Original Research Article

CLINICAL SIGNIFICANCE OF T LYMPHOCYTE SUBSETS AND HLA-DR IN PERIPHERAL BLOOD OF PATIENTS WITH SEVERE PREECLAMPSIA

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Abstract

Objective: To observe the variation of T lymphocyte subsets and human histocompatibility antigen (HLA-DR) in peripheral blood of patients with severe preeclampsia（SPE）.

Method: Peripheral blood from 30 patients with SPE (SPE group), 30 healthy pregnant women (healthy pregnant group) and 18 healthy un-pregnant women (healthy un-pregnant group) was a collected. The percentage of $CD4^+T$, $CD8^+T$ and HLA-DR in each group were compared by Flow cytometry (FCM).

Results: There was no difference in the expression of $CD4^+T$ in each group ((35.6 ± 5.4)%VS (37.8 ± 8.9), (35.6 ± 5.4)%VS(37.1 ± 9.6)%). The expression of $CD8^+T$ in SPE group was significantly higher than those in the healthy pregnant group ((32.1 ± 7.4)% VS(26.9 ± 7.7)%, $P < 0.01$) and in the healthy un-pregnant group ((32.1 ± 7.4)% VS(25.4 ± 7.6)%, $P < 0.01$). The expression of HLA-DR in SPE group was significantly lower than that in healthy pregnant group ((43.7 ± 12.7)% VS(58.3 ± 13.9)%；$P < 0.01$).

Conclusions: Higher expression of $CD8^+T$ was found in peripheral blood of SPE patients which lead to T lymphocyte subsets imbalance. The decrease of HLA-DR may contribute to the pathologic process of SPE.

Keywords: Severe Preeclampsia; Immunity; T lymphocyte subsets; HLA-DR

Introduction

Preeclampsia and eclampsia all belong to the category of hypertensive disorder complicating pregnancy, which often occurs more than 20 weeks after pregnancy. Preeclampsia and eclampsia take high blood pressure and proteinuria as the main feature, but also often involve the liver, kidney, placenta and other important organs, and are an important cause of maternal and perinatal morbidity and mortality[1].The occurrence rate in our country is 2%~6%[2], and with the release of China's two-child policy, maternal death rate caused by eclampsia increases[3,4]. The etiology and pathogenesis of preeclampsia are not completely clear, the

most accepted causes are placenta shallow implantation and oxidative stress[5,6]. The current research focus on the relationship between immune factors and preeclampsia/eclampsia, and find that the T cell subsets and human histocompatibility antigen (HLA-DR) are related to the occurrence and development of preeclampsia[7,8].Therefore, this study observed the changes of T cells and HLA-DR levels in peripheral blood of patients with severe preeclampsia. The results are as follows.

Materials and methods

Clinical materials: We enrolled 30 cases of severe preeclampsia patients admitted to Liaocheng People's Hospital from March 2014 to December 2015 as the observation group, the age (29.6 + 7) years old, pregnant (33.9 + 4.5) weeks. Diagnostic criteria for severe preeclampsia accorded to the guidelines for the diagnosis of pregnancy-induced hypertension published by the American College of Obstetrics and gynecology (ACOG) in 2002[9]. In the same period, 30 normal pregnant women were taken as normal pregnancy group, the age (29.2 + 5.3) years old, pregnant (37.1 + 3.9) weeks; 18 healthy non-pregnant women were selected from the control group, the age (26.8 + 2.6) years old. Both of the two groups of women have no obstetric complications, hypertension, diabetes, heart disease, renal insufficiency, infection, allergic disease, autoimmune disease, tumor, and recently didn't take any medication. There was no significant difference in age among three groups. The observation group and normal pregnancy group were the end of pregnancy with cesarean section. This study was approved by the clinical ethics committee of our hospital, and all the participants signed the informed consent.

