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Evaluation of the effect of vaginal infections with Mycoplasma genitalium on antimullerian hormone (AMH) levels and its relationship with abortion by molecular methods

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Abstract

Introduction:
Mycoplasma genitalium is a sexually transmitted bacterium that causes 15 to 25% of male nongonococcal urethritis and is associated with cervicitis and pelvic inflammatory disease in women.The aim of this study was to investigate the effect of vaginal infections with Mycoplasma genitalium on the level of antimullerian hormone (AMH) and its relationship with abortion by molecular methods in Urmia.
Methods: In this study, microbiological methods were used to culture samples suspected of mycoplasma and also to increase the sensitivity to diagnose mycoplasma infections and possible association with the amount of antimullerian hormone in women in the age range of delivery. Genus and species of Mycoplasma RFLP-PCR was used.
Results: From 500 intrauterine samples of pregnant women samples, extracted DNA, after PCR, 125 sample were positive. The culture results of the samples also showed that 80 cases grew after 2 weeks. 70% of PCR-confirmed samples had a history of miscarriage and 25% of antimullerian hormone levels were abnormal. Genotyping results indicated that the dominant mycoplasma was Mycoplasma genitalium strain G37 of the type.

Conclusion:
In general, it can be said that Mycoplasma genitalium It can be one of the possible causes of abortion. PCR assay based on 16S rRNA gene sequences a valuable and reliable technique for identification Mycoplasma genitalium to find the cause of spontaneous abortions is on the other hand, the present study showed that all Mycoplasma genitalium isolates were identical differences in enzyme patterns of this bacterium after PCR-RFLP was not observed and all were of the Mycoplasma type the genitalia were G37.
Key words:
vaginal infections, Mycoplasma genitalium, antimullerian hormone, abortion

Introduction:
Mycoplasma genitalium is a sexually transmitted bacterium that causes 15 to 25% of male nongonococcal urethritis and is associated with cervicitis and pelvic inflammatory disease in women(1). It can also be associated with cervicitis, pelvic inflammatory disease, and tubular factor infertility in women(2, 3). Women may also experience bleeding after sex and is also associated with infertility factor tube(4). For men, the most common symptom is painful urination or watery discharge from the penis. The disease is strongly associated with persistent and recurrent nongonococcal urethritis (NGU), which accounts for 15 to 20% of all symptomatic NGU cases in men(5-7). The genome of M. genitalium consists of 525 genes. in one circular DNA of 580,070 base pairs(8). There is a persistent association between M. genitalium infection and female reproductive system syndromes. The 16 mycoplasmal /ureaplasma species considered to be of human origin, respectively, according to the first discovery or naming report. Most of them are in the respiratory tract or genitourinary tract, at least four of them, including M. genitalium, show pathogenic properties(1, 9). M. genitalium infection was significantly associated with an increased risk of preterm birth, spontaneous abortion, cervicitis and pelvic inflammatory disease. In addition, this pathogen may lately infect the placental villi tissue of pregnant women, thus affecting the outcome of pregnancy(10). The risk of infertility is also strongly associated with infection with M. genitalium, although evidence suggests that it is not associated with male infertility(11). Recent research shows that Mycoplasma genitalium is currently more prevalent than other mycoplasmas (sexually transmitted infections)(1). The currently recommended treatment for M. genitalium infection is a long course of oral macrolide. Azithromycin Single-dose treatment for M. genitalium infection is not optimized with a therapeutic response and, if treatment is unsuccessful, is associated with the development of M. genitalium macrolide resistance(12, 13). NGU-positive therapeutic trials have shown that only one gram dose of azithromycin is more effective than doxycycline when administered at a dose of 100 mg twice daily for 7 days. However, a single dose of azithromycin is associated with 15% -30% of treatment failure(14-19). The aim of this study was to investigate the

effect of vaginal infections with Mycoplasma genitalium on the level of antimullerian hormone (AMH) and its relationship with abortion by molecular methods in Urmia.

Methods:

Culture

After patient satisfaction, endocervical sampling was performed. After swab sampling, they were placed in PPLO broth medium. After 2-3 days incubation, the samples were removed by syringe. Then pass through 0.2 micron filters and a few drops of the liquid were poured On PPLO agar medium containing 15% yeast extract, 20% horse serum, penicillin G(Sigma), and cycloheximide(Sigma). They were incubated for several weeks at 35.5 ° C inside the candle jar. After examination of the specimens, we observed the growth of Nebula-shaped colonies in some specimens (Figure1).

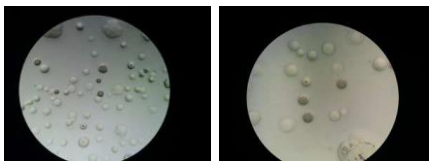

Figure 1: Mycoplasma-shaped hemispherical colonies

Hormoneology

On the other hand, the levels of Anti-Müllerian hormone (AMH) in positive samples were also measured by Electro Chemi Luminescence (Rosch, Cobas e411). Molecular detection The PCR reaction was considered at a final volume of 30 µl. Each sample includes: 15µL mix Master 2X 20 pmol, DNA template containing 1 µgr, 1.5mM MgCl2 from each primer F, R, Taq polymerase enzyme and distilled water double sterile distillation was up to 30 µl. Primers used in our laboratory 16S rRNA genes were selected. The PCR process in a thermocycler consists of 5 minutes 95 degrees Celsius, followed by 30 cycles, 30 seconds at 94 degrees Celsius, 60 seconds at 56 degrees celsius primer binding step and 60 seconds at 72 degrees Celsius and finally 5 minutes at 72 degrees celsius was done.

