

Application of plasma D-dimer detection in ovarian hyperstimulation syndrome

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Abstract

Background: This study investigated the changes of plasma D-dimer level and the role of its test in the pathogenesis of ovarian hyperstimulation syndrome (OHSS). Overall, 140 patients were divided into four groups based on OHSS status: normal, mild, moderate, and severe. All patients were hospitalized because of embryo transfer or OHSS. We retrospectively analyzed their clinical data, hormone levels, ovulation induction agents, and plasma D-dimer levels during the occurrence of OHSS and improved stages.

Results: Plasma D-dimer levels were significantly higher in the four groups with early onset of symptoms ($P < 0.05$; reference value, $0-0.5 \mu\text{g/mL}$) than in the normal group. With increasing symptoms of OHSS, D-dimer levels gradually increased in all four groups. There was a significant positive correlation between symptoms and D-dimer levels ($\gamma = 0.575$; $P < 0.05$), and the 95% confidence intervals of the three OHSS groups were $0.71-1.41 \mu\text{g/mL}$ (mild group), $1.65-2.26 \mu\text{g/mL}$ (moderate group), and $2.24-3.62 \mu\text{g/mL}$ (severe group). The cross-interval between the different groups of OHSS symptoms was minimal. When OHSS symptoms improved or healed based on the patient's clinical manifestations and laboratory parameters, the D-dimer levels of patients with moderate and severe symptoms remained significantly higher than the normal range ($P < 0.05$). The D-dimer levels in the improved stage in moderate and severe groups were significantly higher than those in the early stage ($P < 0.05$).

Conclusions: According to elevated D-dimer levels, we can more accurately predict the severity of OHSS in the early stage. Additionally, with further increases in D-dimer levels along with symptoms, we can more precisely determine the prognosis of patients.

Introduction

In the in vitro fertilization and embryo transfer (IVF-ET) process, controlled ovarian hyperstimulation (COH) is a routine step. However, ovarian hyperstimulation syndrome (OHSS) is a critical complication of COH. The incidence of moderate to severe OHSS is approximately 0.3–5%, and severe cases can be fatal. However, the pathogenesis of OHSS remains unclear. At present, it has been found that renin-angiotensin and other ovarian-derived renin-angiotensin system factors, inflammatory cytokines such as interleukins (interleukin-1, 2, 6, 8, 10), tumor necrosis factor (tumor necrosis factor- α), and vascular endothelial growth factor play important roles in the pathogenesis of OHSS¹⁻³.

From the current clinical application viewpoint, the detection of aforementioned cytokines or biologically active substances is still in the experimental stage. The lack of practiced large-scale clinical application and lack of experiments based on large sample size limit the application of a diagnostic criteria.

To find suitable laboratory test indicators to assist clinicians in making better accurate assessments of the prognosis and efficacy of OHSS, we reviewed and analyzed several laboratory test indicators, including plasma D-dimer levels of patients who were hospitalized because of embryo transfer or OHSS. We aimed to analyze the difference in plasma D-dimer levels with different degrees of OHSS, whether it

changes after different outcomes of OHSS and whether it can be used as a reference indicator for evaluating the prognostic efficacy of OHSS.

Materials And Methods

Research object

A retrospective analysis of 140 patients was performed. These patients were hospitalized for observation after embryo transfer or OHSS after undergoing IVF-ET stimulation cycle treatment at the reproductive medicine center of Ningbo women and children's hospital from January 2018 to December 2019. Based on the OHSS classification proposed by Golan⁴ in 2009 after summarizing the current classification of OHSS, patients were divided into four groups: normal, mild, moderate, and severe. The normal group included patients who have been assessed and have a high risk of ovarian hyperstimulation and have undergone relevant laboratory tests and preventive expansion treatments but have not shown clinical symptoms of OHSS. This study was approved by the Ethics Committee of Ningbo Women and Children's Hospital (No. 20183282). Written informed consents were obtained from all participants before the commencement of the study.

Laboratory index measurement method

To avoid the influence of infusion or coagulation and other factors on hematology test results, disposable blood collection tubes containing 109 mmol/L sodium citrate anticoagulant (BD Medical Equipment Co., Ltd., New York, USA) were used to collect blood samples in the morning, with the patient in a fasting state. The infusion was stopped for at least 5 hours. After the specimen was centrifuged at 3500 rpm for 10 minutes, plasma was separated and placed in the STA-R automatic coagulation analyzer (Diagnostica Stago, Paris, France) for detection. The reagents were imported from Diagnostica Stago (Paris, France), and plasma D-dimer was determined using the immunoturbidimetric method.

