

Predictive Values of Serum Progesterone, Human Chorionic Gonadotropin and CA-125 for Pregnancy Outcomes of Threatened Abortion and Subchorionic Hematoma

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Objective: We aimed to study the predictive values of serum progesterone (P), human chorionic gonadotropin (HCG) and CA-125 for the pregnancy outcomes of threatened abortion complicated with subchorionic hematoma (SCH).

Materials and methods: Sixty women with threatened abortion and SCH in 6-10th gestational week, 60 with threatened abortion only and 60 healthy pregnant women were selected as groups A-C respectively. Levels of β -HCG, P, estradiol (E2) and CA-125 in peripheral venous blood were measured by chemiluminescence assay. Serum levels of INF- γ , TNF- α , IL-2, IL-4 and IL-10 were detected by ELISA.

Results: Serum β -HCG, P, CA-125 and E2 levels of group A were significantly lower than those in group C ($P < 0.05$). Groups A and B had significantly higher INF- γ , TNF- α and IL-2 levels but lower IL-4 and IL-10 ones than those of group C ($P < 0.05$). The hospitalization and vaginal bleeding times of group A were significantly longer than those of groups B and C ($P < 0.05$). The success rate of fetal protection, neonatal body weight and gestational age of groups A and B were significantly lower than those of group C ($P < 0.05$). Serum P, E2 and HCG levels were low in women with threatened abortion and SCH in early pregnancy, but CA-125 level was high, accompanied by increased INF- γ , TNF- α and IL-2 together with decreased IL-4 and IL-10.

Conclusion: The onset and progression of SCH may be related to imbalance between T lymphocyte factors towards Th1 direction.

Keywords: threatened abortion; subchorionic hematoma; progesterone; human chorionic gonadotropin; CA-125

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Threatened abortion refers to vaginal bleeding with or without lower abdominal pain before 28 weeks of gestation, most commonly manifested as vaginal bleeding. Clinically, serum progesterone (P), estradiol (E2), β -human chorionic gonadotropin (β -HCG) and ultrasonography were mainly used to evaluate embryo development. B-scan ultrasonography of some patients can disclose an anechoic zone between the gestational sac and intrauterine wall, referred to as subchorionic hematoma (SCH). The persistence of SCH can cause repeated vaginal bleeding and even uterine

contractions, resulting in inevitable abortion or evolution into placental abruption, with adverse pregnancy outcomes. SCH has been positively correlated with the spontaneous abortion rate of patients with threatened abortion, also negatively affecting pregnancy outcomes¹. Pearlstone and Baxi found in 14 SCH cases that compared with other high-risk factors, SCH more easily induced miscarriage². SCH increases not only the risk of miscarriage, but also the difficulty of fetal protection. The pathogenesis of SCH is complicated. The pregnancy process is dominated

by the tolerance of T lymphocytes at the maternal-fetal interface, and the abnormal behaviors of these cells are closely related to abortion. In recent years, CA125 has also been closely associated with threatened abortion. A higher level means more severe damage of decidual or chorionic cells and higher possibility of abortion. Therefore, it may be applicable to pregnancy outcome prediction³. In this study, we explored the predictive values of peripheral serum levels of P, HCG, CA125, Th1 cytokines and Th2 cytokines for the pregnancy outcomes of patients with threatened abortion complicated with SCH.

MATERIALS AND METHODS

Baseline clinical data

This study has been approved by the ethics committee of our hospital, and written informed consent has been obtained from all patients. From February 2015 to July 2018, 60 women with threatened abortion and SCH in the 6th to 10th week of pregnancy were selected as group A, with the average age of (26.3 ± 3.6) years old. Another 60 women with threatened abortion only in the 6th to 10th week of pregnancy were selected as group B, with the average age of (25.4 ± 2.6) years old. Meanwhile, 60 healthy pregnant women were selected as group C, with the mean age of (26.2 ± 2.6) years old.

