

Relation between mycoplasma infection and recurrent spontaneous abortion

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Abstract. – OBJECTIVE: This study explores the possible relation between cervical infections with *Ureaplasma urealyticum* (UU) and *Mycoplasma hominis* (MH) and the occurrence of recurrent spontaneous abortion (RSA).

PATIENTS AND METHODS: 132 patients with RSA (observation group) and 96 normal pregnancy volunteers undergoing planned abortions (control group) were selected successively and enrolled in the investigation. Cervical secretion samples were obtained for each subject. Bacterial cultures were started to detect UU, MH and other bacterial infections, and fluorescence quantitative PCR was used to detect gene copy number in chorion and decidual tissues. Additionally, ELISA (enzyme-linked immunosorbent assay) was used to detect anticardiolipin antibody (ACA) to rate positivity of Immunoglobulin M (IgM) and IgG in secretions, and Western blot was applied to quantify the expression levels of Interleukin (IL)-6, tumor necrosis factor- α (TNF- α), prostacyclin (PGI2) and bax/bcl-2.

RESULTS: Our results showed the UU, MH, and overall bacterial infection rate of chorionic and decidual tissues, and the gene copy number of UU, MH were higher in the observation group than those in the control group ($p < 0.05$). Moreover, the ACA-IgM and IgG positive rates in secretions of the observation group were significantly higher than those in the control group ($p < 0.05$). Finally, the expression levels of IL-6, TNF- α , PGI2, and bax/bcl-2 were higher than those in the control group as well ($p < 0.05$).

CONCLUSIONS: Our results support the notion that RSA might be associated with UU and MH infection, could influence the occurrence of other bacterial infections and could stimulate ACA expression, inflammatory response, thrombogenesis, and factors associated with cell apoptosis, increasing the risk for an abortion during pregnancy.

Key Words:

Ureaplasma urealyticum, *Mycoplasma hominis*, Recurrent spontaneous abortion, Bacterial infection, Anticardiolipin antibody, Inflammatory response, Prostacyclin, Cell apoptosis.

Introduction

Recurrent spontaneous abortions (RSA) occur in about 5 to 20% of pregnant women, and 20 to 40% of RSA cases suffer more than 3 abortions. To make matters worse, 10 to 15% of patients cannot get pregnant again after undergoing related treatments, which brings about serious adverse effects on the patients and their families¹. The pathogenesis of RSA consists of multiple mechanisms involving genetic or immunological abnormalities, embryonic development disorders, secondary inflammatory responses, cell apoptosis stimulation, genital tract malformations, infections, and more². It has been proposed that *Ureaplasma urealyticum* (UU) and *Mycoplasma hominis* (MH) infection may be a common cause of RSA³. In a previous study⁴, it was found that the rate of UU and MH colonization in patients with RSA was higher than that in normal pregnancy groups, and the infection rate increases in the patients experiencing higher numbers of spontaneous abortions. UU and MH infection have also been related to an arrest of embryonic development⁵. UU and MH are opportunistic pathogens of the urogenital tract that can coexist with multiple bacterial infections and alter the microenvironment in the uterine cavity affecting the normal development of the embryo⁶. This work further analyzed if UU and MH infection coexisted with embryonic inflammation, and looked for signs of immune, thrombotic and apoptotic mechanisms, providing a theoretical basis for RSA *Mycoplasma* infection theory.

Patients and Methods

Patients

132 patients diagnosed with RSA in our hospital from June 2012 to June 2016 were selected successively for the observation group, and 96

normal pregnant volunteers with normal abortion plans during the corresponding period were enrolled for the control group. Those patients whose pregnancies were complicated with a definite pathogene, those suffering from *Toxoplasma gondii*, *Cytomegalovirus*, *Herpesvirus hominis* and *Rubivirus* infections, those using topical vaginal medications and those participating in intercourse within three days of the abortion were excluded. All the patients signed the informed consents. The study was approved by the Ethics Committee of Maternal and Child Health Care Hospital.

The average age of patients in the observation group was 25.6 ± 5.7 years. There were 101 cases with 2 abortions and 3 cases with three abortions. And the spontaneous abortions occurred during gestational weeks 6 to 14, with an average of 10.2 ± 3.5 weeks. In the control group, the average age of the individuals was 23.8 ± 5.6 years. The planned abortions took place during gestational weeks of 4 to 13 with an average of 9.5 ± 2.7 weeks. No significant differences were found when comparing the ages and gestational weeks of the patients in the different groups ($p > 0.05$).

Research Methods and Clinical Observations

Cervical Secretion Samples were Obtained for Each Patient.

