

Original Article

Expression of CD11c+HLA-DR+dendritic cells and related cytokines in the follicular fluid might be related to pathogenesis of ovarian hyperstimulation syndrome

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Abstract: Objective: To explore the expressions of CD11c+HLA-DR+dendritic cells in the follicular fluid of patients with OHSS and their significances. Subjects: 100 individuals. Treatment: embryos were observed. The distribution of dendritic cells in follicular fluid and the levels of IL-10, IL-12, IL-18 and IL-23 in follicular fluid were detected. Methods: There were ovarian hyperstimulation syndrome (OHSS) group and control group in this study. The OHSS group consisted of 50 patients with OHSS and the control group consisted of 50 patients who underwent in vitro fertilization-embryo transfer (IVF-ET) only due to male factors. The statuses of embryos were compared between the two groups. The distribution of dendritic cells in follicular fluid was determined with flow cytometry, and the levels of IL-10, IL-12, IL-18 and IL-23 in follicular fluid were detected with enzyme-linked immunosorbent assay (ELISA) in all patients. Results: The two-pronuclear (2PN) fertility rate, high-quality embryo rate and available embryo rate were all significantly lower in OHSS group than in control group (all $P < 0.05$). The number of CD11c+HLA-DR+dendritic cells ($P < 0.05$) and the levels of IL-10, IL-12, IL-18 and IL-23 were all significantly higher in OHSS group than in control group (all $P < 0.01$). Conclusion: The follicular fluid of the patients with OHSS is in an inflammatory status, the inflammatory status may be involved in OHSS and the microenvironment of follicular fluid may affect oocyte quality and embryo development.

Keywords: Ovarian hyperstimulation syndrome, dendritic cells, cytokines, inflammatory reaction

Introduction

Ovarian hyperstimulation syndrome (OHSS), one of the major complications during assisted reproductive technology (ART), is characterized by multiple follicular development, increased ovaries and a series of clinical complications caused by extravasation of body fluid and protein into a third space. The pathogenesis of OHSS is still unclear. OHSS is associated with multiple follicular development and excessively increased ovaries. Follicular development and maturity, ovulation and luteinization are processes of alternating damage and healing occurring in a piece of controlled ovarian tissue [1]. Inflammatory factors in ovarian microenvironment play an important role in follicular and luteal development. Follicular fluid is a main part of ovarian microenvironment, and consists of visible components such as; granular cells and white blood cells, and invisible compo-

nents such as; estrogens, progesterone and some cytokines. The components of follicular fluid may reflect the statuses of ovarian hormone synthesis, oocyte maturity, ovulation and luteinization; so follicular fluid is strongly associated with fertilization and early embryo development [2]. The study on component of follicular fluid will be conducive to exploring the pathogenesis of related diseases. There are natural killer cells in follicular fluid, and the number of natural killer cells is related to the degree of oocyte maturity and the level of ovarian response to gonadotropin (Gn) [3, 4]. The follicular fluid also contains CD11c+HLA-DR+dendritic cells whose maturity degree is associated with the level of serum estrogens [5]. Dendritic cells are the most powerful antigen-presenting cells in human bodies. Mature dendritic cells can effectively activate naïve T cells, playing an important role in inflammatory reaction. Little research has been done on den-

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Table 1. Comparison of embryos between OHSS group and control group (n = 50, % or mean ± SD)

	Number of oocyte retrieval	Rate of mature oocytes (%)	2PN fertility rate (%)	2PN cleavage rate (%)	High-quality embryo rate (%)	Available embryo rate (%)
OHSS group	25.1±7.3	86.9 (1459/1678)	66.1 (964/1459)	96.0 (925/964)	58.2 (538/925)	60 (555/925)
Control group	11.4±7.2	86.7 (499/575)	70.9 (354/499)	97.0 (343/354)	70.8 (243/343)	72.7 (249/343)
P values	0.0000	0.92	0.045	0.43	0.0000	0.0000

Notes: OHSS: Ovarian hyperstimulation syndrome; 2PN: Two-pronuclear.

driftic cells in assisted reproductive technology, and the study on dendritic cells in follicular fluid of patients with OHSS has not yet been reported. It has since been unclear, the relation of the number of dendritic cells to follicular development. Therefore, we observed the expressions of dendritic cells and cytokines in follicular fluid of patients with OHSS in order to explore the pathogenesis of OHSS.

