-4, as well as to investigate the correlation between the cytokine levels and the progression of PIH.

Materials and methods

General information

A total of 50 PIH patients admitted to the No. 8 People's Hospital of Qingdao from January 2015 to January 2016 were recruited in our study.

This study was pre-approved by the ethical committee of Zaozhuang Municipal Hospital. All the patients signed the informed consent before they were recruited in this study.

Inclusive criteria: The patients in the PIH group were diagnosed following the guidelines of gynecology and obstetrics [12]. All included patients had monocyesis without any functional injury of the major organs such as the heart, liver or kidneys, acute/chronic inflammation, a repeated abortion history, hematological or immune related diseases, or immune drugs.

Exclusion criteria: i) Patients who recently had an acute myocardial infarction or recent acute myocarditis were excluded; ii) patients with acute and chronic infections; iii) patients with hyperthyroidism, sepsis, diabetes insipidus and tumors; iv) patients with primary aldosteronism; v) patients with bronchial asthma; and vi) patients with simple gestational hypertension or simple heart failure.

Reagents

ELISA kits for measuring the levels of IL-2, IL-4, and IFN-γ were purchased from Jingmei (China). A centrifuge was purchased from Feige (China). The microplate reader was from TECNA (UK).

Blood sample collection

Fasting blood samples were collected from peripheral veins (about 5 ml) and were centrifuged at 3,000 g to collect the sera which were placed in Eppendorf tubes at -70°C.

Flow cytometry analysis of Th1 and Th2 cells

Peripheral blood cells were isolated followed by addition of 1 mL of RPM I1640 (containing 10% FCS), and PMA (50 ug/L), Ionomycin (250 ug/L), monensin (1:1) (all from Shanghai Shenggong Biological Co., Ltd.) and subsequent incubation in a 5% CO_2, 37°C incubator for 4 h. Single cells were collected to make a single cell suspension, and the cell concentration was adjusted to 5×10^5 cells/ml. 1 mL cell suspension was added to each 12 mm × 75 mm tube followed by being stained with a cytokine antibody (CD4, IFN-γ, IL4, purchased from Mirin Biotech Co., Ltd.) and incubation at 4°C for 30 min in the dark. After being washed twice with a permeate, the cells were resuspended in a staining buffer, followed by a flow cytometry analysis through gating the CD4+ to detect the percentage of Th1 (CD4+IFN-γ+IL-4-) and Th2 cells (CD4+IFN-γ-IL-4+).

Serum IL-2, IL-4 and IFN-γ contents by ELISA

ELISA was used to test the serum contents of IL-2, IL-4, and IFN-γ. In brief, the test kit was placed at room temperature for 30 min, and the contents were diluted in the standard way. At each concentration, five replicated wells were used for mixing with the reaction buffer, washing, developing and quenching. A microplate reader was used to measure the absorbance values at the 450 nm wavelength. A linear regression function was plotted to calculate the sample concentration.

Data processing

SPSS 17.0 software was used for analyzing all the data, which were presented as the mean ± standard deviation (SD). The comparisons of the differences of the enumeration data were tested using a chi-square test, and the measurement data were compared using an analysis of variance (ANOVA). The Pearson coefficient was used to assess correlations between the cytokine ratios and the indexes of MAP and 24 h urea protein. A statistical significance was defined when $P<0.05$.

Results

Patient characteristics

Among the patients, there were 10 cases of gestation hypertension (GH), 17 cases of mild eclampsia (MP), and 23 cases of severe eclampsia (SP). The patients were aged between 25 and 40 years old (average age = 32.1±3.2 years old) (**Table 1**). Another cohort of 30 normal pregnant women in the same period were enrolled as the control group (aging between 25 and 40 years old, average age = 30.1±2.5 years old). No significant differences of age or gestation week were observed between the patients and the controls (P>0.05).

Table 1. Characteristics of the patient and control groups

n	PIH patients	Control	*P* value
	50	30	
Gender (M/F)	26/24	13/17	>0.05[a]
Age (ranges)	32 (25-40)	30 (25-40)	>0.05[b]
Gestation week (ranges)	24 (23-32)	26 (24-33)	>0.05[b]
GH (n)	10		
MP (n)	17		
SP (n)	23		

[a]Chi-square test and [b]Student's *t* test.