Diagnostic criteria

1) With a small amount of vaginal bleeding after menopause, with or without lower abdomen pain, and without vaginal secretion discharge; 2) gynecological examination disclosed that the cervix was not open, the fetal membrane was not broken and the uterus size was consistent with gestational weeks; 3) with positive pregnancy test results; 4) B-scan ultrasonography disclosed a polygonal or irregular anechoic zone between the uterine wall and gestational sac.

Inclusion criteria

Group A: 1) 20~34 years old; 2) with threatened abortion symptoms; 3) ultrasonography revealed intrauterine pregnancy, 6~10 gestational weeks or fetal heartbeat and SCH; 4) without receiving any

medication for threatened abortion before this study.

Group B: 1) 20~34 years old; 2) diagnosed as early threatened abortion; 3) without history of fetal death, stillbirth, spontaneous abortion, autoimmune diseases, or internal/surgical diseases; 4) ultrasonography revealed intrauterine pregnancy, and 6~10 gestational weeks or fetal heartbeat, without SCH; 5) without receiving any medication for threatened abortion before this study.

Group C: 1) 20~34 years old; 2) without signs or symptoms of threatened abortion; 3) ultrasonography revealed intrauterine pregnancy, and 6~10 gestational weeks or fetal heartbeat, without SCH.

Laboratory examinations

On the day of diagnosis, 4 mL of venous blood was collected, from which serum was immediately centrifuged to measure the levels of β -HCG, P, E2 and CA-125 by chemiluminescence assay. All kits were purchased from Roche Diagnostics (USA). The expression of TNF- α , IL-2, IL-4, IL-10 and INF- γ were detected by ELISA as follows. Reaction strips were incubated with controls and serum in microwells for 30 min at room temperature, and rinsed 5 times. Afterwards, 100 μ L of enzyme conjugate was pipetted into each microwell, incubate for 15 min at room temperature, and rinsed 5 times. Then 100 μ L of TMB substrate solution was pipetted into each microwell, incubate for 15 min at room temperature, and added 100 μ L of stop buffer into each well. Finally, the optical density at 450 nm was measured to calculate the results⁴.

Statistical analysis

All data were analyzed by SPSS22.0 software. The categorical data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). Inter-group comparisons were performed by the independent sample t test. $P < 0.05$ was considered statistically significant.

RESULTS

Serum levels of β -HCG, P, CA-125 and E2

The serum levels of β -HCG, P, CA-125 and E2 in group A were significantly lower than those in group C ($P < 0.05$). There were no significant differences in serum β -HCG, P, CA-125 and E2 levels between groups A and B ($P > 0.05$) (Table 1).

Serum levels of TNF- α , IL-2, IL-4, INF- γ and IL-10

Serum INF- γ , TNF- α , and IL-2 levels in groups A and B were significantly higher than those in group C ($P < 0.05$). Serum IL-4 and IL-10 levels in groups A and B were significantly lower than those in group C ($P < 0.05$) (Table 2).

Hospitalization time and vaginal bleeding time

The hospitalization time and vaginal bleeding time of group A were significantly longer than those of groups B and C ($P < 0.05$). The times of groups A and B were also significantly different ($P < 0.05$) (Table 3).

Pregnancy outcomes

The success rate of fetal protection, neonatal body weight and gestational age of groups A and B were significantly lower than those of group C ($P < 0.05$). The success of fetus in group A and group B was successful. The values of groups A and B were also significantly different ($P < 0.05$) (Table 4).

