Predictive value of fetal fibronectin on the embryonic loss of patients with recurrent spontaneous abortion in early pregnancy

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Summary

Objective: This work aims to investigate the predictive value of fetal fibronectin (fFN) in embryonic loss of patients with recurrent spontaneous abortion (RSA) in early pregnancy. *Materials and Methods:* Eighty-four patients with RSA in early pregnancy were selected as the test group and 31 healthy women in early pregnancy were selected as the control group. The ages and number of previous abortions, along with other information, were recorded. These patients underwent a fFN test, and their pregnancy outcome was followed up until 14 weeks. *Results:* The incidence of spontaneous abortion was 20.24% in the test group and 9.68% in the control group. The positive fFN [fFN (+)] rate was 57.14% in the test group and 12.90% in the control group, indicating a statistically significant difference ($p < 0.01$, $\chi^2 = 17.89$). The incidence of spontaneous abortion was 29.17% (14/48) in the fFN (+) group and 8.33% (3/36) in the fFN (-) group, indicating a statistically significant difference ($p < 0.05$, $\chi^2 = 5.53$). The sensitivity, specificity, and positive and negative predictive values in the prediction of abortion in fFN (+) patients of the test group were 82.35%, 49.25%, 29.17%, and 91.67%, respectively. *Conclusion:* If detected at an early stage of pregnancy, fFN in patients with RSA is largely related to the prediction of abortion and facilitates the evaluation of pregnancy outcomes.

Key words: Fetal fibronectin; Recurrent spontaneous abortion; Early pregnancy.

Introduction

Spontaneous abortion is the termination of pregnancy before 28 weeks. The incidence of spontaneous abortion among those with estimated fetal weight < 1,000 g covers about 15% of the total number of pregnancies. The majority of spontaneous abortion occurs before 14 weeks of pregnancy. Spontaneous abortion usually arises within 12 weeks of pregnancy (most within eight weeks) and seldom arises after 12 weeks of pregnancy. Recurrent spontaneous abortion (RSA) refers to two or more consecutive abortions, which has an incidence rate of about one to five percent, and tends to arise within 12 weeks of pregnancy [1-3]. Repeated abortion brings serious adverse effects to women's physical and mental health. Thus, related research on RSA has become one of the hotspots in reproductive medicine.

Few methods for clinically predicting the pregnancy outcome of patients with RSA during early pregnancy are known. The B-type ultrasonic examination of the fetal heartbeat is a reliable index for predicting a favorable pregnancy outcome. However, the fetal heartbeat is only detectable at a specific stage of pregnancy, thereby limiting the capability of the method to predict the pregnancy outcome during early pregnancy. Nevertheless, previous research on fetal fibronectin (fFN) has provided a new concept for the clinical prediction of pregnancy outcomes of patients with RSA during early pregnancy.

fFN is an isoform of the fibronectin secreted by decidua containing the carcinofetal fragment III-CS and identifiable by the monoclonal antibody FDC-6 in an fFN kit. In the embryonic implantation and placentation process, fFN interacts with its receptor, regulates the activity level of proteolytic enzymes, and functions in the implantation of fertilized eggs and in the connection and adhesion of placental villi and decidua [4]. Immunohistochemical analysis showed that fFN usually exists in the interface of the amnion, placental tissue, chorion, and decidua, is an important extracellular matrix, and can reflect the growth of chorionic trophoblast cells and placenta. Throughout pregnancy, fFN has an important role in maintaining the integrity of the extracellular matrix in the placenta and the stability of the maternal–fetal interface. As an independent predictor, fFN does not depend on gestational weeks and has specific predictive value at different stages of pregnancy [5-7]. However, most studies have focused on the application of fFN in the prediction of premature delivery. Under normal conditions after 24 weeks of pregnancy, fFN should not be detected in the secretions of the posterior fornix (fibronectin < 0.5 μg/ml). A fFN-positive [fFN (+)] result suggests increased risk of premature delivery [8]. Few applications of fFN research during early pregnancy are known.

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Previous studies have shown that the positive rate of FFN is very high in patients with early spontaneous abortion, which is probably due to an immune dysfunction mediated by abundant lymphocytes in decidualized interstitial cells; and by abnormal changes in the decidua and cytokines secreted by the decidua. The latter, in turn, causes proteolytic destruction at the chorion-decidua interface, as well as the release of extracellular matrix protein of the chorion and deciduas into the cervical and vaginal secretions [9, 10]. These findings prove the possibility of predicting abortion using fFN. However, previous studies either used a small sample size or lacked a control group, the opposites of which being necessary in further deepening related research. The present research was conducted to investigate the predictive value of fFN on the embryonic loss of patients with RSA in early pregnancy using a control study, thereby providing clinical evidence for early prevention and individualized treatment.