Th1 and Th2 cells

Our results showed that the SP1 group had the highest content of Th1 cells, followed by the MP group, the GH group, and the control group, and there were statistical differences among them (P<0.05) (**Figure 1**). Meanwhile, the content of the Th2 cells was the highest in control group, and Th2 cells in the GH group were significantly higher than they were in the MP and SP groups, and there was a statistical difference (P<0.05).

Serum level of IL-2, IL-4 and IFN-γ

We analyzed the serum levels of IL-2, IL-4 and IFN-γ in both the patients and the healthy group. The results showed significantly increased levels of IL-2 and IFN-γ, along with a reduced level of IL-4 in the PIH group, compared to the levels in the control group (P<0.05). Among all patients, the SP population had significantly higher levels of IL-2 and IFN-γ but a lower level of IL-4, compared to the GH or MP population (P<0.05, **Table 2**).

IFN-γ/IL-4 and IL-2/IL-4 comparison

We further compared the IL-2/IL-4 and IFN-γ/IL-4 ratios between the patients and the healthy controls. Our data exhibited significantly elevated ratios in individuals with PIH compared to those in the control group (P<0.05). Notably, the SP patients had significantly higher ratios of IL-2/IL-4 and IFN-γ/IL-4 compared to the GH or MP group (P<0.05) (**Table 3**).

Correlation between Th1/Th2 cytokine ratio and MAP

The IL-2/IL-4 ratio presented a significantly positive correlation with MAP (r = 0.529, P<0.01). Similarly, the IFN-γ/IL-4 ratio was also positively correlated with MAP (r = 0.647, P<0.01) (**Figure 2**).

Th1/Th2 cytokine ratios and 24 h urea protein

In PIH patients, the index of 24 h total urea protein showed a positive correlation with the IL-2/IL-4 and IFN-γ/IL-4 ratios (r = 0.640 and 0.738, P<0.01, **Figure 3**).

Discussion

Modern reproductive immunology indicates the hemi-autograft of pregnancy inside the body. For normal pregnant women, the maintenance of pregnancy depends on the precise and dynamic balance between the maternal and fetal immune systems [13, 14]. T cells mainly come from bone marrow lymphocyte stem cells, and the specific differentiation relies on a Th1 or Th2 type immune response [15]. This study aims to investigate the changes of immunity in PIH patients. In the present study, we found that levels of IL-2 and IFN-γ were significantly increased and the IL-4 level was significantly decreased in patients with PIH compared with the healthy controls, suggesting that an abnormal immune response in PIH and Th1 and Th2 cytokines might be involved in the pathogenesis and development of PIH.

As it has been demonstrated that Th1 type immune cells mainly secrete cytokines including IL-2 and IFN-γ, which play important roles in the regulation of the body's cellular immune response, but Th2 cells primarily secret cytokines including IL-4 and IL-10, which participate in the body's humoral immune response [16]. During pregnancy, IFN-γ can damage placental tissues and inhibit embryonic and fetal growth and development, mainly by modulating NK cell activation and proliferation. Previous studies showed that the abortion rate of pregnant mice was significantly elevated after treatment with IFN-γ, demonstrating the retarding effect of IFN-γ on trophocyte proliferation and placental growth, which eventually leads to abnormal embryonic development [17]. IL-4 is a typical member of Th2 cell-secreted cytokines that induces the proliferation of Th2 immune cells and inhibits the secretion of pro-inflammatory cytokines. IL-4, therefore, mainly exerts an