Materials and methods

This study involving the use of human tissue specimens was approved by Review Board of the First Affiliated Hospital of Zhengzhou University. The patient samples used in this study were obtained with informed consent.

Subjects

All subjects in this study were from the patients undergoing *in vitro* fertilization-embryo transfer (IVF-ET) in our reproductive center between May and November, 2014. The study included experimental group (OHSS group) and control group. The OHSS group consisted of 50 patients with OHSS who belonged to the patients with high ovarian response and had corresponding symptoms or signs. The diagnostic criteria of high ovarian response were either E2>3000 pg/ml on the day of human chorionic gonadotropin administration (hCG day) or oocyte retrieval >15. The corresponding signs or symptoms included abdominal distension, nausea, vomiting, diarrhea, rapidly increased body weight, oliguria or anuria, pachemia, hypovolemia, electrolyte disturbances, pleural effusion, hydropericardium, seroperitoneum, respiratory distress syndrome, thrombosis, even multiple organ failure [6]. The control group consisted of 50 patients who underwent IVF-ET only due to masculine factors. The mean age was 31.5±5.6 years in the OHSS group and 31.6±5.7 years in control group.

Collection of follicular fluid

On the day of oocyte retrieval, the first tube of follicular fluid obtained by transvaginal ultra-

sound-guided aspiration, was collected. The follicular fluid had no red blood cells to the human eye, and was not washed with G-MOPS. The follicular fluid was centrifuged at 350 g for 10 min. The supernatant fluid were respectively placed 2 ml-EP tubes for storage at -40°C. The cell component was used for analysis of flow cytometry.

CD11c+HLA-DR+dendritic cells in follicular fluid detected with flow cytometry

The samples were suspended at a concentration of $1-2 \times 10^6$ cells. Fluorescence-labeling monoclonal antibodies CD11c-PE, HLADR-ECD and CD45-PE-CY5 (Beckman Coulter, Cincinnati, USA) were respectively added into 100 µl of cell suspension. Correspondingly, control antibodies IgG1-PE, IgG1-ECD and CD45-PE-CY5 (Beckman Coulter, Cincinnati, USA) were respectively added as well into 100 µl of cell suspension. After 15 min-incubation in the dark, the samples were washed with PBS, underwent flow cytometry (Epics XL 4CLR flow cytometer, Beckman Coulter, Cincinnati, USA), and then analyzed with FlowJo software (Tree Star, USA).

Levels of IL-10, IL-12, IL-18 and IL-23 in follicular fluid determined by enzyme-linked immunosorbent assay (ELISA)

The levels of IL-10, IL-12, IL-18 and IL-23 were achieved according to the instructions of enzyme-linked immunosorbent kits (RD Company, Minnesota, USA).

Statistical analysis

Statistical treatment was performed with SPSS17.0 software. Measurement data were expressed as mean ± standard deviation, and were compared between the two groups with *t* test. Numeration data were expressed as rate (%), and were compared between the two groups with Chi square test or Fisher-exact probability test. Statistical significance was