Statistical analysis

The experimental data were expressed as mean±standard deviation, and GraphPad 5.0 software (La Jolla, CA, USA) was used for statistical analysis of data. One-way analysis of variance was adopted for comparisons of data in line with normal distribution among groups; $P < 0.05$ was considered statistically significant.

Results

Analysis of clinical indicators

Among the four groups of patients, age, years of infertility, body mass index (BMI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), LH/FSH, the number of antral follicles in the basal state, gonadotropin (Gn) medication days and dosage in the ovulation induction cycle, E2 level on

the day of egg harvesting, and human chorionic gonadotropin (HCG) before egg retrieval were analyzed. There was no significant difference observed (Table 1).

Analysis of plasma D-dimer levels

Analysis in the early stage of OHSS:

The plasma D-dimer levels of each group were significantly higher than the normal high value (0.5 µg/mL) (Table 2). With the aggravation of OHSS, its level gradually increased, and there was a significant positive correlation between the two (correlation coefficient, $\gamma = 0.575$; $P < 0.05$). The comparison between groups showed that there was no significant difference between the normal and mild groups and that there were significant differences between the mild, moderate, and severe groups (Figure 1).

Analysis in the improvement stage of OHSS:

The plasma D-dimer levels in the normal and mild groups were higher than the normal range (0.5 µg/mL); however, there was no significant difference from the high value of normal range; those of moderate and severe groups were significantly higher than that in the normal group (0.5 µg/mL) (Table 2). With the aggravation of OHSS, the plasma D-dimer levels in each group gradually increased, and there was a significant positive correlation between the two ($\gamma = 0.333$; $P < 0.05$). The comparison between groups showed that there was a significant difference between severe and normal groups; there was no significant difference between the mild, moderate, and severe groups (Figure 1).

Analysis of early and improvement stages of OHSS:

The plasma D-dimer levels of the normal and mild groups showed no significant difference in early and improvement stages. The plasma D-dimer levels of moderate and severe groups were significantly higher in the improvement stage than in the early stage (Table 2).

Discussion

Owing to the development of multiple follicles after the use of ovulation drugs, blood E2 increased, plasma thrombin and fibrin levels increased, antithrombin level decreased, and blood vessel permeability increased, causing body fluid extravasation and increase in blood concentration. The patient was in a hypercoagulable state that could easily lead to deep thrombosis⁵. A study by Kodama et al.⁵ found that during the IVF-ET treatment cycle, the activation of the coagulation system occurred within 2 days after HCG injection, whereas the activation of the fibrinolytic system occurred later. The activation of these systems continued after OHSS, and the blood coagulation system returned to normal earlier than the fibrinolysis system. Therefore, the occurrence, development, and outcome of OHSS are accompanied by changes in coagulation function.

D-dimer is the final product of cross-linked fibrin after plasmin action. When blood clotting occurs in the body, thrombin acts on fibrinogen to convert it into cross-linked fibrin, and the fibrinolytic system is

activated to degrade cross-linked fibrin to form various fibrin degradation products. Due to cross-linking of the γ chain, two D-fragments containing the connected γ -chains are produced, namely, the formation of D-dimer fragments. Lowe⁶ pointed out that since D-dimer has good stability in plasma, it can be used as a sensitive and specific marker to confirm the presence of a hypercoagulable state and secondary fibrinolysis in the body. Therefore, D-dimer levels can be used as a significant indicator to observe changes in OHSS coagulation function.

This study found that the plasma D-dimer levels of each group in the early stages of disease increased significantly with the severity of OHSS. This suggested that with the aggravation of symptoms, the activation of coagulation and fibrinolytic systems in the body significantly increased. Moreover, the patients' risk of developing thrombotic diseases was significantly increased and was consistent with the severity of OHSS symptoms. With the relief of symptoms, the plasma D-dimer levels of patients in the moderate and severe groups became significantly higher than those in the early stages of disease. This result indicated that the coagulation system in the body has gradually returned to normal with the relief of OHSS symptoms, and the fibrinolytic system was continuously activated. The continuous increase in D-dimer levels indicated that the recovery of the fibrinolytic system had a lag period when compared with the symptoms.