UuF 5-TGG AGT TAA GTC GTA ACA AG-3´

UuR 5-CTG AGA TGT TTC ACT TCA CC-3´

To evaluate PCR products from gel electrophoresis 1.5% agarose and DNA staining with sybr green dye were used. Gels using the device documentation Gel were examined (Figure 2) and finally the PCR product is obtained 465 bp fragment (Mycoplasma genitalium for confirmation).Sequencing was performed. Also finally using descriptive statistics methods were analyzed.

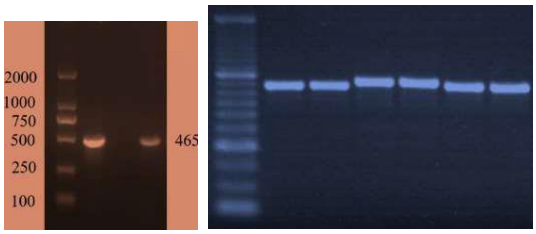

Figure 2: 465 bp fragment of Mycoplasma genitalium

PCR-RFLP

After receiving Mycoplasma DNA sequence genitalium, using its BLAST genotype program alginated with sequenced genome sequences. Sequence the study was given to Cutter Web software and list of enzymes plus locus of effect of enzymes different constraints were obtained PCR products to perform RFLP-PCR, PCR products obtained from Mycoplasma genitalium underthe influence of enzymes Cac8I, BbsI, EcoRI, AluI, TaqI limited effect were located. To perform an enzymatic cleavage, first react 15µL below for positive samples prepared: 6.3µL sterile distilled water, 1/5 µL buffer enzyme,0.2 µL limited effect of enzyme and 7 µL PCR product. After preparing the above reactions separately, microtypes containing all but one enzyme Taq I at 37 °C and microtubes contains Taq I enzyme in thermocycle at 65 ° C were exposed for 2 hours. Next from 2 hours of RFLP product on 3% agarose gel comes with marker and PCR product without enzyme limited effect Taq I were electrophoresed

Results:

From 500 intrauterine samples of pregnant women samples, extracted DNA, after PCR, 125 sample were positive. The culture results of the samples also showed that 80 cases grew after 2 weeks. 70% of PCR-confirmed samples had a history of miscarriage and 25% of antimullerian hormone levels were abnormal. Which RFLP-PCR results showed then from enzymatic sections of PCR product clinical samples positive, a difference in the enzyme cleavage patterns obtained did not exist and all had a pattern. Genetically heterogeneous Mycoplasma genitalia and infections can occur in different people on the one hand, they are Mycoplasma genitalium G37. necessary It is mentioned that the obtained sequence is the product of PCR by genome sequencing of *Mycoplasma genitalium* strain G37 The title of the reference prototype sequence are the same, therefore It can be said that the most common species is Mycoplasma genitalium G37.

Conclusion:

In general, it can be said that Mycoplasma genitalium It can be one of the possible causes of abortion. PCR assay based on 16S rRNA gene sequences a valuable and reliable technique for identification Mycoplasma genitalium to find the cause of spontaneous abortions is on the other hand, the present study showed that all Mycoplasma genitalium isolates were identical differences in enzyme patterns of this bacterium after PCR-RFLP was not observed and all were of the Mycoplasma type the genitalia were G37. Also, to find out the relationship between antimullerian hormone and mycoplasma infection, the level of this hormone was measured in all patients, but there does not seem to be a relationship between this hormone and mycoplasma infection. In our study, 70% of people with Mycoplasma infection had a history of miscarriage. Some also had a history of multiple miscarriages. Using quantitative real-time PCR, the prevalence of M. genitalium was 1.5% in 473 women at low risk for sexually transmitted diseases. These studies show that M. genitalium is a common cause of sexually transmitted diseases in men and women and is a cause of infertility in infected people(20). In Iran, Amir Mozafari et al. Reported that in 210 genital swabs taken from 210 patients, mycoplasma strains were isolated from 39.5% of patients using a selective mycoplasma separator medium, but using PCR in 57.1% of patient samples. Mycoplasma is positive. In our study, PCR showed that the overall prevalence of M. genitalium infection in patients was (25%). PCR is very sensitive because sex-specific primers are used to detect genital mycoplasma compared to culture(21).

In our study, valuable mycoplasma 16S rRNA genes were used, which can be used to achieve good results. In Cuba, Mondeja et al using a culture method in Vero cell suspensions, 16S ribosomal RNA, and MgPa1–3 PCR showed that all 11 isolates that were detected as M. genitalium with the culture method were also positive for M. genitalium with PCR(22). PCR is commonly used to diagnose M. genitalium infections because it has been shown to be more sensitive, specific, and faster than conventional culture methods(23, 24). In Mirnejad et al's study, genital swabs were taken from 210 women in Tehran, Iran, and, using PCR the frequency of M. genitalium was 3.3%. The prevalence of C. trachomatis and M. genitalium in pregnant women of Sabzevar (north-east of Iran) in Haghighi Hasanabad et al's study was determined in 196 urine specimens by duplex PCR. A total of 31 (15.81%) specimens were positive, with C. trachomatis in 27 (13.7%), M. genitalium in two (1.02%), and coinfection with both in two (1.02%) specimens. Also, significant correlation was found between preterm labor and infection(23). Differences of the results in some studies may be due to the type of population (pregnant vs. nonpregnant), sample types (urine vs. swab), date of study (recent vs. past), as well as the detection methodologies.

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