DISCUSSION

Threatened abortion is a common complication of women during pregnancy. It usually occurs before 28 weeks of gestation, and its incidence is between 16% and 25%. Once a pregnant woman has a threatened abortion, a small amount of vaginal bleeding will occur, and there will be mild pain and backache in the lower abdomen. Vaginal bleeding and abdominal pain are the two main clinical manifestations. If not treated in time, it may lead to miscarriage. According to the survey, nearly half of the threatened abortion patients will eventually have miscarriage. There are many causes for vaginal bleeding, such as cervical cervix, ectopic pregnancy, trauma, cervical polyps and malignant tumors⁵. SCH is more common in patients with threatened abortion, mainly due to the separation of the chorionic villus and the sacral membrane, resulting in hemorrhage. A large amount of blood flows out between the chorion and the sacral membrane to form a hematoma, multiple hematoma margins. Both are connected to the intrauterine mouth^{6,7}. Therefore, the subdural hematoma, due to

hematoma rupture and bleeding, vaginal bleeding and other clinical symptoms, with or without abdominal pain, a small number of patients may have no obvious symptoms, and was missed. SCH can be seen under ultrasound. There is an anechoic zone between the patient's wall and the membrane, which is triangular, polygonal or crescent shaped. In recent years, the development of clinical medical technology, ultrasound examination technology has been continuously improved and popularized. Generally, patients with threatened abortion with vaginal bleeding symptoms will take B-ultrasound as a routine examination, so it is easy to find patients with SCH. The pathophysiological basis of early threatened abortion combined with SCH is still unclear. At present, clinical research remains at the high incidence of SCH and the observation of the influence of SCH on pregnancy prognosis. How to rise to the prevention and treatment level remains to be studied in large samples. This study explored the relationship between early threatened abortion and the relationship between INF- γ , IL-10 and serum β -HCG, P, CA-125, and E2 levels in early threatened abortion. Changes provide a basis for improving the efficacy of this type of refractory threatened abortion.

The serum β -HCG, P, E2 concentrations in group A and group B were similar, being significantly lower than those in group C. It is well known that among the endocrine factors, serum β -HCG, P, and E2 are important endocrine regulatory hormones in early pregnancy. Serum β -HCG is the main factor for maintaining normal pregnancy. It can promote the secretion of steroid hormones from the gestational corpus luteum of the ovary. E2 can promote blood supply, promote uterine myocyte proliferation and hypertrophy, and increase the synthesis of P in the placenta⁸. P can inhibit the maternal immune response to fetal antigens and maternal rejection of trophoblasts, and can reduce uterine smooth muscle excitability, inhibit uterine contraction, and facilitate embryo growth and development^{9,10}. CA125 is a tumor antigen marker derived from ovarian epithelial cells, genital tract mucosa and body cavity epithelium. Clinically, patients with ovarian cancer, endometriosis and endometrial cancer can detect elevated levels of CA125. At the same time, a large amount of CA125

is present in maternal decidua, amniotic fluid, and fetal chorion. The destruction and separation of decidual cells and chorion cells are the main sources of maternal serum CA125^{11,12}. The higher the serum CA-125 level, the greater the likelihood of miscarriage. This study found that serum CA125 levels in group A and group B were significantly higher than group C.

CD⁺ T cells divide two functional subgroups of Th1 and Th2 according to different types of secreted cytokines. Th1 cells secrete IL-2, IFN- γ , TNF- α , etc., which mainly mediate cellular immunity and produce immune damage to embryos, while Th2 cells secrete IL-4, IL-5, IL-6, IL-10, etc., assists B cells to produce antibodies, mediates humoral immunity and allograft rejection, inhibits the production of Th1 cytokines, and plays a role in maintaining pregnancy and promoting fetal growth and development¹¹⁻¹³. The Th1/Th2 balance has a great influence on the pregnancy outcome. The establishment of Th1/Th2 balance is beneficial to the implantation of fertilized eggs and the development of embryos and fetuses, so that pregnancy is normal¹⁴. The level of Th1 cytokines in recurrent abortion women is significantly higher than that in normal pregnant women, while the level of Th2 cytokines is significantly decreased, and the balance of Th1/Th2 is moved toward Th1. Unexplained recurrent spontaneous abortion patients with peripheral blood and decidual tissue IL-2 and other Th2 cytokines were significantly lower than the normal pregnancy group¹⁵. The Th1 cytokines TNF- α , IL-2 and IFN- γ in group A and group B were significantly higher than those in group C ($P < 0.05$). Th2 cytokines IL-4 and IL-6 in group A and group B were significantly lower than those in group C ($P < 0.05$). Thus, abortion in patients with SCH is related to the imbalance of Th cells, moving in the direction of Th1, and deviating from Th2, but the imbalance of Th1/Th2 may affect the occurrence of SCH or *vice versa*. The causal relationship of Th1/Th2 imbalance needs further research.