Bacterial cultures were used to detect UU and MH in cervical secretions. Bacterial culture reagents were bought from Jiangsu Beyotime Science and Technology Ltd (Jiangsu, China). For *Mycoplasma* cultures each sample was inoculated into a culture bottle, which was sealed and placed in the isolated constant temperature incubator to culture for 48 hours. The nutrient solution was yellow in negative samples and turned clear or translucent red in positive samples. For other germs, a rapid detection kit was bought from Guangdong Zhongshan Tianyang Bioelectronics Ltd (Guangdong, China). The saline pendant-drop method was used to screen for *Trichomonas* and *Candida* under the microscope. A swab was used to inoculate the reaction liquid tube; after a 10 min reaction time at 37°C , the result was read using a standard color card: yellow and green colors were negative non-neuraminidase reactive samples, blue showed the positive elevated neuraminidase activity samples. The *Mycoplasma* gene detection kit was bought from Sigma-Aldrich (St. Louis, MO, USA).

Fluorescence quantitative Polymerase Chain Reaction (PCR) was performed to measure the

gene copy number of the pathogens in chorion and decidua tissues. The PCR thermocycler used was bought from Applied Biosystems (Foster City, CA, USA), and the protocol was run according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay (ELISA) detected Immunoglobulin M (IgM) and IgG anticardiolipin antibodies (ACA) in secretions. An ELISA kit was bought from Golden Bridge Biology (Beijing Zhongshan, Beijing, China), and the microplate reader from Bio-Rad (Hercules, CA, USA).

Finally, Western blot was used to quantify the expression levels of Interleukin (IL)-6, tumor necrosis factor- α (TNF- α), prostacyclin (PGI₂), and bax/bcl-2 in secretions. Briefly, a radioimmuno-precipitation assay (RIPA) lysate was made out of each sample to extract the proteins. Coomassie brilliant blue was applied to quantify the total protein content. Detection of β -actin with antibody for each sample was used to standardize the amount of each protein detected by Western blot. 30 μg of total protein were used for electrophoretic separation by 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins in the gel were transferred to a polyvinylidene difluoride (PVDF) membrane. Mouse anti-human IL-6, TNF- α , PGI₂, bax, and bcl-2 monoclonal antibodies (1:2000, R&D Systems, Minneapolis, MN, USA) were incubated with the membrane overnight. Next day, after washing, rabbit anti-mouse polyclonal antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was incubated for 4 hours at room temperature. Then, after the final wash with phosphate-buffered saline (PBS), Enhanced chemiluminescence (ECL) was used for coloration and detection. The results were scanned to save the evidence, and the Lab Works4.5 gel-imaging software (Invitrogen, Carlsbad, CA, USA) was used for semiquantitative analysis measuring the integral optical density (IOD) of each sample and normalizing to the β -actin content.

Statistical Analysis

The SPSS20.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) software was applied to conduct the statistical analysis. Measurement data is shown by mean \pm Standard Deviation, and independent-samples *t*-test was applied for comparison among groups. Enumeration data was expressed by case number or rate (%), and χ^2 -test was used for comparison among groups; $p < 0.05$ was taken to mean that a given difference had statistical significance.

Results

Detection of UU, MH and Bacterial Infection in Tissues

The positive rate of bacterial infections with UU or MH in chorion and decidual tissues and the gene copy numbers for UU and MH were significantly higher in the observation than in the control group, and the average difference found had a statistical significance ($p < 0.05$) (Table I).

Detection of ACA-IgM and IgG Positivity Rate in Secretions

The positivity rates for ACA-IgM and IgG of secretions in the observation group were significantly higher than that in the control group ($p < 0.05$) (Table II).

Detection of IL-6, TNF- α , PGI2 and bax/bcl-2 Expression Level in Secretions

The expression levels of IL-6, TNF- α , PGI2, and bax/bcl-2 were also significantly higher than those in the control group, and the differences had a statistical significance ($p < 0.05$) (Table III).

Discussion

According to Verteramo et al⁷, about 50% of pregnant women present bacterial vaginal canal

infections, and in about 80% of those, UU or MH are the causative agents. Due to the atypical clinical symptoms present in most cases, the infections remain undiscovered or get misdiagnosed. Some colonizing bacteria may induce abortion by generating mucinase or fumarase enzymes, which can alter the function of the non-specific immune system locally and increase reproductive tract infections and an inflammatory response⁸. Lipoidase and protease enzymes secreted by some bacteria may dissolve the cow's lipid and protein components, resulting in a thinner membrane that may result in a spontaneous abortion⁹. Finally, other bacteria can invade into the embryonic tissues, resulting in deciduitis, chorioamnionitis, cowl fragility, and abortion¹⁰. UU can metabolize urea to generate NH₄ that disturbs the acidic genital tract environment, creating more favorable conditions for ascending bacteria to infect the uterine cavity and the fetus¹¹. UU can also change the cellular morphology of the placenta by inducing mitochondrial dysfunction, bringing about an abnormal placental energy metabolism and nutrient transport leading to ischemia and anoxia of the fetus¹².