Materials and Methods

Objects and grouping

Eighty-four patients diagnosed with RSA during early pregnancy from August 2008 to February 2009 were selected as the test group. Thirty-one healthy women diagnosed in the present hospital during early pregnancy were selected as the control group. The pregnant women were grouped according to the following criteria: (1) < 12 weeks pregnancy; (2) no symptoms of threatened abortion, such as abdominal pain and colporrhagia; (3) no obvious abnormalities, such as subchorionic hematoma, as observed through B-type ultrasonic examination; (4) no abnormal findings in the bacteriological examination of vaginal secretion; (5) no sexual activity and gynecological examination within 24 hours; (6) no developmental malformation of the genital system; (7) no gynecological diseases, such as chronic cervicitis and cervical polyps; (8) no severe multi-system diseases; (9) no bad habits such as smoking and drinking; and (10) no history of taking prescription medicine. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Sun Yat-sen University. Written informed consent was also obtained from all participants.

Sample collection

The vaginas of the pregnant women in lithotomy position were opened with disposable vaginal speculums. A special swab provided in the fFN kit was gently dipped in the cervicovaginal secretions in the posterior fornix for about ten seconds. The swab was removed and its head was inserted and fully mixed into the buffer for ten to 15 seconds. The fFN test bar was removed from the aluminum foil bag and the marking end of the test bar was directly inserted into the buffer. Ten minutes later, the test bar was removed and the results were read. A negative result was indicated by one line, whereas a positive result was indicated by two lines with a deep or shallow color, suggesting that the fFN content in the sample was higher than 50 ng/ml. However, if the quality control line did not appear, the test failed and would have to be repeated.

Index recording

In general, the age, gestational week of sampling, number of pregnancies, and fFN test results were recorded. Pregnancy outcome: all pregnant women were followed up until 14 gestational weeks, and their nuchal translucency (NT) color Doppler ultrasound results for the 12th and 13th gestational weeks were followed up by telephone. A favorable heartbeat obtained during B-type ultrasonic examination suggested that the pregnant women would successfully tide over early pregnancy, and fetal blastocolysis results during B-type ultrasonic examination suggested spontaneous abortion and pregnancy failure. The sensitivity of fFN in predicting abortion in fFN (+) patients (true positive rate) in the test group was determined by calculating the proportion of fFN (+) patients to the total number of abortions. The specificity of predicting abortion in fFN (+) patients (true negative rate) refers to the proportion of fFN (-) patients to the total number of regular pregnant women. The positive predictive value (accuracy of positive predictive results) refers to the proportion of practical abortions to the number of fFN (+) patients. The negative predictive value (accuracy of negative predictive results) refers to the proportion of normal pregnancies to the number of fFN (-) patients.

Statistical analysis

SPSS 13.0 for Windows was used to conduct statistical analysis. The incidence and morbidity rates were expressed as percentages (%). The t-test was used for data measurement; and the chi-square test was used to compare the ratios. A $p < 0.05$ denoted statistical significance.

Results

General data

Test group: The samples were aged 24 to 43 years, with the average number of pregnancies at 3.57 ± 0.66. The fFN was tested at the gestational age of 35 to 66 days. The control group was composed of patients aged 20 to 39 years, with the average number of pregnancies at 3.24 ± 0.47. The fFN was tested at the gestational age of 39 to 67 days. No statistically significant differences were found for age, gestational age, and number of pregnancies ($p > 0.05$, Table 1).

Pregnancy outcome and fFN test

Seventeen pregnant women in the test group had spontaneous abortions, which brought the incidence rate to 20.24%. On the other hand, three pregnant women in the control group had spontaneous abortions, bringing the incidence rate to 9.68% in the group. The difference was not statistically significant.

Forty-eight patients (57.14%) from the test group were fFN (+), whereas four patients (12.90%) were fFN (+) in the control group. A statistically significant difference was found between the positive fFN rates in the two groups ($p < 0.01$, $\chi^2 = 17.89$).

In the test group, the incidence of spontaneous abortion was 29.17% (14/48) in patients with fFN (+), but only 8.33% (3/36) in patients with fFN (-), denoting a statistically significant difference ($p < 0.05$, $\chi^2 = 5.53$). In the control group, the incidence of spontaneous abortion was 25% (1/4) in patients with fFN (+) but only 7.41% (2/27) in patients with fFN (-), as shown in Table 2.