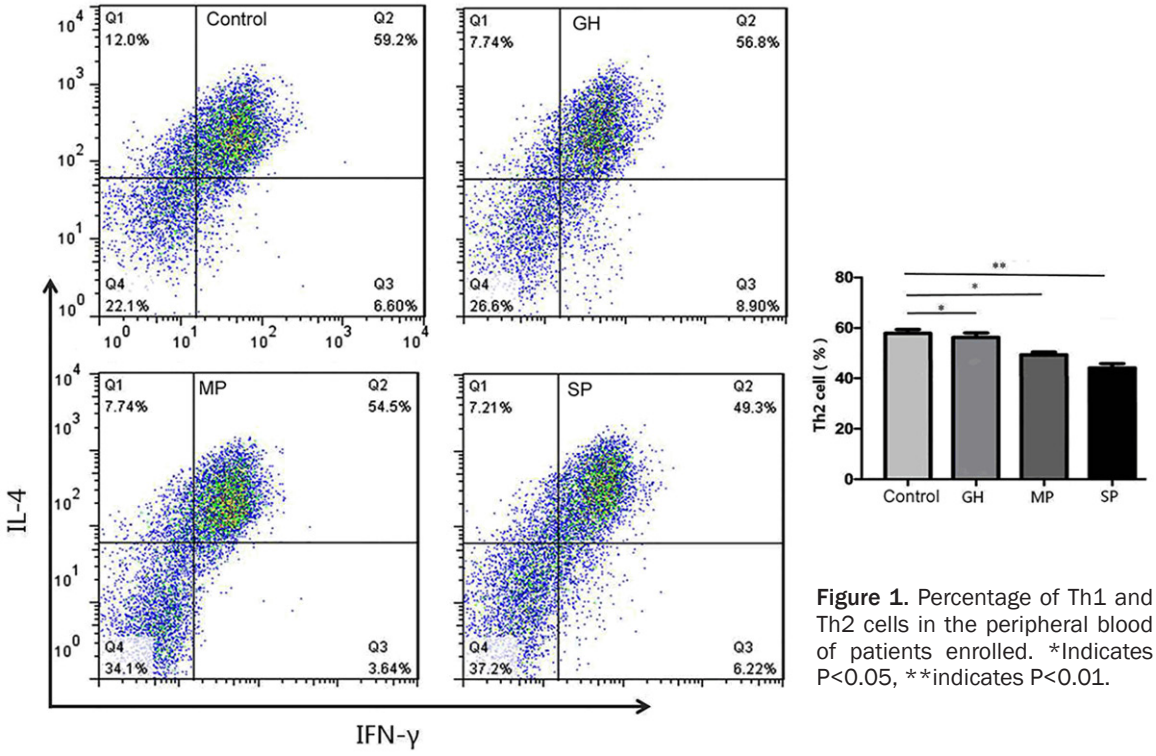

Figure 1. Percentage of Th1 and Th2 cells in the peripheral blood of patients enrolled. *Indicates P<0.05, **indicates P<0.01.

Table 2. Patient serum levels of IL-2, IL-4 and IFN-γ (ng/ml)

Group	N	IL-2	IFN-γ	IL-4
Disease	50			
GH	10	183.2±78.5	334.1±113.9	39.1±14.5
MP	17	243.1±99.2$^{*,\&}$	430.1±132.8$^{*,\&}$	32.3±8.8$^{*,\&}$
SP	23	234.7±107.3$^{*,\&,\#}$	466.9±165.4$^{*,\&,\#}$	26.4±8.9$^{*,\&,\#}$
Control	30	94.2±73.3	107.6±90.1	62.1±11.5

Note: Data were analyzed using a one-way ANOVA. *, P<0.05 compared to the control group; and &, P<0.05 compared to the GH group; #, P<0.05 compared to the MP group.

Table 3. IFN-γ/IL-4 and IL-2/IL-4 ratios

Group	N	IL-2/IL-4	IFN-γ/IL-4
Disease	50		
GH	10	5.5±3.6	8.2±3.2
MP	17	7.9±3.2$^{*,\&}$	12.9±4.2$^{*,\&}$
SP	23	10.1±3.8$^{*,\&,\#}$	17.4±5.3$^{*,\&,\#}$
Control	30	3.5±1.8	7.6±2.5

Note: The data were analyzed using a one-way ANOVA. *, P<0.05 compared to the control group; and &, P<0.05 compared to the GH group; #, P<0.05 compared to the MP group.