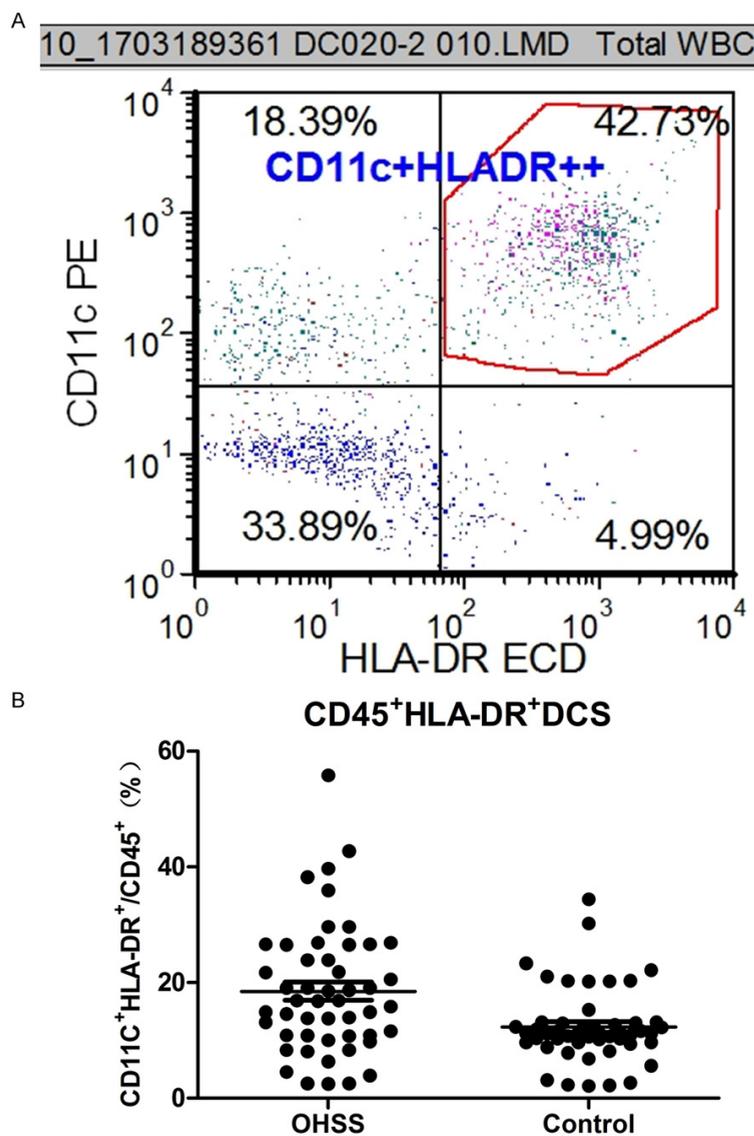


Figure 1. Distributions of CD11c+HLA-DR+dendritic cells in OHSS group and control group. A: The scatterplot of flow cytometry indicates CD11c+HLA-DR+dendritic cells accounts for proportion of CD45+leukocytes in the follicular fluid of the patients with OHSS. B: The scatterplot indicates the difference between OHSS group and control group in the number of CD11c+HLA-DR+dendritic cells ($P<0.05$). OHSS: Ovarian hyperstimulation syndrome.

established at $P<0.05$. Diagrams were performed with GraphPad Prism 5.0 software.

Results

Comparison of embryos between OHSS group and control group

Compared with the control group, the number of oocyte retrieval significantly increased in OHSS group ($P = 0000$). There was no statistical difference between OHSS group and control

group in mature oocyte rate and two-pronuclear (2PN) cleavage rate (all $P>0.05$). However, 2PN fertility rate, high-quality embryo rate and available embryo rate were all significantly lower in OHSS group than in control group (all $P<0.05$) (Table 1).

Distributions of CD11c+HLA-DR+dendritic cells in follicular fluid

The CD11c+HLA-DR+dendritic cells accounted for $18.68\pm 2.2\%$ in OHSS group and for $13.32\pm 1.6\%$ in control group. There was a statistical difference in the number of CD11c+HLA-DR+dendritic cells between OHSS group and control group ($P<0.05$) (Figure 1).

Levels of IL-10, IL-12, IL-18 and IL-23 in follicular fluid

The levels of IL-10, IL-12, IL-18 and IL-23 were all significantly higher in OHSS group than in control group (all $P<0.01$) (Table 2).

Discussion

With the further development of assisted reproductive technology, people have come to know much more about the reproductive process. The reproductive process is regulated by hormones and cytokines which form a complex

network system. Some cytokines influence on follicular development, oocyte maturation, ovulation, fertilization, and embryo development and implantation.