Table 1. — *Comparison of general clinical data.*

	Test group (n = 84)	Control group (n = 31)	*p*
Age (years)	31.55 ± 5.00	27.90 ± 5.87	0.122
Gestational age (days)	51.10 ± 11.01	56.0 ± 7.13	0.254
Number of pregnancies (times)	3.57 ± 0.66	3.24 ± 0.47	0.196

Table 2. — *fFN results and pregnancy outcome in two groups.*

fFN test result	Abortion ratio in the test group	Abortion ratio in the control group
Positive	29.17 (14/48)	25.00 (1/4)
Negative	8.33 (3/36)	7.41 (2/27)

Table 3. — *Sensitivity and specificity in prediction of early abortion in fFN-positive pregnant women.*

Group	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Test group	82.35 (14/17)	49.25 (33/67)	29.17 (14/48)	91.67(33/36)
Control group	33.33 (1/3)	89.29 (25/28)	25.00 (1/4)	92.59(25/27)

Table 4. — *The pregnancy outcome, age, and number of previous abortions in patients with recurrent spontaneous abortion.*

Items	Number (n)	Abortion ratio (%)	*p*
Age ≤ 35 (years)	70	18.57 (13/70)	> 0.05
Age > 35 (years)	14	28.57 (4/14)	
Number of previous abortions ≤ 2	73	19.18 (14/73)	> 0.05
Number of previous abortions > 2	11	27.27 (3/11)	

Prediction of abortion in fFN (+) patients

The sensitivity, specificity, and positive and negative predictive values in the prediction of abortion for the fFN (+) early pregnancy group with RSA were 82.35%, 49.25%, 29.17%, and 92.67%, respectively. Those for the normal early pregnancy group were 33.33%, 89.29%, 33.33%, and 92.59%, respectively, as shown in Table 3.

Relationship between the pregnancy outcome and age and number of previous abortions in patients with RSA

After a systematic examination of the cause of spontaneous abortion and treatment for the RSA group, no statistically significant difference was found between the incidence of abortion in pregnant women with RSA aged ≤ 35 years and > 35 years ($p > 0.05$, 18.57% vs. 28.57%, respectively). No statistically significant difference was also observed between the incidences of abortion in pregnant women with two previous abortions and in pregnant women with more than two previous abortions ($p > 0.05$, 19.18% vs. 27.27%, respectively; Table 4).

Discussion

RSA is one of the frequently observed diseases in obstetrics. Multiple previous abortions increase the risk of abortion of the subsequent pregnancy. A recent large sample study on Scottish women showed that the risk of abortion in the present pregnancy is increased among women with one and two previous abortions. On the contrary, the incidence of three previous abortions does not increase the risk of abortion during the next pregnancy [11]. The use of indexes that can effectively predict the risks of embryonic loss will facilitate the clinical diagnosis and treatment of RSA.

Previous studies showed that fFN has a great potential and application value in the prediction of embryonic loss. As an important extracellular matrix secreted by decidua, fFN plays an important role in the implantation of fertilized eggs, as well as in the connection and adhesion of placental villi and decidua [12].

Furthermore, a study found that the dynamic expression of fibronectin presented in early pregnancy plays an important role in the morphological differentiation of the endometrial matrix in mice [13].

In the present study, the positive rate of fFN in patients with RSA in the early pregnancy group was significantly higher than that in the control group. The sensitivity and negative predictive value for predicting abortion among fFN (+) patients were as high as 82.35% and 91.67%, respectively. Consequently, fFN, which has a lower likelihood of providing a missed diagnosis, was used as an index to predict the occurrence of abortion in the next pregnancy of pregnant women with a history of abortion. A negative result has great significance for the exclusion of the probability of abortion. Conducting a fFN test on RSA patients in the early stage of pregnancy, facilitates the assessment of the pregnancy outcome. fFN (+) results during early pregnancy possibly indicate poor uterine receptivity and suggest higher risks of adverse pregnancy outcome.

Previous studies have indicated that the incidence of abortion is related to the age and number of previous abortions of pregnant women. Munnes *et al.* proved through a multicenter retrospective study of the age and number of abortions of pregnant women that the risk of abortion increases with age [14]. An epidemiological study conducted in Japan also showed that both the age and number of previous abortions of pregnant women affects subsequent pregnancy outcomes [15]. After in vitro fertilization and embryo transplantation, the incidence of early abortion was also found to increase with age [16]. Most scholars consider that age reduces the reproductive capacity of women mainly because of the effect of age on the oocytes [17], namely, 1) the number of oocytes; and 2) the quality of oocytes gradually decreases with age (i.e., the ovum is aging). Furthermore, intracytoplasmic peroxidation is enhanced, and oxygen free radicals increase with age, thereby damaging the nuclear and mitochondrial DNA. This

process affects meiosis and chromosome assortment, resulting in chromosomal abnormalities in the spermatozoon, ovum, or embryo, as well as increasing the ratio of clinical spontaneous abortions [18-21].

In the present study, the analysis of the age and number of previous abortions of pregnant women showed that after the systematic examination of the cause of abortion and comprehensive treatment of patients with RSA, the abortion ratio of the test group was 18.57% in pregnant women aged ≤ 35 years and 28.57% in pregnant women aged > 35 years, for which no statistically significant difference was found. However, the findings show that the abortion ratio in pregnant women aged > 35 years significantly increased. The incidence of abortion in pregnant women with two previous abortions was 19.18% in pregnant women with two previous abortions and 27.27% for those with more than two previous abortions, suggesting that the risk of abortion slightly increases with age and number of abortions, which is consistent with the findings of previous studies [22].

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