immune protective role. Moreover, IL-4 has an anti-abortive role in early pregnancy by suppressing the IL-2-induced activation of NK cells and trophocytes [18-20]. In the present study, we showed significantly increased levels of Th1 cells and IL-2 and IFN-γ, along with reduced Th2 cells and IL-4 in the PIH group, compared to those in the control groups, indicating that the imbalance of Th1 and Th2 cells might be involved in the pathogenesis of PIH, further confirming the contributing role of Th1 and Th2 cells to the development of PIH. Previous evidence indicated that the Th2 type immune response was mainly involved in the immunity of pregnant women, manifesting that the humoral immune response plays a critical role in the maintenance of homeostasis during normal pregnancy [21]. If the Th1/Th2 balance is disrupted, the maternal immune response enters a regulatory adaption period. Due to the bias of Th1/Th2 balance towards the Th1 immune response, a series of disorders in the body might develop with a loss of antagonizing ability and enhanced invasion. During the early phase of pregnancy, due to the effect of invasion, trophocytes suffer from immune injury, leading to the occurrence and progression of PIH.

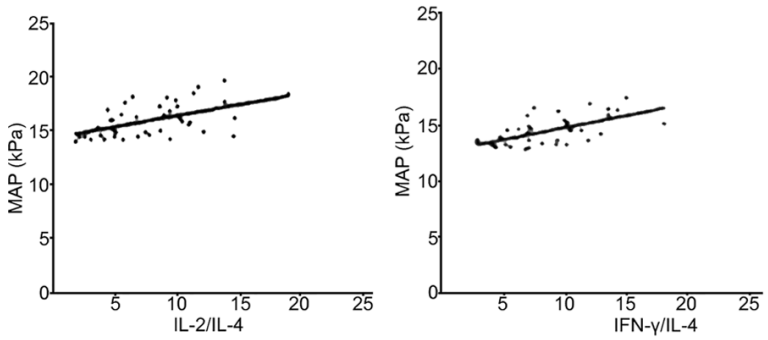

Figure 2. The correlation between Th1/Th2 cytokine ratios and MAP.

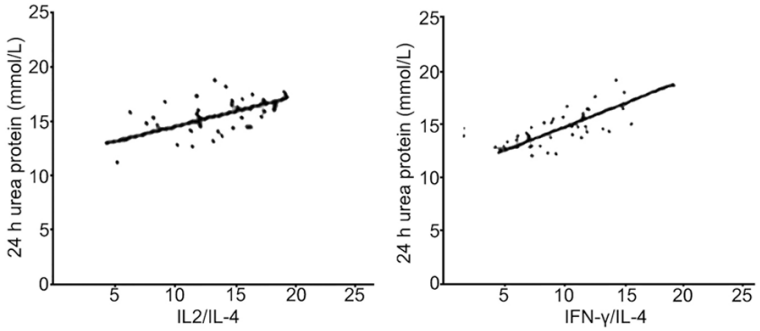

Figure 3. The correlation between Th1/Th2 cytokine ratios and 24 h urea protein in PIH patients.

PIH is actually a dysfunction of the immune regulatory paradigm inside the body environment. The Th1 cell-induced immune response functions as a major tool for pregnant women to confer immunity against the xenograft antigen, the fetus. It impedes the immune tolerance of a mother to her fetus, and it causes a series of complications [22, 23]. In the pregnancy process, the orchestration of both humoral and cellular immunity from the beginning to the end guarantees normal life indexes. However, the imbalance of Th1/Th2 affects the immune tolerance of the mother to her fetus through the recognition of the embryo as a xenograft antigen and induces rejection of the antigen. In this study, through analyzing the relationship between Th1/Th2 secreted cytokine ratios and MAP or 24 h urea protein, we found that PIH patients had positive correlations of IL-2/IL-4 or IFN-γ/IL-4 ratios with MAP or 24 h urea protein, which provides new insights for clinical treatment of PIH. The limitation of this study still exists, namely that the investigation mainly was focused from the perspective of cytokines, so further research is required to elucidate the molecular and genetic mechanisms involving how Th1/Th2 cytokines are involved in the progression of PIH.

In conclusion, in PIH patients, Th1 cells and the expression levels of the associated cytokines IL-2 and IFN-γ were significantly increased, but the Th2 cells and the associated cytokine IL-4 was significantly reduced, leading to an elevated Th1/Th2 cytokine ratio and the subsequent transition to a Th1 immune response in PIH.

Disclosure of conflict of interest

None.

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