Detect T lymphocytes and HLA-DR in peripheral blood: All subjects do not take immune suppressive drugs before collect samples. We drew 2ml blood from median cubital vein disinfected at 8 am., then placed blood in heparinized tubes and detected in less than 4 h. Took 100 uL anticoagulant whole blood, CD3-FITC, CD4-FITC and CD8-PE fluorescent monoclonal antibody were added to each of the 10 uL anticoagulant whole blood, then kept them in dark place at room temperature 15 min after mixing. After lysed erythrocyte, the supernatant was discarded after centrifugation. Then cells were suspended in PBS, centrifuged it, discarded supernatant, added sheath liquid, detected it with the machine. Calculated the ratio of CD4+ and CD8+T lymphocyte. Similarly, took 100 uL anticoagulant blood, added CD14-PE and HLA-DR-FITC 10 uL respectively, then protected from light at room temperature 15 min after mixing. Lysed erythrocyte, discarded the supernatant after centrifugation, suspended cells with PBS, centrifuged it, and then discarded supernatant, added sheath liquid, after that detected it with the machine. Finally, calculated percentage of HLA-DR in white blood cells in peripheral blood.

Statistical methods: Our data was analyzed by SPSS13.0 statistical software. The normal distribution of measurement data was expressed by means of $\bar{x} \pm s$. Single factor analysis of variance was used to compare among groups, and the two-two comparisons were done by LSD method. P < 0.05 was statistically significant.

Results

Levels of T-lymphocyte subsets in peripheral blood of the three groups are compared in table 1. In the observation group, normal pregnancy group and control group, the percentages of HLA-DR respectively were 43.7% + 12.7%, 58.3% + 13.9%, 70.41% + 11.3%. Compared with the normal pregnancy group and the control group, the observation group was less than 0.01 P.

"Clinical significance of T lymphocyte subsets and HLA-DR in peripheral blood of patients with severe preeclampsia"

Table 1: Comparison of T lymphocytes in peripheral blood of three groups ($\bar{x} \pm s$)

Group	T lymphocytes			
	CD3$^+$（%）	CD4$^+$（%）	CD8$^+$（%）	CD4$^+$/CD8$^+$
Observation group	68.2±7.8	35.6±5.4	32.1±7.4★	1.18±0.43▲
Normal Pregnancy group	66.1±8.4	37.8±8.9	26.9±7.7	1.56±0.66
Control group	68.4±7.0	37.1±9.6	25.4±7.6	1.51±0.48

Note: ★Compared with the normal pregnancy group and control group, P < 0.01.
▲Compared with normal pregnancy group and control group, P < 0.05.

Discussion

In a normal pregnancy, matrix highly mediate immune tolerance by reducing the expression rate of lymphocytes in peripheral blood [10, 11]. T lymphocyte subsets are one of the most important components of our immune defense system. CD3+ is a differentiation antigen expressed on the surface of T cells, represents the total T lymphocytes which include CD4+T cell subsets(helper T cell subsets) and CD8+T cell subsets(suppressor T cell subsets). CD4+T cells are mainly responsible for the regulation of the immune response activity, enhance and expand the immune response process through the secretion of lymph factors, and then induce immune cells such as B helper cells, killer T cells to play a role in common. CD8+T cells mainly inhibit B cells to produce antibodies and the killing function of cytotoxic T cells. Normally CD4+T and CD8+T cells maintain the body in a stable dynamic equilibrium and maintain immune homeostasis, which will lead to various kinds of immune damage and immune related diseases if this steady state was broken. A wide range of studies[12,13] has confirmed that the balance of T cell subsets in favor of moderate immunosuppression has important significance to maintaining maternal-fetal interface immune balance of the normal state pregnancy. This experiment found that:

There was no significant difference in the expression rate of CD3+T lymphocytes between severe preeclampsia and normal pregnant women; the expression rate of CD4+T was slightly lower than that of normal pregnant women, and the expression rate of CD8+T cells was significantly higher than that of normal pregnant women, which were consistent with part of previous research[14~16], but different with the results of Qin Caijuan[17], Zhong Xiaohong and other people[18].In normal pregnancy women, C D8+T cells are in a dominant position, which slightly suppresses the maternal immune system. CD8+T cells increase significantly and CD4+/CD8+ decrease in preeclampsia, which suggests that the onset of preeclampsia may be related to the imbalance of CD4+T and CD8+T cells. The significant decrease of CD4+/CD8+ will abnormally activate B cells, and stimulate humoral immunity to produce IgG and other products, which form a membrane to attack complex that activates the complement system in an active state. The increase of Pyrolysis products such as C5a, C3a will result in the imbalance of the maternal immune balance, enhance the rejection of the fetal as allograft antigen, and weaken protective reaction. Under the action of a large number of abnormal cytokines, the maternal vascular endothelial cells are

damaged, showing the clinical symptoms such as hypertension, proteinuria and so on. Human leukocyte antigen (HLA) is a group of high polymorphism gene complex encoded by HLA gene complex, which conditions special reactions and allograft rejection. HLA also is a sensitive index to reflect the immune function of the body. The allogeneic antigen is presented by antigen presenting cells (APC) cells to CD4+T cell surface, which express on monocytes, activation of T lymphocytes, B lymphocytes, macrophages and other cells, initiate adaptive immune responses through different mechanisms. Pregnancy is a successful "semi-self" transplantation. The embryo begins to express HLA antigen about 5 weeks from fertilization. The immune system is formed at 20 weeks of the embryo. It plays cellular immunity function by producing a synthesis of immunoglobulin, induces the immune tolerance between mother and fetus, allows the fetus to avoid immune rejections which from maternal B lymphocytes and NK cells, blocks the lymphocyte pathway between maternal and fetal, then blocks lymphocyte mediated cytotoxicity and graft versus host reaction thereby maintains normal pregnancy. The lower expression rate of HLA-DR can cause the immune function disorder and even lead to immune paralysis. Proper expression of HLA-DR during normal pregnancy enhances the infiltration of trophoblast cells in the process of placental implantation and induces immune tolerance, which is beneficial to the trophoblast cells to avoid the recognition and attack of the parent body and then complete the vascular remodeling. However, the protective effect of blocking antibodies cannot be used in preeclampsia; it is vulnerable to the attack of cytotoxic T cells and NK cells, and then causes rejection to the fetus [19]. Poor expression of HLA takes part in the disorder of placenta formation in patients with eclampsia, causes the abnormality of 'vascular remodeling' of the uterine spiral artery and leads to placental ischemia and hypoxia. Therefore, HLA-DR is one of the factors that are closely related to the occurrence and development of preeclampsia. Oggé and other tests [20] showed that there was no significant difference between the level of HLA-DR in peripheral blood mononuclear cells of preeclampsia and healthy pregnant women. Lv Lianzheng's and others' studies[21] showed that the positive rate of HLA-DR expression in an intermediate mononuclear cell of preeclampsia was significantly higher than that in healthy pregnant women. And this study found that: The level of HLA-DR in peripheral blood of patients with severe preeclampsia significantly decreased, which suggested that maternal immune tolerance decreased in preeclampsia and inflammatory reaction at the maternal-fetal interface caused pathological pregnancy. The ability to tolerate immune recognition and the immune attack was weakened, so that abnormally invaded the uterine spiral artery, resulted in the placenta shallow implantation, caused a series of pathological changes in eclampsia. Previous studies have focused on the relationship of HLA-D / HLA-G antigen sharing rate and the occurrence and development of preeclampsia between the mother and the fetus. There is less study on the expression of HLA-DR in mononuclear cells of preeclampsia patients. We speculate that the reasons for the low level of HLA-DR are mainly because the study subjects we selected were in a serious condition, especially some of them had suffered from multiple organ dysfunction syndromes before entering the ICU, moreover, they were in a serious imbalance of immune balance.

In summary, higher expression of CD8$^+$T was found in peripheral blood of SPE patients which lead to T lymphocyte subsets imbalance The decrease of HLA-DR may contribute to the pathologic process of SPE.

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