We found that the positivity rate for UU, MH, and other bacterial cultures of chorion and decidual tissues and the gene copy numbers of UU and MH in the same tissues for the patients in

Table I. Detection of UU, MH and bacterial infection in tissues.

Groups	Observation group (n=132)		Control group (n=96)	
	Chorion	Decidua	Chorion	Decidua
UU [cases (%)]	59 (44.7)	52 (39.4)	8 (8.3)	6 (6.3)
MH [cases (%)]	44 (33.3)	46 (34.8)	4 (4.2)	5 (5.2)
Bacteria [cases (%)]	30 (22.7)	25 (18.9)	2 (2.1)	3 (3.1)
UU ($\times 10^6$ /ml)	5.2 \pm 1.3	5.4 \pm 1.5	0.5 \pm 0.2	0.6 \pm 0.2
MH ($\times 10^6$ /ml)	4.1 \pm 1.4	3.7 \pm 1.2	0.3 \pm 0.1	0.2 \pm 0.1

Table II. Detection of ACA-IgM and IgG positive rate in secretions [number of cases (%)].

Groups	Observation Group (n=132)		Control Group (n=96)	χ^2	p
IgM	50 (37.9)	6 (6.3)	30.007	0.000	
IgG	55 (41.7)	8 (8.3)	30.883	0.000	

Table III. Detection of IL-6, TNF- α , PGI2 and bax/bcl-2 expression levels in cervical secretions.

Groups	Observation Group	Control Group	t	p
IL-6	0.44 \pm 0.15	0.16 \pm 0.07	5.236	0.000
TNF- α	0.38 \pm 0.09	0.12 \pm 0.06	5.124	0.000
PGI2	0.35 \pm 0.13	0.08 \pm 0.02	5.526	0.000
bax/bcl-2	0.72 \pm 0.24	0.34 \pm 0.11	5.857	0.000

the observation group were significantly higher than those for patients in the control group. *Mycoplasma* infection is associated with a weak immune function in its host. Spontaneous abortion, premature delivery, premature rupture of membranes, and pregnancy complications can all occur at increased rates in cases of *Mycoplasma* and bacterial infections¹³. RSA increases damage to the endometrium and may lead to metrorrhagia and chronic inflammation. The defense capability of the organism declines with each occurrence of spontaneous abortion, due to the inflammatory response that ensues; a large number of mononuclear macrophages induce activation of T lymphocytes leading to apoptosis stimulation and an autoimmune response generating multiple inflammatory cytokines and resulting in many instances in a spontaneous abortion¹⁴. The positivity rate in secretions for ACA-IgM and IgG in the observation group was significantly higher than that in the control group, and the expression levels of IL-6, TNF- α , PGI2, and bax/bcl-2 were significantly higher in the observation group as well. ACA is closely related to the antiphospholipid antibody (APS), which plays an important role in RSA¹⁵. ACA can combine with phospholipids in the membranes of endothelial and platelet cells inducing platelet aggregation and thrombus formations¹⁶, which in turn can inhibit the generation of PGI2 and cause placental circulation disturbances¹⁷. UU and MH have a strong adsorption capacity that can allow them to colonize the female's genital tract. The incidence of RSA is related to an abnormal inflammatory response in the uterine cavity and embryonic tissues¹⁸. Elevated levels of TNF- α can result in the inhibition of the corpus luteum function and the activation of nitric oxide synthases (NOS). NO synthesis increases, and NO excess damages endothelial cells and inhibits the generation of decidual blood vessels, leading to ischemia in the embryo¹⁹. In addition, UU can produce phospholipase A2 that can disintegrate embryonic cells and generate prostaglandins, inducing the release of multiple inflammatory factors and can also result in a spontaneous abortion²⁰. There is at least one report²¹ on the literature associating abortions to the effects of apoptosis of chorion and decidual tissues. The balance of bax/bcl-2 can stimulate or inhibit cell apoptosis. With the apoptosis-promoting factor bax increasing, the NF- κ B gets activated, and the resulting induced caspase cell signaling transduction pathways lead to apoptosis^{22,23}.

Conclusions

To sum up, based on our results, RSA may be associated with UU and MH infection. We propose a mechanism in which the *Mycoplasma* infection generates a cascade of events including increased ACA expression, inflammatory response, thrombogenesis, and cell apoptosis leading to the occurrence of a spontaneous abortion. To reduce the incidence of RSA, it would be very helpful to use a sensitive and stable screening method for *Mycoplasma* that would help identify pregnancies in a high-risk group of RSA in the early stages, ensuring the adoption of (like *Mycoplasma* infection eradication) prompt preventive measures.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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