This study indicated that dendritic cells were present in follicular fluid of both OHSS group and control group, and the number of CD11c+HLA-DR+dendritic cells was significantly higher in OHSS group than in control group, suggesting that the CD11c+HLA-DR+dendritic

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Table 2. Levels of IL-10, IL-12, IL-18 and IL-23 in follicular fluid (n = 50, mean ± SD)

	Control group	OHSS group	P values
IL-10 (pg/ml)	16.76±3.13	23.00±2.14	0.000
IL-12 (pg/ml)	16.41±2.05	21.60±2.56	0.000
IL-18 (pg/ml)	145.04±18.22	183.92±15.24	0.000
IL-23 (pg/ml)	17.11±2.71	23.00±2.14	0.000

Notes: OHSS: Ovarian hyperstimulation syndrome.

cells are a part of microenvironment of preovulatory follicular fluid. Follicular fluid contains about 10% natural killer (NK) cells derived from CD45+leukocytes in the bone marrow [3]. In assisted reproductive process, the number of CD16+CD56+NK cells significantly increases, but the number of CD56+CD3+NKT cells significantly decreases in the follicular fluid of pregnant women, demonstrating that the distribution of lymphocytes in the follicular fluid is associated with oogenesis, oocyte maturation and pregnancy outcomes [7]. The proportion of CD45+dendritic cells in this study is higher than that of NK cells reported by Fainaru et al [3]. Dendritic cells are innate immune cells [8], and mature dendritic cells control the initial inflammatory reaction [9]. Dendritic cells regulate some ovulation-related inflammatory genes [10]. Under the stimulation of human chorionic gonadotropin (hCG), dendritic cells gather in preovulatory follicles, participate in the formation of corpus luteum, and up-regulate these genes which are necessary for hCG-induced ovulation such as *Star*, *Prg*, *Vcan* and *Adamts1* genes, and angiogenesis-related genes such as *VEGFa* and *VEGFc* genes [11]. This study also indicated that the number of CD11c+HLA-DR+dendritic cells and the levels of their related cytokines including IL-10, IL-12, IL-18 and IL-23 in follicular fluid were all significantly higher in OHSS group than in control group. Dendritic cells can effectively activate T cells and are involved in ovulation. Ovulation is also an inflammation-related process and the patients with OHSS manifest excessive ovulation, so dendritic cells may be involved in the pathophysiological process of OHSS. This provides a new idea and theoretical basis for the treatment of OHSS.

In this study, there were expressions of IL-10, IL-12, IL-18 and IL-23 in follicular fluid of all patients, and their expressions were higher in OHSS group than in control group. Cytokines

IL-10 and IL-12 are important for immunoregulation. IL-10 exhibits its depressive effect on immunity mainly through acting on T cells or dendritic cells [12]. IL-12 is mainly produced by dendritic cells. The functions of IL-23 are similar to that of IL-12. CD11c+HLA-DR+dendritic cells may promote the activation of effector T lymphocytes through releasing IL-23 and other inflammatory factors [14]. It has been reported that IL-12 is negatively correlated with oocyte maturity, fertility rate and embryo development [14, 15]. This is consistent with our results. Our results indicated that the fertility rate, high-quality embryo rate and available embryo rate were all lower in OHSS group than in control group, but the levels of IL-12 and IL-23 were all higher in OHSS group than in control group. This may be associated with the fact that IL-12 and IL-23 can promote the activation of T cells and activate the lethal effect of T cells. IL-18, also a factor of immunoregulation, is conducive to the production of some inflammatory cytokines, and promotes inflammatory reaction [16]. High IL-18 in follicular fluid is associated with OHSS, and the level of IL-18 in preovulatory follicular fluid is positively correlated with the number of oocyte retrieval [17]. Barak et al [18] have also found that IL-18 in both serum and follicular fluid of the patients with OHSS all increase, and can be used as a marker to predict the occurrence of OHSS. These are consistent with our results. This may be that in the patients with OHSS, ovarian inflammatory reaction is aggravated by development of excessive ovarian follicles, the aggravated inflammatory reaction increases immunoregulation-related factors and the increased immune factors in turn aggravate ovarian inflammatory reaction.

In summary, dendritic cells and their related inflammatory cytokines significantly increase in follicular fluid of the patients with OHSS, suggesting that the follicular fluid of the patients with OHSS is in an inflammatory status, the inflammatory status may be involved in OHSS and the microenvironment of follicular fluid may be associated with oocyte quality and embryo development.

Disclosure of conflict of interest

None.

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