Thrombosis is a serious complication of OHSS, which can be fatal. Thrombosis is a small portion of blood clot that forms on the surface of the exfoliation or repair of the inner surface of the cardiovascular system. It is composed of insoluble fibrin, deposited platelets, white blood cells, and trapped red blood cells.

During OHSS, when HCG is injected, the blood coagulation function in the body is activated, but not the fibrinolytic system. A large amount of fibrinogen in the body forms soluble cross-linked fibrin under the action of thrombin. When the delayed fibrinolytic system is activated, the cross-linked fibrin will be degraded while continuing to form, resulting in many D-dimer fragments in the patient's plasma, and the coagulation and fibrinolytic systems remain relatively balanced in the activated state. When OHSS enters the prognosis period, the coagulation system returns to normal before the fibrinolytic system, and the fibrinolytic system continues to activate, causing the patient's plasma D-dimer level to further increase. The hysteresis of this activation varies with the severity of OHSS.

The presence of OHSS in patients causes massive neovascularization, leading to massive vascular endothelial damage, increased vascular permeability, and increased blood concentration causing the concentration of platelets, white blood cells, and red blood cells in the blood to exceed the normal range. When the fibrinolytic system fails to activate on time or during clotting during OHSS, the relative balance between the system and fibrinolytic system in the activated state is damaged. This results in a large amount of cross-linked fibrin that cannot be degraded in time, which can easily lead to thrombotic diseases.

A study by Rao et al.⁷ showed that 74% of the patients who developed thrombosis during IVF treatment had OHSS symptoms. This suggests a change in coagulation function in OHSS patients. Plasma D-dimer is the most valuable indicator for observing the effect of fibrinolysis in vivo. The plasma D-dimer levels of patients in each group in this study were significantly higher than the normal range. Therefore, the large amount of cross-linked fibrin in the body cannot be easily ignored.

Targeted prevention of thrombotic complications in patients with OHSS were according to the following three major conditions of thrombosis: repair the vascular endothelial wound; volume expansion therapy to reduce the concentration of blood cells such as platelets, white blood cells, and red blood cells; and anticoagulation therapy accelerates the degradation of cross-linked fibrin in the blood.

According to the recommendations of the Royal College of Obstetricians and gynecologists⁸, preventive use of heparin is recommended for OHSS patients. There have been reports⁹ stating that anticoagulation therapy may be safe for continued pregnancy and embryos. However, there are reports¹⁰ suggesting that after the emergence of OHSS, intracranial venous thrombosis still occurs despite the preventive use of heparin.

This study has several limitations. First, selection and surveillance biases in our analysis could not be controlled owing to the retrospective study design of only 140 patients from a single academic institution. Second, although we excluded patients with inflammatory conditions, some hematological biomarkers may have been affected by the presence of unrecognized systemic inflammatory diseases.

Conclusions

In conclusion, even if anticoagulant drugs are used prophylactically during OHSS, the detection of coagulation function indicators, especially plasma D-dimer, cannot be ignored. This can be based on the degree of increase of D-dimer in the early stage of OHSS for more accurate detection of changes in coagulation function, assessment of the severity of overstimulation, and early application of anticoagulant drugs for preventive treatment. During the return period of OHSS, according to the further increase of D-dimer, combined with the patient's symptoms, the prognosis of the patient can be more accurately determined and the treatment plan can be adjusted on time. The increase in D-dimer indicates the hypercoagulable state in the patient, and other indicators need to be integrated to assess the risk of thrombosis in the patient.

For example, when OHSS patients have D-dimer $>1.65 \mu\text{g/mL}$ in the early stage of onset, they should be highly vigilant that they may have moderate or more severe OHSS symptoms in the later stage. These patients should postpone the subsequent embryo transfer surgery and implement whole embryo freezing to avoid possible pregnancy after embryo transfer, which will further aggravate OHSS symptoms. When OHSS patients have D-dimer $>2.25 \mu\text{g/mL}$ in the early stage of onset, it is necessary to be highly vigilant in the later stages of severe OHSS symptoms such as a large amount of pleural fluid and ascites, and early treatment measures should be taken to help the patient through the entire course of the disease.