We also found that group A had lower success rate of fetal protection and neonatal body weight than those of groups B and C, suggesting that SCH increased natural abortion rate. Moreover, the

hospitalization time and vaginal bleeding time of the three groups of patients were studied. It was shown that the hospitalization time and vaginal bleeding time of group A patients were significantly longer than those of groups B and C, indicating that the patients with threatened abortion and SCH have uterine hemorrhage. This leads to an increase in vaginal bleeding time, which in turn increases hospital stay. Hence, patients with threatened abortion should be examined by ultrasound first, so as to detect the subdural hematoma early and actively stop bleeding, actively carry out the treatment of the fetus, prevent the subdural hematoma from further aggravating, thereby improving the success rate of the fetus and promoting a good pregnancy outcome.

In summary, close detection of serum progesterone, CA-125 and β -HCG levels in early pregnancy has predictive value for pregnancy outcomes. Furthermore, the balance of Th1/Th2 cytokines is necessary to maintain pregnancy physiology. Balance imbalance affects the maintenance and development of normal pregnancy, and its balance to Th1 development may lead to miscarriage.

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Table 1.
Serum levels of β -HCG, P, CA-125 and E2

Group	n	β -HCG (IU/mL)	P (ng/mL)	E2 (pg/mL)	CA-125 (U/mL)
A	60	1252 \pm 52.34	18.53 \pm 2.15	201.23 \pm 10.12	58.53 \pm 5.43
B	60	1318 \pm 51.23	19.23 \pm 3.12	213.52 \pm 10.23	45.52 \pm 5.12
C	60	6952 \pm 60.25*#	27.66 \pm 2.85*#	879.35 \pm 15.64*#	18.52 \pm 2.15*#

*Compared with group A, P<0.05; #compared with group B, P<0.05.

Table 2.
Serum levels of TNF- α , IL-2, IL-4, INF- γ and IL-10 (x \pm s, pg/mL)

Group	n	Th1 cytokine			Th2 cytokine	
		TNF- α	IL-2	INF- γ	IL-4	IL-10
A	60	4.57 \pm 0.14	0.91 \pm 0.03	2.52 \pm 0.12	0.52 \pm 0.03	4.51 \pm 1.23
B	60	4.11 \pm 0.13	0.85 \pm 0.02	2.48 \pm 0.02	0.50 \pm 0.02	4.50 \pm 1.13
C	60	1.12 \pm 0.12*#	0.15 \pm 0.01*#	0.13 \pm 0.03*#	2.85 \pm 0.05*#	13.52 \pm 3.54*#

*Compared with group A, P<0.05; #compared with group B, P<0.05.

Table 3.
Hospitalization time and vaginal bleeding time

Group	n	Vaginal bleeding time (d)	Hospitalization time (d)
A	60	18.52 \pm 3.25	29.52 \pm 4.52
B	60	17.52 \pm 3.51*	28.56 \pm 3.52*
C	60	1.52 \pm 2.51*#	3.59 \pm 2.51*#

*Compared with group A, P<0.05; #compared with group B, P<0.05.

Table 4.
Pregnancy outcomes

Group	n	Fetal protection		Gestational age (week, x \pm s)	Neonatal body weight (kg, x \pm s)
		Case No.	Rate (%)		
A	60	29	48.33	33.21 \pm 4.51	2.13 \pm 0.01
B	60	35	58.33	36.24 \pm 3.52*	3.02 \pm 0.03*
C	60	58	96.67	39.17 \pm 5.21*#	5.63 \pm 0.12*#

*Compared with group A, P<0.05; #compared with group B, P<0.05.