Declarations

Ethics approval and consent to participate: The study was approved by the Ningbo Women and Children's Hospital review board Hospital (No. 20183282), and the requirement for written informed consent was waived owing to its retrospective design.

Consent for publication: Not applicable

Availability of data and materials: The dataset supporting the conclusions of this article is available upon request. Please contact Dr. Minbo Zhu (zhuminbo@163.com).

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Authors' contributions

LC performed data curation and formal analysis, obtained funding, conducted the investigation, planned the methodology, administered the project, and wrote the original draft of the manuscript. MZ performed data curation and formal analysis, conducted the investigation, planned the methodology, administered the project, and reviewed and edited the manuscript. MH conducted formal analyses and investigations, obtained resources, and performed validation. ZS conducted formal analyses and investigations, obtained resources, and performed validation. All the authors contributed to the conception and design of the study. All authors read and approved the final manuscript.

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Tables

Table 1: Comparison of clinical indicators of the four groups of patients (mean \pm standard deviation)

		Normal	Mild	Moderate	Severe	<i>P</i>
Number of cases	140	29	32	50	29	
Age	Years old	28.9 ± 3.7	28.7 ± 3.3	29.8 ± 3.6	28.8 ± 4.0	0.509
Years of infertility	Years	4.5 ± 2.1	3.9 ± 2.6	5.0 ± 3.3	4.3 ± 2.4	0.414
BMI	kg/m ²	21.5 ± 2.6	20.6 ± 2.5	21.2 ± 2.6	21.0 ± 2.1	0.555
Basic state FSH	IU/L	6.6 ± 1.4	7.1 ± 1.9	6.6 ± 1.7	7.1 ± 1.4	0.354
Basic state LH	IU/L	5.4 ± 5.5	5.5 ± 3.0	5.1 ± 3.1	5.8 ± 2.4	0.852
Basic state LH/FSH		0.792 ± 0.655	0.849 ± 0.592	0.819 ± 0.561	0.835 ± 0.369	0.976
Basic state E2	pmol/L	139.1 ± 45.7	159.0 ± 68.5	152.1 ± 60.7	118.4 ± 49.2	0.032
AFC		14.4 ± 4.5	13.7 ± 7.0	14.7 ± 6.0	14.5 ± 5.3	0.892
Gn medication days	Days	9.0 ± 1.3	9.5 ± 1.4	9.2 ± 2.3	8.3 ± 2.8	0.151
Gn dosage	U	1548.3 ± 415.6	1673.8 ± 466.0	1645.3 ± 552.9	1519.4 ± 379.7	0.512
Number of follicles (>14 mm)		14.0 ± 6.0	15.9 ± 5.6	13.7 ± 3.9	15.9 ± 6.9	0.183
HCG day E ₂ level	pmol/L	16268 ± 7946	20199 ± 6226	17730 ± 5733	18429 ± 6555	0.125
HCG day P level	nmol/L	4.4 ± 2.2	5.5 ± 1.8	4.9 ± 1.8	5.6 ± 2.8	0.112
Total number of eggs		17.8 ± 7.1	20.5 ± 8.1	18.3 ± 7.3	19.2 ± 8.1	0.499

Table 2: Measurement results of plasma D-dimer levels in the four groups (µg/mL, mean ± standard deviation)

		Normal	Mild	Moderate	Severe
	Number of cases (early/improvement)	29/6 [#]	32/13	50/43	28/28
Early stage of OHSS	139	0.65 ± 0.38*	1.06 ± 0.96*	1.95 ± 1.07**	2.93 ± 1.79***
<i>P</i> (0.5 µg/mL)		0.039	0.002	0.001	0.001
95% confidence interval: Early stage of OHSS		0.51– 0.79	0.71– 1.41	1.65– 2.26	2.24– 3.62
Improvement stage of OHSS	90	0.73 ± 0.69*	2.92 ± 5.18*	3.32 ± 1.92*	5.55 ± 5.00**
<i>P</i> (0.5 µg/mL)		0.450	0.118	0.001	0.001
<i>P</i> (early stage to improvement stage)		0.186	0.259	0.001	0.001

#: The results of the improvement stage of the normal group are the results obtained after the patients were hospitalized for observation and rechecked before being discharged.

*/**/***: The different “*” marks indicate that there is a significant difference between each group.

P (0.5 µg/mL): indicates the *P* value obtained by comparing with “0.5 µg/mL”

Figures

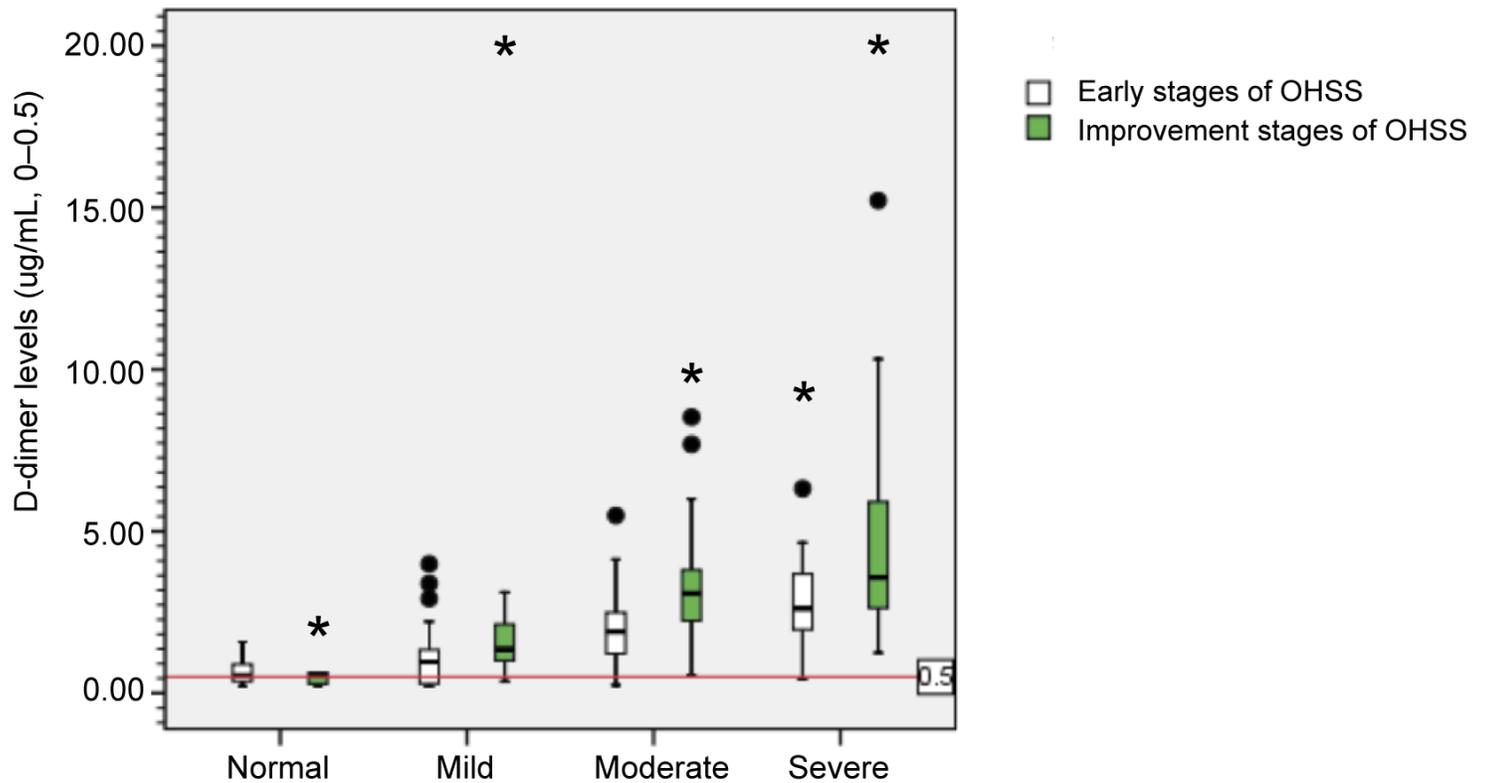


Figure 1

Measurement of plasma D-dimer level in four groups of patients (ug/mL) The top and bottom line segments in this box chart represent the maximum and minimum values of the data, respectively. The thick line segment in the middle of the box plot represents the median of the data, the upper and lower limits of the box plot are the first and third quartiles of this set of data, the top and bottom line segments respectively represent the normal distribution interval of this set of data, ● is an outlier in this set of data, ☒ is the extreme value generated in this set of data, and the red line is the high value of the normal range of this test item.